Theme:

Food Innovation: Trends, Impacts and Solution for a Sustainable Future

13 - 14 June 2024

PROCEEDINGS

26

FOOD INNOVATION ASIA CONFERENCE 2024 (FIAC2024)

DRAFT

Food Innovation Asia Conference (26th FIAC 2024)

Food Innovation: Trends, Impacts and Solution for a Sustainable Future

DRAFT

13 – 14 June 2024

BITEC, Bangkok, THAILAND

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CONFERENCE PROGRAM

Day 1: June 13, 2024 @ GH202

Time	Program
09:00-09:50	**Welcome Remark** By *Associate Professor Dr. Suwit Wuthisuthmethavee* *Vice President, Walailak University, Thailand* **Welcome Remark** By *Associate Professor Dr. Yuthana Phimolsiripol* *Dean of Faculty of Agro-Industry, Chiang Mai University, Thailand* **Welcome Remark** By *Assistant Professor Dr. Arunya Mingmuang* *Dean of Faculty of Agricultural Product Innovation and Technology, Srinakharinwirot University, Thailand* **Opening Ceremony** By *Assistant Professor Puangpetch Nitayanont* *President of the Food Science and Technology Association of Thailand (FoSTAT)* **Awards Presentation** **Ajinomoto - FoSTAT Young Food Scientist Award** *Associate Professor Dr. Thunnalin Winuprasith* *Deputy director of the Institute of Technology and Innovation Management (iNT), Mahidol University*
	Group Photo
09.50-10.35	**Keynote Speech 1:** **Phenolic and bioactive ingredients from valorized plant products-The importance of regenerative food innovation** By *Professor. Dr. Charles Brennan* *Chief Scientific Director – Food & Nutrition Innovation Hub STEM College, Royal Melbourne Institute of Technology, Australia*
10.35-10.50	**Break 15 min**
10.50-11.20	**Keynote Speech 2:** **Innovation for Asia based premier oils: case studies on rice bran oil and fragrant oils** By *Professor. Dr. Xuebing Xu* *Chief Scientist - Wilmar (Shanghai) Biotechnology Research and Development Center Ltd., China*

Time	Program		
11.25-11.55	**Keynote Speech 3:** **Utilization of food hydrocolloids and colloidal systems for food texture modification and nutrient delivery** *By Associate Professor Dr. Thunnalin Winuprasith* *Deputy director of the Institute of Technology and Innovation Management (iNT), Mahidol University, Thailand*		
12:00-13:00	**Lunch**		
13:15-16:30	**Oral Presentation** **Division A-1:** Food Chemistry, Nutrition, and Analysis (Room: MR218-219) **Division D:** Food Microbiology, Food Biotechnology, Fermentation (Room: MR215) **Division E:** Related Topics (Food Packaging, Food Safety & Quality, Food Laws & Regulations, Food Policy, etc.) (Room: MR216) **Division F:** Molecular Sensory Science for Food Industry (THAI language) (Room: MR217)	13:00-16:30	**Poster Session 1** **Poster Presentation Competition**
16:00-18:00	**FIFSTA Meeting** (Room: MR220)		

Day 2: June 14, 2024

Time	Program		
08:45-12:30	**Oral Presentation** **Division B:** Food Processing and Engineering (Room: MR213) **Division C:** Food Product Development, Sensory, and Consumer Research (Room: MR218-219) **Division F:** Hygienic Design for Improving the Productivity of Food Processing (THAI language) (Room: MR217) **Oral Presentation Competition** (Room: MR215)	09:00-12:00	**Poster Session 2**
09:00-12:00	**FoSTAT Food Innovation Contest 2024** "Food for Crisis" (Room: Grand Hall 202)		
12:00-13:00	**Lunch**		
13:00-15:00	**AIAC Meeting** (Room: MR217) **Oral and Poster Awards Presentation**		
13:00-16:30	**Oral Presentation** **Division A-2:** Food Chemistry, Nutrition, and Analysis (Room: MR218-219)	13:00-16:30	**Poster Session 3**
13.30-13.45	**Special Sharing**: Coffee with editor – Maybe eating more local food is what we need: qualitative views on plant-based food among Thai consumers. *By Professor. Dr. Charles Brennan* *Editor in Chief of the International Journal of Food Science and Technology, RMIT University, Australia* (Room: MR222)		
14.00-15.30	**Special Sharing**: How to write good research papers and How it could boots H-index? *By Professor. Dr. Mohsen Gavahian* *National Pingtung University of Science and Technology, Taiwan* (Room: MR222)		
13:00-16:30	**FoSTAT Food Innovation Contest 2024** "Food for Crisis" (Room: Grand Hall 202)		

Oral Presentation Program

Division A: Food Chemistry, Nutrition, and Analysis

Day 1: June 13, 2024 (Room: MR218-219)

Time	Code	Topic
13:15-13:25		**Introduction of the Division A – Food Chemistry, Nutrition, and Analysis** **Chair:** Associate Professor Dr. Utai Klinkesorn (Kasetsart University, Thailand) **Co-Chair:** Assistant Professor Dr. Suphat Phongthai (Chiang Mai University, Thailand) Dr. Prae Charoenwoodhipong (Srinakharinwirot University, Thailand)
13:25-13:55	Invited speaker #1	**Bioactive Peptides with Antihypertensive and Antibacterial Activity Derived from Food Industrial By-Products** *By Associate Professor Dr. Jirawat Yongsawatdigul (Suranaree University, Thailand)*
13:55-14:25	Invited speaker #2	**Edible Insects: Decoding the Chemical Compositions for Sustainable Nutrition** *By Professor. Dr. Sirithon Siriamornpun (Mahasarakham University, Thailand)*
14:25-14:40	AB-O088	**Unravelling the Phytochemical Composition and Antioxidant Activity of Ethanolic Kratom Leaf Extract** *By Udana Eranda (Walailak University, Thailand)*
14:40-14:55	AB-O194	**Gelation-Related Biochemical Parameters for Myofibrillar Proteins of Thai Ligor Hybrid Chicken and Broiler Meats** *By Ngassa Julius Mussa (Walailak University, Thailand)*
14:55-15.10	AB-O113	**Unraveling the Bioactive Content, Antioxidant Capacity, and Anti-Diabetic Activity of Three Papaya Leaf Extracts at Different Stages of Maturity** *By Sirinet Chaijan (Walailak University, Thailand)*
15.10-15:20	**Break 10 min**	
15.20-15.35	AP-O212	**Impact of Protein Concentration on the Stability of Emulsions and Characteristics of Plant-Based Fat Analogs** *By Sophana Pech (Ubon Ratchathani University, Thailand)*
15:35-15:50	AP-O208	**Effects of Banana Flour as Wheat Flour Substitution on Physicochemical Properties in Financier** *By Passachol Atthakorn (King Mongkut's Institute of Technology Ladkrabang, Thailand)*
15:50-16:05	AB-O036	**Profiling of Free and Glycosidically Bound Volatile Compounds and Food Components in Shiikuwasha (*Citrus depressa*) Fruits from Okinawa, Japan** *By Yonathan Asikin (University of Ryukyus, Japan)*
16:05-16:20	AB-O027	**Genetic Characteristics and Volatile Aroma Compounds of Leaf and Fruit Edible Part of *Citrus depressa* from Kagoshima, Okinawa, and Taiwan** *By Moena Oe (University of Ryukyus, Japan)*

Day 2: June 14, 2024 (Room: MR218-219)

Time	Code	Topic
13:00-13:05		**Introduction of the Division A – Food Chemistry, Nutrition, and Analysis** **Chair:** Associate Professor Dr. Utai Klinkesorn (Kasetsart University, Thailand) **Co-Chair:** Professor. Dr. Manat Chaijan (Walailak University, Thailand) Assistant Professor Dr. Suphat Phongthai (Chiang Mai University, Thailand)
13:05-13:20	AP-O080	**Exploring the Functional and Structural Properties of Four Types of Pulse** *By Phuriwat Tuiman (Ubon Ratchathani University, Thailand)*
13:20-13:35	AB-O091	**A Comparative Study on Quality Evaluation of Selected Edible Oils in the Sri Lankan Market** *By Chamila Madushani Ranasinghe (University of Ruhuna, Sri Lanka)*
13.35-13:50	AJ-O216	**Changes in Nutritional and Anti-Nutritional Compounds During the Formation of Lamtoro Gung (*Giant Leucaena*) ACE-I Peptide through Germination** *By Aprilia Fitriani (Universitas Ahmad Dahlan, Indonesia)*
13.50-14:05	AB-O177	**Production of Egg Yolk Hydrolysate with Multifunctional Activities and Their Stability** *By Natcha Tanyakaew (Suranaree University of Technology, Thailand)*
14:05-14:20	AP-O175	**Enhancing Okara Protein Concentrate Production using Ultrasonic Pretreatments** *By Athippat Thanaratkuljarern (Naresuan University, Thailand)*
14.20-14.30	**Break 10 min**	
14:30-14:45	AJ-O231	**Physicochemical Characteristics of Catfish (*Clarias Batrachus*) Bone Powder Induced by the Addition of Maltodextrin and Gelatin** *By Amalya Nurul Khairi (Universitas Ahmad Dahlan, Indonesia)*
14:45-15:00	AP-O180	**Valorization of Fish Head By-Product through Enzymatic Hydrolysis** *By Poowanat Surachaisatikul, Photawan Chaodee, Polchanok Methakulanart (Thammasat university, Thailand)*
15.00-15.15	AP-O055	**Effect of Protein Glutaminase Deamidation on Functional Properties of Pea Protein Isolate** *By Panattida Siripitakpong (Chulalongkorn University, Thailand)*
15.15-15.30	AB-O051	**Physicochemical Properties and Storage Stability of OSA Taro and Potato Modified Starch-Stabilized Pickering Emulsions in Chocolate Spread** *By NABILAH BINTI Abdul Hadi (Universiti Malaysia Terengganu, Malaysia)*
15.30-15.45	AJ-O220	**Fatty Acid Profile, Physical Properties and Organoleptic Acceptance of Chocolate Spread with Varying Types and Volumes of Oil as Cocoa Butter Substitutes** *By Titisari Juwitaningtyas (Universitas Ahmad Dahlan, Indonesia)*

Division B: Food Processing and Engineering

Day 2: June 14, 2024 (Room: MR213)

Time	Code	Topic
08:50-09:00		**Introduction of the Division B – Food Processing and Engineering** **Chair:** Associate Professor Dr. Weerachet Jittanit (Kasetsart University, Thailand) **Co-Chair:** Assistant Professor Dr. Yongyut Chalermchat (Chiang Mai University, Thailand) Assistant Professor Dr. Srivikorn Ditudompo (Srinakharinwirot University, Thailand)
09:00-09:30	Invited speaker #1	**Biopolymeric Encapsulation System to Enhance the Stability: Oral Bioaccessibility and Bioavailability of Bioactive Food Components** *By Professor. Dr. Anil Kumar Anal (Asian Institute of Technology, Thailand)*
09:30-10:00	Invited speaker #2	**Valorization of Agricultural By-Products though Emerging Extraction Technique** *By Associate Professor Dr. Mohsen Gavahian (National Pingtung University of Science and Technology, Taiwan)*
10:00-10:15	BP-0040	**Encapsulation of Betacyanin from Red Dragon Fruit Peels Using Maltodextrin and Pectin: Impact on Physicochemical Properties** *By Rahmat Widianto (Chulalongkorn University, Thailand)*
10:15-10:30	BP-0136	**Effect of Particle Size Classification on Pasting Properties of Cassava Starch** *By Phumpitug Rakrueang (Chulalongkorn University, Thailand)*
10.30-10.40	**Break 10 min**	
10:40-10:55	BB-0141	**Effect of Steaming and Chilling Processes on Quality of Dried Gluten-Free Mung Bean Pasta** *By Soklay HENG (Kasetsart university, Thailand)*
10:55-11:10	BB-0142	**Egg White Powder Production and Characterization: A Foam Mat Drying Approach** *By Narisara Janpleng (Mahasarakham University, Thailand)*
11:10-11:25	BP-0229	**Effects of Ultrasound and High-Pressure Pretreatments Combined with Supercritical CO_2 Extraction on Oil Yield and Properties of House Cricket (*Acheta domesticus*)** *By Nantawan Boonmee (Naresuan University, Thailand)*
11:25-11:40	BP-0228	**Chemical Compositions and Functional properties of Raw, Roasted and Autoclaved Tiger Peanut (*Arachis hupogaea L.*) Powder from Mae Hong Son, Thailand** *By Nichanan Kaewnualpan (Srinakharinwirot university, Thailand)*
11:40-11:55	BJ-0223	**Utilization of Viscozyme L as Pre-treatment during Production of Quinoa Protein Isolate** *By Desak Putu Ariska Pradnya Dewi (Indonesia International Institute for Life Sciences, Indonesia)*

Division C: Food Product Development, Sensory, and Consumer

Day 2: June 14, 2024 (Room: MR218-219)

Time	No.	Topic
08:50-09:00		**Introduction of the Division C – Food Product Development, Sensory, and Consumer Research** **Chair:** Assistant Professor Dr. Piyaporn Chueamchaitrakun (Mae Fah Luang University, Thailand) **Co-Chairs:** Associate Professor Dr. Niramon Utama-ang (Chiang Mai University, Thailand) Associate Professor Dr. Narumol Matan (Walailak University, Thailand)
09:00-09:25	Invited speaker #1	**Aroma of Change, Flavor of Responsibility, Sip Sustainability** *By Miss Naruemon Taksaudom (Hillkoff Co., Ltd., Thailand)*
09:25-09:50	Invited speaker #2	**New Trends and Challenge in the Global Tea Industry** *By* Assistant Professor Dr. *Piyaporn Chueamchaitrakun (Mae Fah Luang University, Thailand)*
09.50-10:15	Invited speaker #3	**Science Based Technology: Sensomics Approach: Case Studies on Kokumi Substances in Thai Traditional Fermented Freshwater Fish (Pla-ra)** *By Dr. Apiniharn Phewpan (CPF Food Research and Development Center Co., Ltd., Thailand)*
10.15-10.30	CB-O134	**Physicochemical Evaluation and Sensory Profiling of Protein-Crosslinked Instant Noodles Enriched with Rice Bran at Varying Particle Sizes** *By Kurnia Ramadhan (Universitas Bakrie, Indonesia)*
10:30-10:40	**Break 10 min**	
10.40-10.55	CB-O178	**An Innovative Fermented Beer from Premature Bamboo Shoots (*Bambusa vulgaris*)** *By Sathmi Amasha Amaradisi (University of Ruhuna, Sri Lanka)*
10.55-11:10	CJ-O238	**The Potential of Fishbone Flour from Indian Mackerel in Improving the Calcium Content of Fish Sausage** *By Zuraidah Nasution (IPB University, Indonesia)*
11:10-11:25	CJ-O203	**The Application of Coconut-Based Double Emulsions in the Development of Low Fat Salad Dressings** *By NUR SUAIDAH MOHD ISA (Universiti Malaysia Terengganu, Malaysia)*

Division D: Food Microbiology, Food Biotechnology, and Fermentation

Day 1: June 13, 2024 (Room: MR215)

Time	No.	Topic
13:15-13:25		**Introduction of the Division D – Food Microbiology, Food Biotechnology, and Fermentation** **Chair:** Associate Professor Dr. Phisit Seesuriyachan (Chiang Mai University, Thailand) **Co-Chair:** Assistant Professor Dr. Nisa Saelee (Walailak University, Thailand) Assistant Professor Dr. Paramaporn Kerdsup (Srinakharinwirot University, Thailand)
13:25-13:55	Invited Speaker #1	**Integrated Biofuel and Valued-Added Product Production from Co-Digestion of Sugarcane Leaves and Microalgal Biomass: A Circular Economy Approach** *By Professor. Dr. Alissara Reungsang (Khon Kaen University, Thailand)*
13:55-14:25	Invited Speaker #2	**Bacteriophage: Effective Tool for Food Safety and Sustainable Food Production** *By Associate Professor. Dr. Kitiya Vongkamjan (Kasetsart University, Thailand)*
14:25-14:40	DB-O128	**Antibacterial Activity and Mode of Action of PLLAKVTKLWKKF, a Novel Modified Peptide Derived from Corn Gluten Meal on Bacillus Cereus DMST5040** *By Edhi Nurhartadi (Suranaree University of Technology, Thailand)*
14:40-14:55	DB-O137	**Effect of Passion Fruit Peel-Extracted Pectin and Probiotic-Supplemented Synbiotic Ice Cream in the In Vitro Fecal Culture on Human Intestinal Microbiota Composition** *By Rattanaporn Pimisa (Naresuan University, Thailand)*
14:55-15.10	DP-O202	**Evaluation of Prebiotic Activity Score of Konjac Glucomannan Hydrolysate and Commercial Prebiotic** *By Pichamon Namkieat (Chulalongkorn University, Thailand)*
15.10-15:20	**Break 10 min**	
15.20-15.35	DP-O024	**Comparative Study of the Mutagenic Potential of Conventionally and Organically Grown Chinese Kale: A Preliminary Study in Nakhon Pathom Province** *By Suttida Sukshapsri (Mahidol University, Thailand)*
15:35-15:50	DP-O236	**Application of Food Grade Shrimp Chitosan as a Preservative in Squeezed Orange Juice** *By Naphatsorn Kamsod (Kasetsart University, Thailand)*

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Division E: Related Topics

Day 1: June 13, 2024 (Room: MR216)

Time	No.	Topic
13:15-13:25		**Introduction of the Division E – Related Topics (Food Packaging, Food Safety & Quality, Food Laws & Regulations, Food Policy, etc.)** **Chair:** Professor Dr. Pornchai Rachtanapun (Chiang Mai University, Thailand) **Co-chairs:** Associate Professor Dr. Patcharakamon Nooeaid (Srinakharinwirot University) Assistant Professor Dr. Tanong Aewsiri (Walailak University, Thailand)
13:25-13:55	Invited speaker #1	**Biopolymer Packaging: Tasting the Future of Biopolymer Films and Coatings in Sustainable Food Packaging** *By Professor Dr. Rungsinee Sothornvit (Kasetsart University, Thailand)*
13:55-14:25	Invited speaker #2	**Innovative Trends Shaping the Future of Smart Food Packaging for Sustainable Living** *By Professor Dr. Panuwat Suppakul (Kasetsart University, Thailand)*
14:25-14:40	EB-O174	**Deep Learning to Predict Aquaculture Conditions from Multispectral Images of Raw Pacific White Shrimps** *By Bo-Kai Liao (National Taiwan Ocean University, Taiwan)*
14:40-14:55	EB-O215	**Physicochemical Properties of Corn Starch and Pectin-Based Biofilm with The Peel of Red Dragon Fruit Extract** *By Safinta Nurindra Rahmadhia (Universitas Ahmad Dahlan, Indonesia)*
14:55-15.10	EP-O103	**Plastic Bag Bans Policy: Behaviour and Attitude of People in Bangkok Metropolitan Region, Thailand** *By Weeraya Thongon (Srinakharinwirot university, Thailand)*
15.10-15:20	**Break 10 min**	
15.20-15.35	EB-O090	**Innovative System and Method for a Startup-Centric Degree / Diploma Curriculum in Food Innovation and Entrepreneurship** *By Lahiru prabodha Lande Bandarage (University of Ruhuna, Sri Lanka)*
15:35-15:50	EB-O009	**The Effects of Quality Changes in Vegetable Oils on the Formation and Absorption of Monochloropropanediol Esters (MCPDE) and Glycidyl Esters (GE) During Deep-frying of Potato Chips** *By Nur Azwani Ab Karim (Sime Darby Plantation Research Sdn Bhd, Malaysia)*
15:50-16:05	EB-O219	**Nutritional Content of Commercial Infant and Toddler Complementary Foods in Indonesia** *By Widya Indriani (Indonesia International Institute for Life Sciences, Indonesia)*
16:05-16:20	EB-O092	**Widya Ruhuna "Govi-Nena" Home Gardening Mobile App** *By Lahiru prabodha Lande Bandarage (University of Ruhuna, Sri Lanka)*

Note:
1. Invited speaker 25 min presentation and 5 min Q&A
2. Speaker 12 min presentation and 3 min Q&A

Poster Presentation Competition

Date: June 13, 2024

Time	Code	Program
13.00-13.15	-	Introduction - All finalists must attend
13.15-13.30	AB-P056	**Biochemical Characterization of Bromelain from Pattawia (*Ananas comosus* (L.) Merr.) Pineapple Peel Waste** *By Montita Kamwisaet (Thaksin University, Thailand)*
13.30-13.45	AB-P063	***Polygonum minus* Extract: A Potential Functional Food and Dietary Supplement with Diverse Activities** *By Xi Deng (Universiti Putra Malaysia, Malaysia)*
13.45-14.00	AB-P087	**Gel-Forming Ability and Oxidative Stability Improvement of Mackerel (*Auxis thazard*) through Antioxidant-Infused Soda-Saline Washing** *By Porntip Thongkam (Walailak University, Thailand)*
14.00-14.15	AB-P112	**Protein Recovery from Hatchery By-Products using pH-Shift Processing** *By Supalak Ruangprom (Walailak University, Thailand)*
14.15-14.30	AB-P115	**Role of Skipjack Tuna (*Katsuwonus pelamis*) Blood Protein Hydrolysate as a Natural Cryoprotectant on Threadfin bream Surimi Protein after Freeze-Thaw Cycles** *By Patawee Maliwan (Chiang Mai University, Thailand)*
14.30-14.45	AB-P147	**Effect of Different Drying Methods and Temperatures on the Physicochemical Qualities and Antioxidant Activities of *Gymnema inodorum* Leaves** *By Natthanicha Banpacha (Chiang Mai University, Thailand)*
14.45-14.55	Break 10 min	
14.55-15.10	BB-P098	**Field-Assisted Green Extraction of Betalains from the Amaranthus Employing Natural Deep Eutectic Solvents** *By Vivek Gupta (Indian Institute of Technology Tirupati, India)*
15.10-15.25	CB-P148	**Factors affecting Physicochemical and Textural Properties in the Development of Analogue Cream Cheese from Watermelon Seed** *By Hiran Janpeng (Chiang Mai University, Thailand)*
15.25-15.40	EB-P124	**A Study of Food Poisoning Incidences Among In-Bound Exchange Students at a Local College in Thailand** *By Pornkaeo Sae-Chong (Mahidol University, Thailand)*
15.40-15.55	EB-P179	**Volatile Sulfur Compound Indicator Label for Fresh-Cut Monthong Durian** *By Piyapong Sonkaew (Kasetsart University, Thailand)*
15.55-16.10	EP-P018	**Detecting Chlortetracycline Residues in Milk Using a Lateral Flow Immunoassay** *By Kittiwara Tharmmawongkarn (Chulalongkorn University, Thailand)*

Oral Presentation Competition

Date: June 14, 2024 (Room: MR215)

Time	Code	Program
8.45-9.00	**-**	Introduction - All finalists must attend
9.00-9.20	BP-O204	**Effect of Plant Proteins Mixing Ratios on Free-SH/S-S Bond Change and Functional Properties of High Moisture Meat Analogues via Extrusion** *By Lymeng Roeun (Ubon Ratchathani University, Thailand)*
9.20-9.40	AB-O028	**Optimizing Microencapsulation of Green Tea and Turmeric Extracts through Freeze-drying: Impact of Wall Materials on Encapsulation Efficiency, Bioactive Compounds, and Sensory Enhancement** *By Kanjana Singh (Chiang Mai University, Thailand)*
9.40-10.00	EB-O054	**Green Synthesis of ZnO Nanoparticles and Extraction of Mucilage from Different Parts of *Dillenia indica* and Its Combined Application in Fruit Preservation** *By Akuleti Saikumar (Tezpur University, India)*
10.00-10.20	AB-O079	**Characterization and Storage Stability of Microwave Extracted Red Palm Oil Based Nanoliposomes** *By Tanatchapond Rodsamai (Walailak University, Thailand)*
10.20-10.30	Break 10 min	
10.30-10.50	DB-O128	**Antibacterial Activity and Mode of Action of PLLAKVTKLWKKF, a Novel Modified Peptide Derived from Corn Gluten Meal on *Bacillus cereus* DMST5040** *By Edhi Nurhartadi (Suranaree University of Technology)*
10.50-11.10	AB-O133	**Enhancing the Functional Properties of Mantis Shrimp (*Oratosquilla nepa*) Protein Isolate through pH Treatment** *By Kanchanaphon Chumthong (Walailak University, Thailand)*
11.10-11.30	DB-O137	**Effect of Passion Fruit Peel-Extracted Pectin and Probiotic-Supplemented Synbiotic Ice Cream in the In Vitro Fecal Culture on Human Intestinal Microbiota Composition** *By Rattanaporn Pimisa (Naresuan University, Thailand)*
11.30-11.50	AB-O146	**The Fatty Acid Esterification of Policosanol Increases Hypocholesterolemic Action in Mice** *By Sudthida Kamchonemenukool (Naresuan University, Thailand)*
11.50-12.10	AB-O156	**Properties of Bromelain from Pineapple Stem obtained by Continuous Ethanol Precipitation** By Suteera Srisuk (Silpakorn University, Thailand)
12.10-12.30	DB-O093	**Optimizing Irradiation Doses for Enhanced Microbial Safety and Quality Preservation of Dehydrated Waraka and Carrot in Food Processing** *By Chamila Madushani Ranasinghe (University of Ruhuna, Sri Lanka)*

ABOUT THE CONFERENCE

The conference will provide opportunity to meet and share experiences as well as strengthen networking among international food scientists and scientists in related fields from academia, government and food industries. The objective is to highlight significant developments in research and innovations in food science and technology with an emphasis on innovative ASEAN food research towards the World. The conference will feature a series of presentations and discussions in plenary, concurrent and poster sessions, informal gatherings, competitions and exhibitions.

Organisers:

- Food Science and Technology Association of Thailand (FoSTAT)
- Agro-Industry Academic Council Association (AIAC)
- Walailak University
- Chiang Mai University
- Srinakharinwirot University

Support by:

- Informa markets
- The Federation of the Institute of Food Science and Technology in ASEAN (FIFSTA)

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OPENING REMARK

FoSTAT PRESIDENT

Food Innovation Asia Conference 2024

Opening Remarks by Asst. Prof. Puangpetch Nitayanont
President of Food Science and Technology Association in Thailand (FoSTAT)

Assoc. Prof. Dr. Sakarindr Bhumiratana, Chairman of Ajinomoto Foundation
Assoc. Prof. Dr. Anuvat Jangchud, President of Agro Industry Academic Council Association,
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Associate Professor Dr. Worawan Panpipat,Dean of School of Agricultural Technology and Food Industry, Walailak University,
Assistant Professor Dr. Arunya Mingmuang,Dean of Faculty of Agricultural Product Innovation and Technology, Srinakharinwirot University,

Informa Markets (Asia) Thailand, and All distinguished guests.

On behalf of the Food Science and Technology Association of Thailand as the abbreviation is FoSTAT., I am pleased to welcome you to the Food Innovation Asia Conference 2024. The conference and competition programs will be convened during 13-15 June in concurrent with ProPak Asia 2024.

In year 2024, we gather for a conference emphasizing the significance of innovation in the food industry under the title "Food Innovation: Trends, Impacts and Solutions for a Sustainable Future". Today, we could come together to interpret and exchange knowledge regarding the trends in the food industry, the changes occurring, and the methods to address related challenges for the sustainable conservation and as an opportunity to foster connections and collaboration among researchers, students, entrepreneurs, and those with interest in the food industry. It is our expectation that FIAC will serve as a significant catalyst for fostering innovations that promote economic, social, and ecosystem in our food industries.

This year, it's our great pleasure that FoSTAT collaborates with the Agro-Industry Academic Committee (AIAC), Chiang Mai University, Walailak University, Srinakharinwirot University and the Federation of the Institute of Food Science and Technology in ASEAN (FIFSTA) to organize the scientific events where there will be numerous technical papers presentation, specialty seminar and workshops, FoSTAT-Nestlé Quiz Bowl with 60 university teams around the country in the competition this year, and FoSTAT Food Innovation Contest under the theme “Food for Crisis” that more than 100 fruitful innovations from all universities with Food Science and Technology.

I would like to congratulation to winner of Ajinomoto-FoSTAT Award, which contributes to the advancement of food science and technology. This award recognizes outstanding young researchers in the field of food science and technology, and we are thrilled to present it at this event.

Finally, highly appreciation to thanks Informa Markets Thailand who has been the important one of the key partners in supporting our activities. Thanks also go to the organizing committee of all

activities. We are certain that this year will be another successful year bringing all science, technology, and innovation to the food professional community. I wish you all successful deliberation with warmest regards from Food Science and Technology Association of Thailand.

WELCOME MESSAGE

Walailak University

Associate Professor Dr. Suwit Wuthisuthmethavee
Vice President, Walailak University, Thailand

Your Excellency,

President of the Food Science and Technology Association of Thailand (FoSAT),
President of the Agro-Industry Academic Council Association (AIAC),
Keynote and invited Speakers,
Ladies and Gentlemen,

Good morning, and a warm welcome to the Food Innovation Asia Conference 2024! On behalf of Walailak University and our co-hosts, it is both an honor and a privilege to host such a distinguished assembly of experts, innovators, and visionary leaders in the vibrant and ever-evolving field of food science and innovation.

This year's theme, "Food Innovation: Trends, Impacts, and Solutions for a Sustainable Future," reflects the urgent need to address the pressing challenges and opportunities that lie ahead in our quest for sustainable food systems. As we gather here today, we are reminded of the vital role that innovation plays in shaping a future where food security, environmental sustainability, and human health are harmoniously balanced.

We are delighted to welcome participants from around the globe, representing a diverse array of disciplines, including agriculture, food science, technology, policy, and business. Your presence here signifies a shared commitment to exploring and advancing the frontiers of food innovation.

Over the next few days, we will engage in a series of stimulating discussions and insightful presentations. Our esteemed speakers will share cutting-edge research, groundbreaking technologies, and transformative ideas that are poised to revolutionize the way we produce, process, and consume food.

This conference would not be possible without the hard work and dedication of our organizing committee, sponsors, partners, and volunteers. Your efforts have ensured that we can come together to foster collaboration, inspire innovation, and ignite positive change in the food industry.

As we embark on this journey of discovery and learning, let us remember that the solutions to our global food challenges lie within our collective ingenuity and determination. Together, we can pave the way for a sustainable future where everyone has access to nutritious, safe, and affordable food.

Thank you for being a part of the Food Innovation Asia Conference 2024. I wish you all a fruitful and inspiring conference.

Welcome!

WELCOME MESSAGE

Chiang Mai University

Associate Professor Dr. Yuthana Phimolsiripol
Dean of Faculty of Agro-Industry, Chiang Mai University, Thailand

Esteemed President of the Food Science and Technology Association of Thailand, distinguished speakers, delegates, and participants.

It is with great pleasure that I welcome you to the Food Innovation Asia Conference 2024 (FIAC2024). This prestigious event is brought to you by the Food Science and Technology Association of Thailand (FoSTAT), in collaboration with the Agro-Industry Academic Council Association (AIAC), Chiang Mai University, Srinakharinwirot University and Walailak University. This year, our theme is "Food Innovation: Trends, Impacts, and Solutions for a Sustainable Future," highlighting the crucial contributions of food science and technology to our global community.

The pursuit of sustainability in food production, processing, and distribution is essential to minimizing environmental impact and ensuring food security. Innovators in food technology are constantly working to advance nutritional quality, reduce harmful substances, and offer healthier alternatives, contributing significantly to societal well-being. By embracing these sustainable practices, professionals in the field can help foster a healthier planet and population.

This conference aims to provide an opportunity for academics, researchers, and students from national and international institutions to present and share their new knowledge, ideas, applications and experiences leading to research development and collaboration. Throughout this conference, you will have the chance to engage in various activities, including keynote addresses, panel discussions, as well as oral and poster competitions. These sessions are designed to provide you with valuable knowledge, innovative ideas, and opportunities to connect with peers who share your passion for food science and technology.

Finally, on behalf of the Faculty of Agro-Industry Chiang Mai University, I would like to extend our deepest gratitude to the Food Science and Technology Association of Thailand (FoSTAT) and the Agro-Industry Academic Council Association (AIAC) for their unwavering support. I also want to thank the scientific committee, organizing committee and staff for their exceptional efforts in making this conference possible.

We warmly welcome you to what promises to be an enriching and transformative experience at FIAC2024. Thank you for joining us.

WELCOME MESSAGE

Srinakharinwirot University

Associate Professor Dr. Arunya Mingmuang
Dean of Faculty of Agricultural Product Innovation and Technology,
Srinakharinwirot University, Thailand

Greeting to the President of the Food Science and Technology Association of Thailand, honorable speakers, distinguished guests, and fellow participants.

It is with great honor and excitement that I welcome you all to the 26th Food Innovation Asia Conference 2024 (FIAC 2024), organized by the Food Science and Technology Association of Thailand (FoSTAT), Srinakharinwirot University, Chiangmai University and Walailak University. This year, we gather under the compelling theme: "Food Innovation: Trends, Impacts, and Solutions for a Sustainable Future."

Over the past 26 years, FIAC has been a beacon of knowledge, innovation, and collaboration in the food industry. Our conference has grown to become a premier platform where scientists, industry leaders, policymakers, and entrepreneurs converge to share insights, forge partnerships, and drive progress in food innovation.

This year's theme underscores the critical importance of our mission. In a world grappling with environmental challenges, health crises, and a growing need for sustainable solutions, our role as innovators has never been more vital. The future of food is not just about feeding a growing population; it is about nourishing people in a way that promotes health, ensures sustainability, and enhances overall well-being.

We have an inspiring lineup of keynote speakers, panel discussions and exhibitions designed to provoke thought and inspire action. From breakthroughs in sustainable agriculture and food technology to innovative approaches to nutrition and health, our program is rich with opportunities to learn, share, and collaborate.

I encourage each of you to take full advantage of the diverse expertise and perspectives gathered here. Engage in meaningful dialogues, challenge conventional thinking, and explore new ideas that

can propel our industry forward. Let us embrace this moment to envision and create a future where our food systems are resilient, sustainable, and capable of promoting the health and well-being of all. On behalf of Srinakharinwirot University and our co-hosts including Chiangmai University and Walailak University, I would like to extend my deepest gratitude to the Food Science and Technology Association of Thailand (FoSTAT) and Agro-Industry Academic Council (AIAC) as well as all scientific committees and organizing staffs for your great support and hard work. Your dedication to advancing the frontiers of food innovation is truly commendable.

Thank you once again for being here. Welcome to FIAC 2024. Let us make this milestone conference a remarkable and transformative experience. Thank you very much.

PROCEEDINGS

Division A

(Food Chemistry, Nutrution and Analysis)

AP-P012

Investigating bioactive compounds in freeze-dried *Piper sarmentosum Roxb.* leave and their anti-inflammatory effect on lipopolysaccharide-induced RAW 264.7 macrophages.

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Keywords

Anti-inflammation
Bioactive compounds
Freeze drying process
Piper sarmentosum

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ABSTRACT

Piper sarmentosum Roxb. leave (PL) which contains nutrients and bioactive compounds, are commonly consumed in Southeast Asian countries. Freeze drying process is one of the most popular drying technology which can extend shelf life and results in high quality product. The aim of this study was to identify bioactive compounds (chlorophyll, phenolic acid and flavonoid) of ethanolic extracts from freeze dried P. sarmentosum leave (FPL) and its anti-inflammatory properties in LPS-induced RAW264.7 macrophage cell line. PL were freeze dried, grinded and extracted with 70% ethanol at room temperature for 24 hr. The extract was evaluated for its components and anti-inflammatory activities. The results demonstrated that FPL extract had chlorophyll a (1129.06 mg/100g), chlorophyll b (489.34 mg/100g), rutin hydrate (554.70 mg/100g), methyl gallate (59.48 mg/100g) and myricetin (58.63 mg/100g,) which had antioxidant activity (452.07 mg Trolox/100g sample). Moreover, FPL showed anti-inflammatory activities by decreasing NO and pro-inflammatory cytokine (IL-6 and TNF-α) levels on lipopolysaccharide-induced RAW264.7 macrophage cells. Our results indicated that the bioactive compounds, antioxidant and anti-inflammatory properties of FPL make them a new prospective functional ingredient for product development.

1. INTRODUCTION

Freeze drying process is a dehydration technique to remove water under low pressure and temperature. This drying method offers shelf-life extension, high-quality, easy-to-handle, and non-perishable food products [1]. Therefore, freeze drying process have become commonly used for preserving fruit and vegetable, which are showed higher nutrients, bioactive compound, antioxidant

and characteristics such as taste, colour or appearance and to minimize the degradation of thermolabile compounds than other drying method in previous study[2, 3]

P. sarmentosum (Chaplu) is a plant tree belonging to the family of Piperaceae used in many countries. With its nutrients and bioactive compounds such as phytochemicals, some vitamins and fibre, *Piper sarmentosum* leave (PL) is a main part which used as food and raw material. According to the previously study, the ethanolic extract from PL has high total polyphenols content (60.61 ± 0.96 µg gallic acid/mg) and flavonoid content (70.14 ± 0.38 µg quercetin/mg). Moreover, the strong antioxidant activity of PL extract using DPPH and ABTS methods has been reported. The antioxidant of ethanolic extract of PL had significantly higher than aqueous extract similar to total phenolic acid and flavonoid content [4]. On the other hand, the bioactive compounds in PL may dissolve effectively in solvent.

It is well known that antioxidant activity may reduce oxidative stress damage, nitic oxide output, blood pressure and cholesterol rates. In addition, bioactive compounds and antioxidants can suppress reactive oxygen species (ROS) which lead to decrease inflammation and multiple chronic diseases. Especially, chronic inflammatory diseases such as cardiovascular, diabetes, auto-immune disease, cancer and Alzheimer disease [5]. Therefore, reducing or suppressing ROS and inflammation could be an alternative approach to preventing and treating the chronic inflammatory-associated diseases.

Although, PL has been studied on total polyphenol compounds (phenolic acid and flavonoid) and antioxidant activity, but the phytochemical profile of PL, as well as chlorophyll, phenolic acid and flavonoid compounds remain unclear. Therefore, the present study was conducted to investigate phytochemical of ethanol extracts from (FPL) and its anti-inflammatory properties in LPS-induced Raw264.7 macrophage cell line.

2. MATERIAL AND METHODS

2.1 Materials and chemicals

PL were purchased from a local market in Ayutthaya, Thailand. gallic acid, methyl gallate, caffeic, syringic acid, rutin hydrate, p-Coumaric, trans-ferulic acid, myricetin, diadzein, quercetin, cinnamic acid genistein synthetic, kaempferol, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2 diphenyl-l-picrylhydrazyl (DPPH), were purchased from Sigma-Aldrich (St Louis, MO, USA).

The murine RAW 264.7 macrophage cell lines were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS) were purchased from GIBCO (Grand Island, NY, USA). All chemicals and reagents were analytical or biological grade.

2.2 Sample preparation

The leaves were washed, separated into 500g per bag, and frozen with rapid freezing process which had more freshness and quality of food, less costs and production time. After that, frozen PL were freeze dried at temperature -80°C for 3 days using Labconco freeze dryer model freezone, ground and extracted by 70% ethanol. The mixture was shaken in an incubator at room temperature for 24 h. and then centrifuged at 5000 rpm and 25°C. The FPL extract were stored at -20°C before testing.

Figure 1. Freeze-dried *Piper sarmentosum Roxb.* leaves (A) and its powder (B)

2.3 Bioactive compounds (chlorophyll, phenolic acid and flavonoid) and antioxidant activity determination.

Chlorophyll content of FPL was analyzed using microplate reader. Chlorophyll were extracted from 0.2 g of the powder by added 5 ml of absolute methanol and shaking for 2 hr. at 60 rpm using rotator (Grant Bio PTR 360° Vertical Multi-Function Tube Rotators, Latvia) The FPL was centrifuged at 2400 rpm for 10 min and the supernatant was transferred to a second centrifuge tube. The absorbance of 200 µl of the extract in microplate was converted to 1-cm pathlength[6] .

$$A_{652,1cm} = (A_{652}, \text{microplate-blank})/\text{pathlength}$$

$$A_{665,1\,cm} = (A_{665}, \text{microplate-blank})/\text{pathlength}$$

Chlorophyll concentration was calculated from 1-cm corrected pathlength using this formula below;

$$\text{Chlorophyll a } (\mu g/ml) = -8.0962\ A_{652,\,1\,cm} + 16.5169\ A_{665,\,1\,cm}$$

$$\text{Chlorophyll b } (\mu g/ml) = 27.4405\ A_{652,\,1\,cm} - 12.1688\ A_{665,\,1\,cm}$$

$$\text{Total chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

For the assessment of the bioactive compounds including phenolic acid and flavonoid, High Performance Liquid Chromatography (HPLC; Model prominence LC20 series, Shimadzu, Kyoto, Japan) equipped with a UV visible detector at wavelength 272 nm was used to determine phenolic acid and flavonoid compounds. In this study, the method was modified from a previous study on quantitative HPLC analysis of polyphenols[7]. Phenolic acid and flavonoid analysis were performed on an Ascentis Express Column (C18, 5.0 µm, 15 cm ×4.6mm) using 1% acetic acid in water and 100% acetonitrile as a Mobile phase A and B, respectively. The flow rate was adjusted to 0.4 ml/min, the column was thermostatically controlled at 28°C. The gradient elution was changed from 10 % to 40% B in a linear fashion for duration of 28 min, from 40 to 60 % B in 39 min, from 60 to 90 % B in 45 min and hold 90% B at 50 min. The mobile phase composition back to initial condition (solvent B: solvent A: 10: 90) in 65 min. The injection volume was 10 µl. Each compound was identified by its retention time of standards under the same conditions. The extract of FPL was analyzed and reported with its average in triplicate.

Antioxidant activity of FPL extract was determined by 2,2 diphenyl-l-picrylhydrazyl (DPPH) assay [8]. This measures the ability of a substance to scavenge DPPH radicals. The decrease in absorbance is measured spectrophotometrically. Higher absorbance reduction indicates higher antioxidant activity. The extract was mixed with a DPPH solution and incubated at room temperature for 30 minutes. Absorbance was measured at 525 nm and expressed as mg Trolox equivalents per g sample.

Effects of PL extract on Raw 264.7 macrophages.

RAW 264.7 macrophage cells were grown in DMEM medium supplemented with 10% FBS and 1% antibiotic in humidified incubator at 37°C and 5% CO_2. After reaching 80% confluence, the cells were passaged.

MTS assay was used to analyze the effect of FPL extract on cell viability. RAW 264.7 macrophage cells were seeded at a density of 1×10^4 cells per well in a 96-well plate and treated with 70% ethanol or FPL extract at concentration of 25, 50, 100, 200, 500, 1000 µg/mL for 24 h. Then, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS reagent) was added and incubated in humidified incubator at 37°C and 5% CO_2. Absorbance was measured at 490 nm using a microplate reader (Omega, BMG labtech, Ortenberg, Germany).

Griess method was used to determine the effect of FPL extract on nitric oxide (NO) production of Raw 264.7 macrophage cells. Cells were seeded at a density of 5×10^4 cells per well in a 24-well plate for 24 h. Cells were then co-treated with 100 ng/ml of lipopolysaccharide (LPS) and FPL extract at concentration of 50, 100, 200 µg/mL for 24 h. Medium supernatants were collected and mixed with Griess reagent. Absorbance was measured at 540 nm using a microplate reader. The NO levels were calculated to nitrite concentration of sample-treated cells using sodium nitrite ($NaNO_2$) standard curve [9]. The medium supernatants were stored at 4°C before cytokine testing.

Enzyme-linked immunosorbent assay of mouse ELISA kit from Invitrogen (Frederick, MD, USA) were used to analyze the effect of FPL extracts on inflammatory cytokine (IL-6 and TNF-α) production. Absorbance was measured at 490 nm using a microplate reader. The concentrations of cytokine levels were calculated from the standard curve.

2.4 Statistical analysis

All treatments were performed using three independent replications. The viability of cell comparison between the control (non-treated) and FPLE-treated group was performed with pair-sample T-Test. Anti-inflammatory effects including NO, IL-6 and TNF-α cytokine levels between the control and LPS stimulated group, or LPS stimulated and FPLE-treated group were performed with pair-sample T-Test. A p-value of 0.05 was considered statistically significant using SPSS software version 22 (IBM, Armonk, New York, USA).

3. RESULTS AND DISCUSSION

FPL were ground to powder as shown in figure 1B. The main pigment in FPL is chlorophyll. Moreover, chlorophylls are among the most prominent bioactive compounds and proven to have many benefits on health through their antioxidant, anti-inflammatory and anti-cancer properties [10]. In this study, the total chlorophyll, chlorophyll a and b content were determined. FPL had 1129.06mg/100g of chlorophyll a, 489.34 mg/100g of chlorophyll b and 1618.40 mg/100g of total chlorophyll as shown in table 1.

Table 1. Bioactive compound and antioxidant activity of freeze-dried *Piper sarmentosum Roxb.* leave extract

Bioactive compounds and antioxidant activity	Content (mg/100 g)
Chlorophyll	
Chlorophyll a	1129.06±41.62
Chlorophyll b	489.34±37.87
Total chlorophyll	1618.40±79.24
Phenolic acid	
Gallic acid	ND
Methyl gallate	59.48±0.96
Caffeic	ND
Syringic acid	ND
Cinnamic acid	ND
p-Coumaric	ND
Trans-ferulic acid	ND
Flavonoid	
Rutin hydrate	554.70±24.14
Myricetin	58.63±0.35
Diadzein	ND
Quercetin	ND
Genistein synthetic	ND
Kaempferol	ND
Antioxidant activity	
DPPH radical scavenging activity (mg Trolox/100g)	452.07±17.27

*ND means not detect in this method, mean ± standard deviation from three different experiment perform in triplicate.

For the assessment of the bioactive compounds and antioxidant activities of the FPL extract, 70% ethanol was used as the solvent. HPLC was used to identify phenolic and flavonoid content, while the DPPH assay was used to analyse the antioxidant activities of the extract. Absorbance was

measured at 525 nm and expressed as mg Trolox equivalents per g sample. Figure 2 displays a typical HPLC chromatrogram of the standard mixture with high value recorded at 272 nm. The assessment of the bioactive compounds of FPL extract in this study consist of phenolics and flavonoids. The results showed that FPL extract had methyl gallate, rutin hydrate and myricetin (59.48, 554.70, and 58.63, respectively), and it exhibited an antioxidant activity of 452.07 mg Trolox/100g (68.18 % inhibition), as shown in Table 1. Previous studies have reported that FPL had % inhibition ranging from 17% to 74%, depending on harvesting maturity, growing region, and extraction method[11, 12]

In the present method, rutin hydrate is one of the main flavonoid compounds in FPL extract which has demonstrated a number of biological activities such as antioxidant, anti-inflammatory protecttive, anticarcinogenic, neuroprotective and cardioprotective activities [13-15]. Moreover, it has been reported that methyl gallate and myricetin also have biological functions including antioxidant, anti-tumor, anti-inflammatory, neuroprotective, hepatoprotective and anti-microbial activities[16, 17].

From the bioactive compounds and antioxidant activity of FPL extract results, we hypothesized that the extract could show anti-inflammatory effects on LPS-induced Raw264.7 macrophage cells.

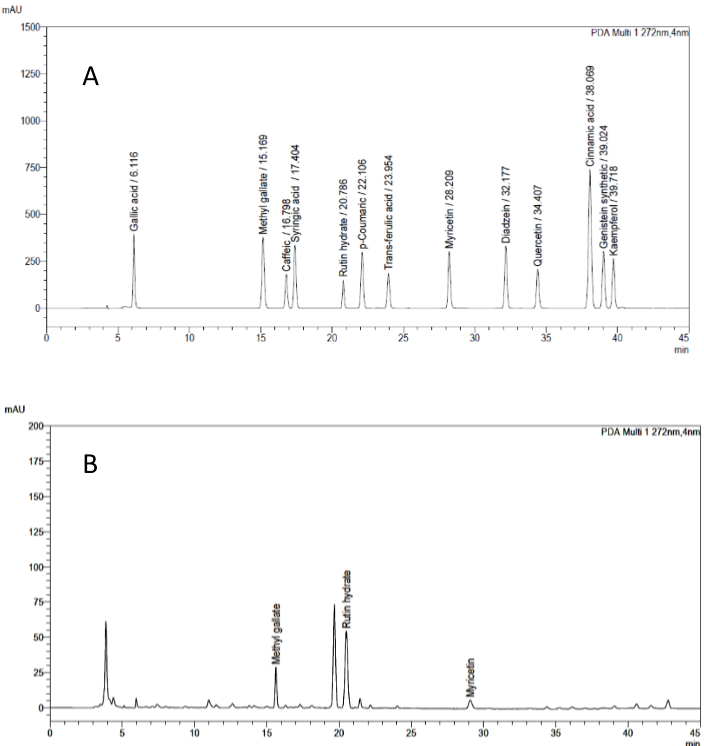

Figure 2. Chromatogram of mixed 13 phenolics and flavonoids standard by HPLC (A), Chromatogram of freeze-dried *Piper sarmentosum Roxb.* leave extract (B)

In this study, Raw 264.7 macrophage cells were co-incubated at LPS concentration 100 ng/ml and the FPL extract concentration 25, 50, 100, 200, 500 and 1000 µg/ml for 24 h. The cell viability values of FPL extract at the concentration 25-200 µg/ml were over 95%. Whereas, at higher concentration (500 and 1000 µg/ml) FPL extract incubation, the cell viability was decreased to less than 90% as shown in figure 4. It showed that at the high concentration of FPL extract may influence

to the cell viability. Therefore, 50-200 µg/mL of FPL were selected to investigate the anti-inflammatory effects via NO production and level of IL-6 and TNF-α cytokine.

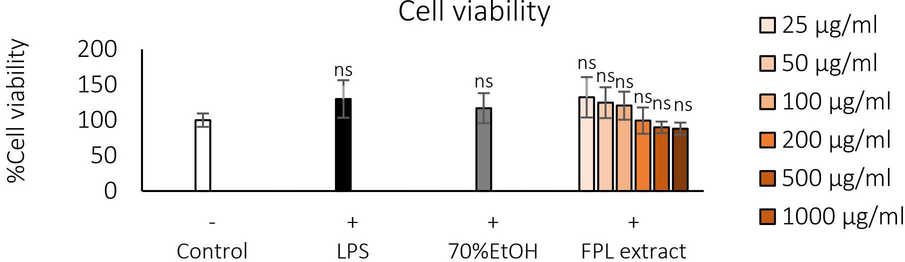

Figure 4. Cytotoxicity of freeze-dried *Piper sarmentosum Roxb.* leave extract on RAW 264.7 macrophages stimulated with lipopolysaccharide for 24 hr. P<0.05 compared with control.

Figure 5 illustrates that the incubation with 100 ng/ml of LPS significantly increased NO levels compared to the control group whereas, 70% ethanol-treated group also significantly produced NO but had no significantly different to LPS group. For the assessment of the anti-inflammatory effects of FPL extract on RAW 264.7 macrophages, the cotreat incubation of LPS and FPL extract at 50-200 µg/ml significantly reduced NO levels in a dose dependent manner (p<0.05).

Further, pro-inflammatory cytokine including IL-6 and TNF-α were used to investigate anti-inflammatory properties of FPL extract on LPS-induced Raw264.7 macrophages. The results illustrated that LPS significantly increased IL-6 and TNF-α production compared to the control group meanwhile, 70% ethanol-treated group also significantly produced NO but had no significantly different to LPS group. However, all treatment of FPL extract significantly reduced IL-6 and TNF-α production as shown in figure 6. Our results supported the data from previous study which showed that *P. sarmentosum* leave water extract has anti-inflammatory activities *in vivo* study[18]. Moreover, the ethanol extract from root also possessed anti-inflammatory [19].

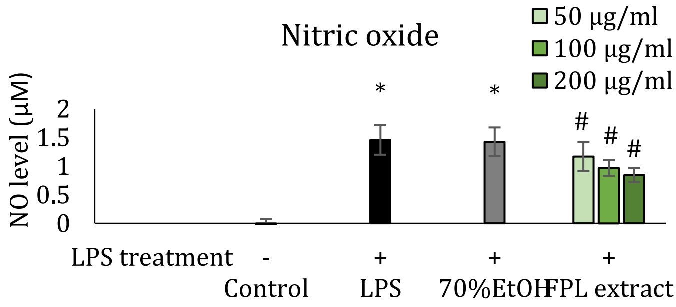

Figure 5. NO level of FPL extract on RAW 264.7 macrophages stimulated with LPS for 24 hr., Control= Untreated, LPS = LPS 100 ng/ml treated, 70%EtOH = 70%ethanol value as same as high value solvent extraction in sample. Value are expressed as the mean ± SD (n=3). P<0.05 compared with control. P<0.05 compared with LPS.

During the inflammatory process, NO, IL-6 and TNF-α cytokine are known as major pro-inflammatory mediators which are produced by macrophages. Phytochemicals have been reported to reduce pro-inflammatory mediators of macrophage cells. In this study, FPL extract at 50-200 µg/ml had phytochemical including rutin hydrate (27.74-110.94 µg/ml), methyl gallate (2.97-

11.90μg/ml) and myricetin (2.93-11.73μg/ml) (The data is obtained from back calculation). From previous studies showed that these polyphenols had anti-inflammatory activity on Raw264.7 macrophage models. A study on anti-inflammatory property of rutin has been reported that with concentration 12.21-61.05 μg/ml of rutin can decrease inducible nitric oxide synthase (iNOS) gene and protein expression levels compared with LPS-treated Raw264.7 macrophage cells [20]. Moreover, pretreatment with methyl gallate at the concentration 1.84-184 μg/ml significantly inhibited the production of IL-6 and CXCL-1 induced by zymosan[21]. In addition, myricetin at the concentration 3.98-7.96 μg/ml significantly inhibited the production of the pro-inflammatory cytokines via the decreasing of mRNA expression level of pro-inflammatory factors (TNF-α, IL-6, IL-1β, COX-2 and iNOS) induced by LPS [22]. However, these polyphenols mixed usage on anti-inflammatory effects had no evidence.

In summary, these results demonstrated that the FPL extract which had bioactive compounds can reduce inflammation on LPS-induced Raw264.7 macrophage cells via of NO, IL-6 and TNF-α level suppression. However, the bioactive absorption, metabolism and mechanism need further clarification.

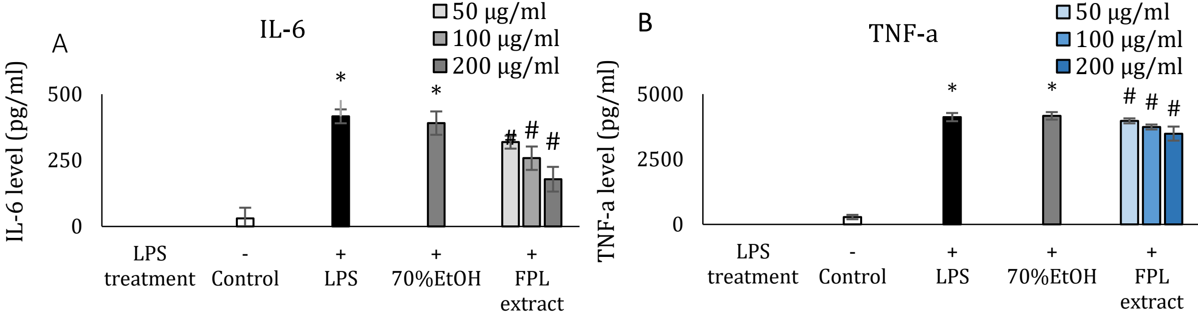

Figure 6. IL-6 (A) and TNF (B) cytokine levels of FLE extract on RAW 264.7 macrophages stimulated with LPS for 24 hr., Control= Untreated, LPS = LPS 100 ng/ml treated, 70%EtOH = 70%ethanol value as same as high value solvent extraction in sample. Value are expressed as the mean ± SD (n=3). P<0.05 compared with control. P<0.05 compared with LPS.

4. CONCLUSIONS

FPL extract had bioactive compounds including chlorophyll, rutin hydrate, methyl gallate and myricetin, which demonstrated antioxidant activity and anti-inflammatory effects. Our results indicated that FPL could be nature antioxidant food by shown antioxidant activity 452.07 mg Trolox/ 100g.

5. ACKNOWLEDGEMENTS

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Free sugar intake in Prince of Songkla University's students (Pattani campus)

AP-P017

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Keywords

Free sugar
Added sugar
Obesity
Food record
Late adolescence
Sugar sweetened beverages

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ABSTRACT

The World Health Organization (WHO) recommends decreasing free sugar intake due to their potential implication in weight gain and dental caries. Students of Prince of Songkla University, Pattani campus (PSU's student) are among the risk population group of high free sugar consumption. The objectives of this study were to evaluate the intake of free sugar, the adherence to the recommendations and the main food sources of free sugar. A cross-sectional study was conducted in Prince of Songkla University, Pattani campus. There were 377 undergraduate students in the 1st - 4th year students were required for the study. General characteristics were collected as well as anthropometric measurements were assessed in the study group. The food consumption data was evaluated by using estimated food record for 3 days (2 days for weekdays and 1 day for weekend). The results showed that free sugar consumption was 37.8 g/day and not significantly different between male and female was observed. Energy from free sugar was 148.4 kcal/day and when compared to the total energy intake/day (1265.6 kcal/day), the proportion of energy from free sugar accounted for 11.7 %. The prevalence of students with free sugar intake lower than 6 teaspoons (~25 g) /day (< 5% of total energy intake) was 31.7%. The major sources of free sugar were sugar sweetened beverage (SSB) such as iced cocoa, iced Thai tea, iced green tea which contributed 55.5% of free sugar intake followed by roti (16%), sauce and dip (8%) respectively.

1. INTRODUCTION

Sugar is widely consumed over the world as one of the main sources of energy for human (1). In term of biochemistry, sugar refer to dietary monosaccharides (glucose, fructose, galactose) and disaccharides for example sucrose and lactose (2). Recently, the decreasing of the trend for sugar consumption has been reported in some developed countries (3-5), however a high sugar intake still prevalent worldwide (3,5-6). Over-consumption of sugar has been proposed as obesogenic by inducing overeating and weight gain (7), increased risk of dental caries (8), cardio-metabolic risk factors as well as mortality (9). However, the adverse health effects are still not conclusive as whether sugar intake has directly impact to such outcomes or rather due to an excessive energy intake from sugar (1). Because of the current obesity epidemic lowering sugar consumption is needed to decrease excessive energy intake.

For preventing and controlling excess body weight, the World Health Organization (WHO) recommend limiting free sugar intake to < 10% of total energy intake (strong recommendation) preferably reduction to <5% (conditional recommendation) (10) to minimize the risk of dental caries.

In 2019 sugar intake in Thai population was 23.5 teaspoons/day which was higher than the recommendation of 6 teaspoons (~ 25 g)/day (5% of total energy intake) (11). The health behavior of population survey reported that the most frequence of sugar sweetened beverage (SSB) consumption of 5 time/week was found in the population aged 15-24 year (late adolescence) (12). Like the other places, changes in eating pattern and nutrition transition have been found in Pattani. Prince of Songkla University, pattani campus (PSU) surrounded by are more than 100 kiosks and shop of SSB, fast food restaurants, café and other fusion food restaurants. Together with the traditional foods and culture of this area are more likely to consume food with high content of fat and sugar such as roti, tea (with sweetened condensed milk), curry with coconut milk as well as traditional desert. Therefore, PSU's students of are at high risk of over consumption of free sugar and this may cause detrimental health effects later in life.

In order to promote dietary patterns fitting with the current recommendations on free sugars consumption, the aims of this study were to determine the intake of free sugars, the adherence to the recommendations of 6 teaspoons (~ 25 g)/day (5% of total energy intake) and the main food sources of free sugars consumption in PSU' s student.

2. MATERIAL AND METHODS

2.1 Study design and subjects

The cross-sectional study was conducted in PSU's students year 1 – 4 from January 2020-March 2020. The inclusion criteria were PSU's student who willing to participate, apparently healthy and not under process of weight loss. Subject who loss appetite or take any medication was excluded. A total of 377 subjects was calculated based on population of PSU's student which was 6,686 (13) and using proportional stratified random sampling from the 8 faculties. Informed consent was obtained from participants. The study protocol was approved by the Research Ethics Committee for Science, Technology and Health Science. Prince of Songkla University, Pattni campus (psu.pn.1-005/62).

2.2 Data collection

2.2.1 General characteristics and anthropometric data

Subjects were asked for general information and socioeconomic status such as age, earning per month, religion, health status, expenses for food, eating habit and family status. Weight and height were measured in duplicate at the department of food science and nutrition by trained staff. The weight of subject was measured using a digital scale (Carmy, USA) which was calibrated daily, and the height were measured with a standinometer (Seca, Japan). According to the Asian-Pacific cutoff points, BMI is categorized into four groups; underweight (≤18.5 kg/m^2), normal weight (18.5–22.9 kg/m^2), overweight (23–24.9 kg/m^2), and obese (≥25 kg/m^2) (14)

2.2.2 Dietary assessment

Dietary intake was assessed by estimated food records for 3 non-consecutive days (2 day for weekdays and 1 day for the weekend). Subjects were trained by the training staffs about food components, cooking method, the amount of consumed portion (size, weight, household measurements) before start doing their food records.

Nutrients intakes were analyzed by INMUCAL V3 programme (Mahidol University, Thailand). The intake of free sugars, defined as sugar added to foods during production or cooking in addition to sugar found in honey, syrup and fruit juices (10). Therefore, calculation of free sugar intake depends on nature of foods and drinks. There were 3 different sources of data for sugar content were used 1) INMUCAL programme 2) Nutrition labelling 3) The standard recipe of the most consumed foods and drinks especially traditional or local foods were collected in order to get the amount of free sugar.

2.3 Statistical analysis

Data for nutrient and free sugar intakes were analyzed with INMUCAL V3 programme (Mahidol University, Thailand) and Microsoft excel (Microsoft office 365). Normality was assessed by Shapiro – Wilks test. Values were reported as mean±SD or medians (Q1, Q3) for normal and nonnormally distributed data respectively. All parameters were compared by sex using unpaired t test or the Mann-Whitney test for continuous variables and Pearson chi square test for proportion. Differences were considered significant at $p < 0.05$. All data were statistically analysed with R programme i386 version 3.5.2.

3. RESULTS AND DISCUSSION

A total of 205 students completed data collection with 79% was female (table 1) most of them were Muslim (92%). Due to the semester break, only 54% of expected sample size (n=377) were recruited for the study. The majority of students were from year 3 and 4, average monthly allowance was 5,147 Baht which 57% and 35% of students spending for food cost of 50-100 Baht/day and 101-150 Baht/day respectively, 84% of students bought snack, desert or drink at least once a day (data do not show). The mean BMI was 21.5± 3.6 kg/m^2 which categorized as normal, 19%, 57.1% and 23.9% of subjects were classified as underweight, normal, overweight (and obesity) respectively. Nutrients intakes of participants is showed in Table 2, energy, macronutrients and micronutrients were consumed lower than Dietary Reference Intake for Thais (Thai DRIs) (15). This related with the time of main meal consumption which showed 56.6 % of student consumed ≤ 2 time/day and underweight have been found commonly (19%). However, the limitation of estimate food record is that underestimate of nutrients intakes could have been found due to burden of the respondent (16). For over 2 decades, the rate of obesity (BMI > 25 kg/m^2) is increased and by 2020 prevalence of obesity of Thai people age > 15 years old was 42.4% (17) this was higher than our population group which was more focus in the late adolescence (age 18-24 years old). For this group of population, the psychological and emotional changes that takes place and shift them form teenage to adult includes the desire for independence as adolescents develop individual identities apart from their families. Late adolescents would like to try and explore to new ideas, which includes trying new kinds of food and experimenting with their diet, skip a main meal and snacking are common. Therefore, the health problem they may face include obesity and eating disorders [18].

Table 1. General characteristics and body mass index of PSU's student

General characteristics	Subjects (n)	%
Total	205	100
Sex		
Male	43	20.9
Female	162	79.1
Religion		
Islam	189	92.2
Buddhism	16	7.8
Body composition (BMI; kg/m^2)		
< 18.5	39	19
18.5-22.9	117	57.1
>23	49	23.9
Main meal consumption (time/day)		
1	2	1.0
2	114	55.6
3	85	41.5
>3	4	2.0

Table 2. Nutrients intakes of PSU's student and Dietary Reference Intake for Thais (Thai DRIs)

Nutrient	**Median**	**Q1, Q3**	**Thai DRI***
Energy (Kcal)	1,265.60	1,027.6, 1,535.8	1,780, 2,260
Carbohydrate (g)	166.6	133.2, 209.0	-
Protein (g)	40.8	32.6, 51.5	53, 61
Fat (g)	34.5	24.0, 46.7	-
Calcium (mg)	208.4	116.0, 330.5	800, 800
Iron (mg)	5.4	4.0, 6.7	20, 11.5
Vitamin A (μg)	221.6	138.0, 298.5	600, 700
Vitamin C (mg)	12.3	6.9, 23.8	85, 100

*Values for (women, men)

Table 3 summarizes free sugar intake and it's contribution to the total energy intake as well as the proportion of people who consumed free sugar < 25 g (or 6 teaspoon)/day (< 5% of total energy intake). Median of daily free sugar intake in student was 37.8 g (~ 9 teaspoons) which contributed to ~12% of total energy consumption. Female rather consumed more free sugar than male however the values did not significantly different (p = 0.52). This value exceeding the 10% of

total energy intake recommended upper limit (10). In addition to that, there was only 31.7% who could adhere to the recommendation for the minimum consumption (25 g or 6 teaspoons/day). The intake of free sugar in PSU's student was comparable to data of Mahidol university 's student (35.2 g/day) and lower when compared to average consumption of Thai population (23.5 teaspoon/day) (11). In the U.S. the term "added sugar" is applied instead of "free sugar" with slightly different for the definition. Diets high in free/added sugar can contribute to adverse health consequences including weight gain/obesity, type 2 diabetes, cardiovascular disease and dental caries. Only 47% of adult aged ≥ 20 years in The U.S. met the recommendation in 2015-2016 which limits calories from added sugar to < 10% of the total daily caloric intake (5).

The main foods contributing free sugar of PSU's student were SSB (55%), roti (16%), sauce and dip (8%) and miscellaneous (21%) (figure 1). The data of standard recipe of the local food and drink showed that SSB such as iced cocoa, iced green tea, iced Thai tea contained 20-22 g of free sugar/serving, roti 12 g/serving, dip 6 g/serving (data not show). Therefore, the main contributor of free sugar of PSU's student was SSB. This finding was in agreement with report from The health behavior of population survey that the most frequence of SBB consumption was found in the population aged 15-24 years (late adolescence) with 5 time/week (12). The main food source of free sugar may vary by age group, culture and socioeconomic factors. For example, cereals and cereal products, non-alcoholic beverages and sugar, preserves, confectionary were the major sources of free sugars in UK population (19). Among younger adults in Switzerland, a bigger amount of free sugars came from SSBs (20%) compared to older adults, whose main source of free sugars came from sweet products (20).

Although, free or added sugar consumption has declined in some developed countries the level consumed still higher than recommendation. Therefore, measures or campaign like recommendations, reformulation programme and tax on SSB have been introduced in several countries as well as in Thailand. From a survey of SSB consumption during 2017 and 2018, it was found that after the implementation of sugar tax on SSB in 2017, the amount of consumption of SSB has decreased more than the consumption of non-SSB beverages (without having to pay sugar tax). Therefore, it could be assumed that the implementation of tax enforcement measures can reduce the amount of SSB consumption (21).

Table 3. Free sugar intake, energy contribution form free sugar and percentage meeting recommendation for free sugar consumption of PSU's student

Subject	Free sugar intake (g) Median (Q1, Q3)	Energy contribution form free sugar (kcal, %)
Total	37.8 (20.6, 54.0)	148.4, 11.7
Male	32.4 (16.6, 57.2)	129.6, 10.1
Female	37.5 (18.3, 55.3)	150, 12.1

Category of free sugar intake (g/day)	%
<25.0	31.7
25.0-50.0	40.5
> 50	27.8

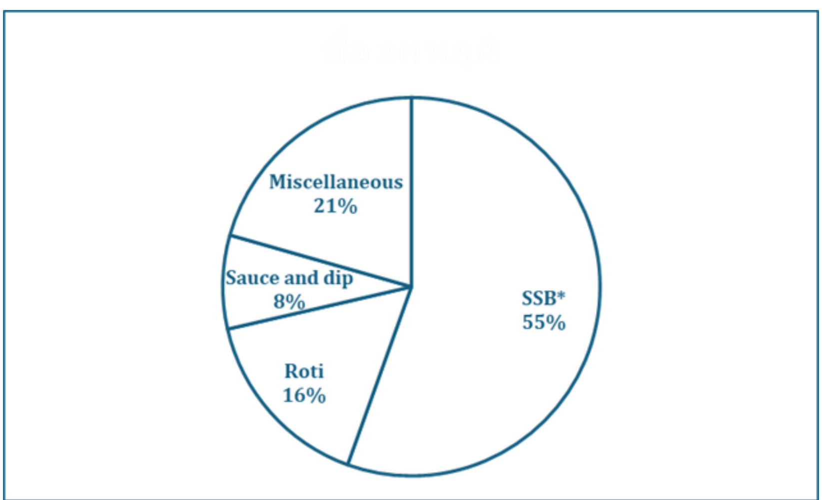

Figure 1. The main food contributed to free sugar intake in PSU's students

4. CONCLUSIONS

Free sugar intake of PSU's student was 37.8 g/d (~ 9 teaspoons) corresponding to 11.7% of total
energy intake and only 31.5% of student consumed < 25 g of free sugar/day. The major sources of free sugar were SBB (55.5%), roti (16%), sauce and dip (8%). The results of this study confirm the strong recommendation of WHO for a reduction of free sugar for preventing and controlling excess of body weight which could happen in this population later in life.

5. ACKNOWLEDGEMENTS

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Inhibition of Melanogenesis in B16F10 Mouse Melanoma Cells by Peptide Derived from Defective Arabica Green Coffee Beans (*CoffeaArabica* L.)

AP-P026

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Peptide
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ABSTRACT

Melanin, the pigment determining human skin color, is synthesized in melanocytes' melanosome *via* melanogenesis. Higher melanin concentrations lead to darker skin tones. Our study focuses on a peptide's inhibitory effect on melanogenesis and its mechanism in B16F10 cells. We evaluate a peptide from defective Arabica green coffee beans by examining its impact on cell viability, melanin content, and gene expression (TYR, TRP-1, TRP-2, and MITF) in B16F10 melanoma cells, all pertinent to melanogenesis. The peptide displays a dose-dependent cytotoxic effect on B16F10 cell proliferation. Interestingly, concentrations ranging from 15.63-1000 µg mL^{-1} show no cytotoxicity on B16F10 murine melanoma cells. At concentrations of 15.63, 62.50, 250, and 1000 µg mL^{-1}, the peptide doesn't affect cell viability, with percentages of 80%, 86%, 92%, and 96%, respectively. Moreover, the peptide reduces melanin content, with percentages of 97%, 94%, 87%, and 79% at the respective concentrations. Real-time PCR reveals down-regulated mRNAs of TYR, TRP-1, TRP-2, and MITF, indicating the peptide's suppression of melanogenesis. These findings suggest potential development of the peptide as a tyrosinase inhibitor for skin-whitening produ

1. INTRODUCTION

Melanin, the end product of melanogenesis, determines the color of human skin, hair, and eyes, and it is synthesized within unique organelles called melanosomes in melanocytes [1,2]. Melanogenesis is a complex process regulated by various environmental, hormonal, and genetic factors, including α-melanocyte stimulating hormone (α-MSH), theophylline, CAMP-elevating agents,

estrogen, and UV light. In this process, tyrosinase plays a pivotal role as the key enzyme in the rate-limiting step, converting L-tyrosine to L-DOPA, which is further oxidized into o-quinone. Tyrosinase activity is modulated by microphthalmia-associated transcription factor (MITF), a master regulator of melanogenesis. Additionally, tyrosinase-related protein (TRP-1) and TRP-2 are major targets of melanogenic enzymes induced by MITF. TRP-2 catalyzes the conversion of dopachrome to 5,6-dihydroxyindole-2-carboxylic acid (DHICA), while TRP-1 oxidizes DHICA to a carboxylate indole-quinone, ultimately leading to melanin formation [3,4]. Therefore, downregulation of tyrosinase activity has been suggested to reduce melanin production.

From an industrial standpoint, cosmetics play a crucial role in depigmenting skin color, addressing a fundamental aspect of various functional cosmetic fields. Particularly, the development of novel whitening phytochemicals from natural sources has recently gained popularity [5]. Numerous studies have focused on inhibiting melanogenesis and preventing abnormal pigmentation for cosmetic purposes [6]. Defective green coffee beans are characterized by higher levels of polyphenols, flavonoids, and caffeine. Enzymatic extraction of these defective green coffee beans yields extracts with a high protein content and a certain amount of polyphenols. These extracts exhibit antioxidant, anti-tyrosinase, and antimicrobial activities, suggesting a promising strategy for repairing defective coffee beans [7].

In our previous study, we introduced a novel heptapeptide with tyrosinase inhibitory activity [8]. This peptide, named SAIYDRP (Ser-Ala-Ile-Tyr-Asp-Arg-Pro), originates from a short sequence found within the protein extracted from defective green coffee beans. Notably, the SAIYDRP peptide showed substantial tyrosinase inhibition activity without causing harm to melanocytes. These findings strongly suggest that the SAIYDRP peptide represents an ideal tyrosinase inhibitor. In this study, we investigated the inhibitory effect of peptides derived from defective arabica green coffee beans on melanogenesis and its mechanism in B16F10 cells. To assess the peptide's impact on melanogenesis, we utilized the B16F10 cell line to measure melanin synthesis. B16F10 cells, possessing melanogenic mechanisms similar to normal human melanocytes and being easily cultured *in vitro*, serve as an appropriate model. Therefore, the regulation of tyrosinase enzymatic activity has attracted significant attention due to its potential applications in medicine, cosmetics, and agriculture.

2. MATERIAL AND METHODS

2.1 Chemicals

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), Dulbecco's modified Eagle medium (DMEM), ethidium bromide, fetal bovine serum (FBS), L-tyrosine, L-DOPA, and tyrosinase from mushrooms were all procured from Sigma-Aldrich Corp., St. Louis, MO, USA. All other chemicals utilized in the investigation were of analytical grade. B16F10 mouse melanoma cells were obtained from the American Type Culture Collection (Manassas, VA, USA).

2.2 Peptide synthesis

The peptide SAIYDRP was synthesized *via* Fmoc solid-phase synthesis using an Applied Biosystems Model 433A Synergy peptide synthesizer (Applied Biosystems, Foster City, CA, USA).

Peptide purity was then confirmed through analytical mass spectrometry employing a quadrupole ion trap Thermo Finnigan™ LXQ™ LC-ESI-MS (San Jose, CA, USA) linked to a Surveyor HPLC (Thermo Fisher Scientific, San Jose, CA, USA). The synthetic peptide was determined to have a purity of 98.1% based on the HPLC analysis.

2.3 *Anti-tyrosinase activity*

The anti-tyrosinase activity was conducted according to a slightly modified version of the method reported by [9], employing L-DOPA as the substrate. Initially, 35 µL of the sample was combined with 15 µL of tyrosinase from mushroom (333 U/mL) and incubated at room temperature for 5 minutes. Subsequently, each mixture was supplemented with 55 µL of substrates (12 mM L-DOPA) and further incubated at room temperature for 30 minutes. The anti-tyrosinase activity was assessed by measuring the absorbance at 490 nm using a microplate reader.

2.4 *Calculating the percentage inhibition*

The percentage inhibition of anti-tyrosinase activity was calculated using the following Equation [1]:

$$\text{Inhibition } (\%) = \frac{[(Abs_{control} - Abs_{blank}) - (Abs_{sample} - Abs_{background})]}{(Abs_{control} - Abs_{blank})} \times 100 \qquad [1]$$

Where $Abs_{control}$ is the absorbance of the control (no sample), Abs_{sample} is the absorbance of the sample, $Abs_{background}$ is the absorbance of the background (color of the sample). Abs_{blank} is the absorbance of the blank (deionized water). IC_{50} represents the concentration of peptide at which 50% of tyrosinase activity is inhibited.

2.5 *Measurement of protein content*

The concentration of the protein content was determined using the method described by [10], employing BSA as a standard. The sample was mixed at a ratio of 1:20 (v/v) with Bradford's working buffer in a 96-well plate and incubated for 20 minutes. The absorbance was measured at 595 nm using a microplate reader.

2.6 *Cell culture*

Cell culture procedures were conducted according to the methodology outlined by [11]. The B16F10 mouse melanocyte cell line was cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 3.7 g/l $NaHCO_3$, 4.5 g/l D-glucose, stable glutamine, Na-pyruvate, and 10% fetal bovine serum. The cells were then maintained at 37°C and 5% CO_2 in a humidified incubator. Sub-culturing was performed at a ratio of 1:3 every third day. Cell harvesting was accomplished through trypsinization. Following cell enumeration using a hemocytometer, the cells were seeded at appropriate densities into wells of a cell culture plate for subsequent analyses, including cell viability assays, melanin content determination, and assessment of cellular tyrosinase activity.

2.7 *Determination of peptide toxicity in melanocyte (MTT assay)*

Cell cytotoxicity was evaluated using B16F10 murine melanoma cells cultured in complete DMEM. Initially, cells were seeded into a 96-well plate at a density of 1×10^4 cells per well and then allowed to incubate for 24 hours at 37°C and 5% CO_2 in a humidified incubator. Following incubation,

the cells were treated with varying concentrations of the peptide (SAIYDRP), ranging from 15.63 to 1000 μg mL^{-1}, alongside a positive control of 250 μg mL^{-1} arbutin. Each well received 100 μL of a 5 mg mL^{-1} MTT solution and was subsequently incubated for an additional 4 hours at 37°C and 5% CO_2 in a humidified incubator. Upon removal of the medium, DMSO was added to dissolve the formazan crystals, at a volume of 150 μL per well, before measuring the absorbance at 540 nm. The percentage of cells displaying cytotoxicity was determined relative to the control. All experiments were performed in triplicate.

2.8 Melanin content measurement

B16F10 cells in complete DMEM were added to cell culture flasks at a density of $1x10^5$ cells per flask and then incubated overnight at 37°C and 5% CO_2 in a humidified incubator. After a 24-hour incubation period, the cells were treated with serial dilutions of peptide and arbutin (used as a positive control) for 48 hours. Subsequently, the treated cells were harvested and washed twice with phosphate-buffered saline (PBS) at pH 7.4. Finally, all cells were lysed with 500 μl of 1N NaOH. After incubating for 1 hour at 90°C, the lysates were centrifuged at 3,000 g for 10 minutes, and the absorbance values were measured at 405 nm using a microplate reader. All experiments were conducted in triplicate. The results were expressed as a percentage relative to the control, where the control was set as 100% melanin content.

2.9 Real-time quantitative polymerase chain reaction (RT-qPCR)

RT-qPCR was performed on B16F10 cells treated with peptide concentrations ranging from 15.63 to 1.000 μg mL^{-1} and arbutin at 250 μg mL^{-1} (used as a positive control) for 48 hours. Total RNA was extracted from the cells using an RNA extraction kit (Invitrogen, USA), and then 1 μg of total RNA was reverse-transcribed using the Precision nanoScript 2 Reverse Transcription kit (PrimerDesign, England) following the manufacturer's instructions. The reverse transcription reaction mixture, containing RT buffer, 10 mM dNTP, RNAse/DNAse-free water, and reverse transcriptase, was added to each sample on ice and incubated at 42°C for 20 minutes, followed by heat activation at 75°C for 10 minutes. The resulting cDNA samples were stored at -20°C until further use. Real-time PCR was conducted using the Mygo Pro instrument (Mygo; Mygo Pro, England) with the primers listed in Table 1. Total RNA concentration was determined by measuring absorbance at 260 and 280 nm, with β-actin serving as the internal reference gene. The Ct (threshold cycle) value was used to calculate the relative gene expression level, employing the formula relative gene expression = $2^{(-\Delta\Delta Ct)}$, where ΔΔCt represents the change in the gene's threshold cycle. A score of 1 indicates no change in gene expression, while scores above 1 indicate increased expression, and scores below 1 indicate decreased expression.

Table 1. Primer sequences for quantitative real-time PCR analysis.

Target genes	Primer sequences (5'-3')	Product sizes (bp)
TYR_FP TYR_RP	CACCGCCCTCTTTTGGAAGT AAAGCCTGGATCTGACTCTTGG	168
TRP-1_FP TRP-1_RP	TTTCATCTGAGCACCCCTGTCT TTGGCACACTCTCGTGGAAA	173
TRP-2_FP TRP-2_RP	GCTGAACAAGGAATGCTGCC AAGTTTCCTGTGCATTTGCATGT	198
MITF_FP MITF_RP	GGTCTCTGCTCGCCTGATCT GTGATGGTACCGTCCGTGAGAT	137
GAPDH_FP GAPDH_RP	CTACCCCCAATGTGTCCGTC GCTGTTGAAGTCGCAGGAGAC	158

2.10 Statistical analysis

All data were collected in triplicate and are presented as the mean ± one standard error of the mean. Statistical comparisons of the mean values were performed using analysis of variance (ANOVA), followed by Duncan's multiple-range test, utilizing SPSS software version 22 (IBM, New York, USA). A significance level of $P<0.05$ was applied. Regression analysis was conducted using GraphPad Prism Version 6.01 for Windows (GraphPad Software Inc., California, USA).

3. RESULTS AND DISCUSSION

3.1 Anti-tyrosinase activity of SAIYDRP peptide

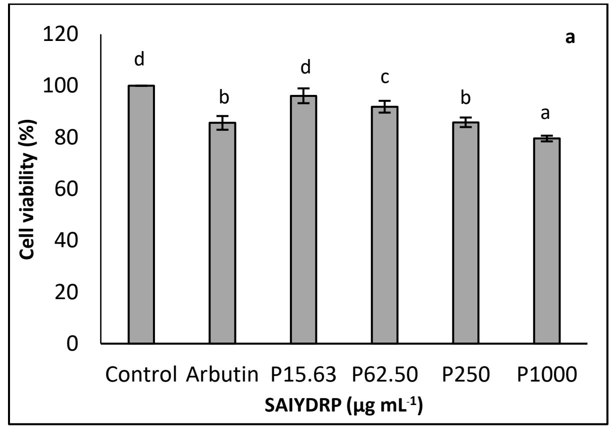
The SAIYDRP peptide demonstrated potent anti-tyrosinase activity, with an IC_{50} value of 1.53±0.01 µg mL^{-1}. This finding indicates that the peptide effectively inhibits the enzyme at a low concentration, highlighting its promising potential as a tyrosinase inhibitor. Consequently, the peptide was chosen for further investigation.

3.2 Effect of SAIYDRP peptide on cell proliferation and melanin content in B16F10 murine melanoma

The cytotoxic effect of serial concentrations of the peptide on B16F10 cell proliferation was investigated using the MTT method. This method relies on the reduction of MTT to a blue formazan by mitochondrial enzymes in viable cells, with the production of the blue formazan product being proportional to the number of viable cells. The peptide was selected to test its cytotoxic effect on B16F10 cells due to its demonstrated potential for inhibiting mushroom tyrosinase activity. Concentrations ranging from 15.63 to 1000 µg mL^{-1} showed no cytotoxic effect on cell viability, with percentages of cell viability measured at 96%, 92%, 86%, and 80%, respectively (Figure 1a).

Melanin is primarily synthesized in two forms: pheomelanin and eumelanin [12, 13]. Pheomelanin ranges from yellow to reddish-brown, while eumelanin ranges from light brown to black. Tyrosinase, the rate-limiting enzyme in melanogenesis, is involved in the early stages of melanin synthesis, including the conversion of L-tyrosine to dopaquinone and the oxidation of L-DOPA to dopaquinone [12]. The inhibitory efficiency of the peptide on melanin production is illustrated in Figure 1b. In this experiment, the control group was left untreated with the peptide, which was

set as 100% melanin content. The data showed that at high concentrations of 1000 μg mL-1, the peptide exhibited inhibitory activity on melanin production, reducing it to 79% of the control level. Therefore, the peptide inhibited melanin production at concentrations that did not affect cell viability. This result suggests that peptide concentrations ranging from 15.63 to 1000 μg mL-1 are capable of inhibiting melanin production. Arbutin was used as the positive control to assess its cytotoxic effect and melanin content, exhibiting a dose-dependent pattern similar to that of the peptide [14].



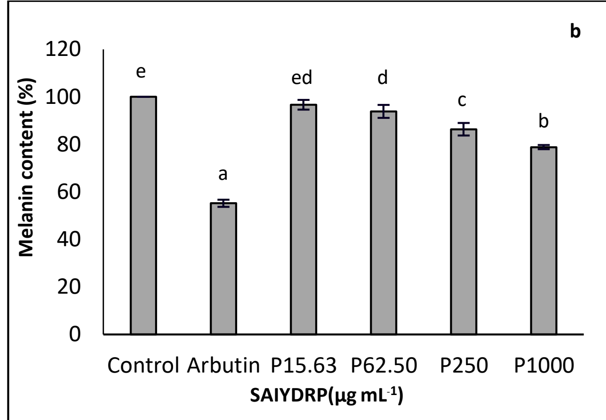
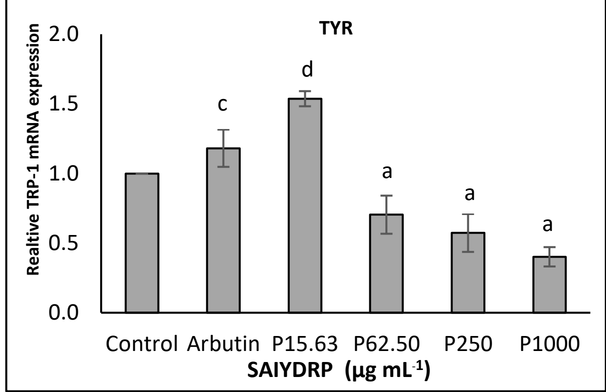
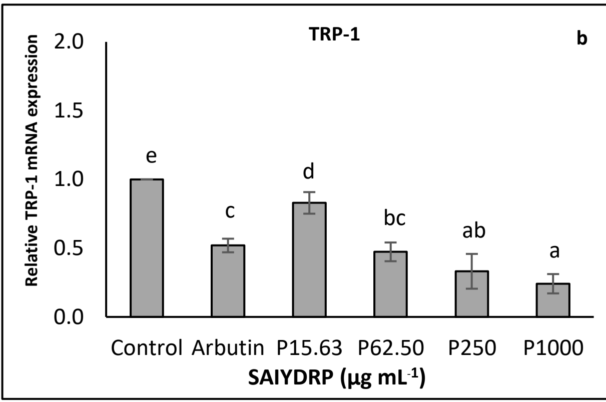


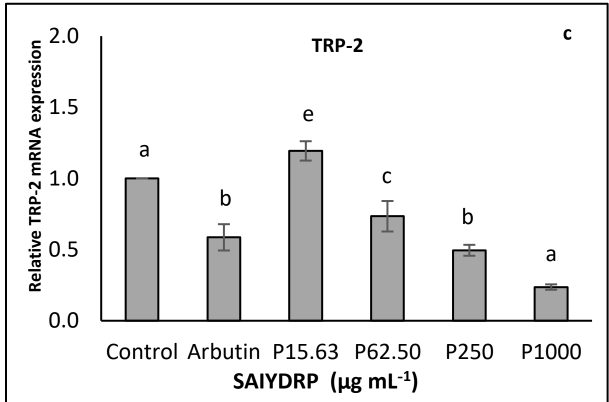
Figure 1. The effect of peptide (SAIYDRP) on B16F10 cell proliferation (a) and melanin production (b). Each experiment was conducted in triplicate.

3.3 Investigating the impact of SAIYDRP peptide on the mRNA expression levels of TYR, TRP-1, TRP-2, and MITF in B16F10 cells

To investigate whether the peptide's ability to inhibit melanin synthesis correlates with changes in melanogenesis-related gene expression, we conducted real-time PCR to evaluate the mRNA levels of TYR, TRP-1, TRP-2, and MITF. Our analysis indicated a consistent decrease in the mRNA levels of all these genes following peptide treatment (**Figure 2a-d**). Specifically, the mRNA levels of TYR, TRP1, TRP2, and MITF were suppressed across a range of peptide concentrations from 15.63 to 1000 μg mL-1. Arbutin, employed as the positive control, is a cosmetic ingredient renowned for its tyrosinase-inhibitory properties [14, 15].







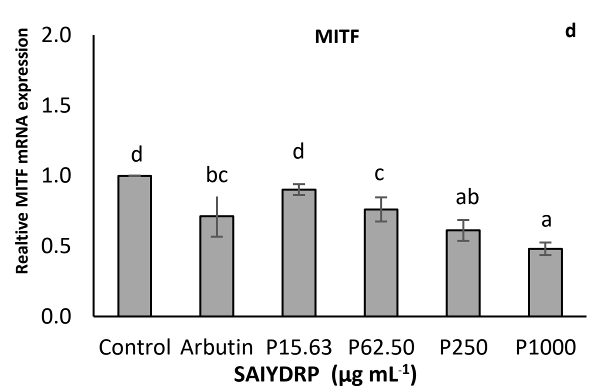


Figure 2. Effects of peptide (SAIYDRP) on mRNA levels of TYR (a), TRP-1(b), TRP-2 (c) and MITF (d) in B16F10 cells.Each bar represents the mean±SD (n=3). Different letters indicate a significant difference according to the ANOVA (P<0.05).

4. CONCLUSIONS

The present study concludes that the peptide derived from defective Arabica green coffee beans effectively suppresses cellular melanin generation and anti-tyrosinase activity in B16F10 murine melanoma cells. These findings suggest that the peptide exhibits promising potential as a potent inhibitor of melanogenesis. Consequently, it could be explored for application in the treatment of skin hyperpigmentation disorders and the development of skin-whitening cosmetic products.

5. ACKNOWLEDGEMENTS

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AP-P029

Fatty Acid Composition and Antioxidant Activity of Watermelon Seed Oil from Two Cultivars: Kinnaree and Yellowstone

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ABSTRACT

Watermelon (*Citrullus lanatus*) is one of the most economically important food crops and highly cultivated in Thailand. Currently, multiple watermelon varieties are being produced and made accessible for commercial cultivation. Fresh watermelon flesh is commonly consumed and processed, producing large amounts of watermelon seeds, which are commonly discarded as waste. The seeds from different watermelon strains contain distinct bioactive compounds and possess different bioactivities with health benefits. The purposes of this research were to evaluate fatty acid composition and to study antioxidant activity of watermelon seed oils derived from two watermelon cultivars with different flesh colors: Kinnaree and Yellowstone. Fatty acids were esterified and analyzed qualitatively and quantitatively by gas chromatography. It was shown that similar fatty acid profiles were observed in seed oils from both cultivars. Eight fatty acids were identified in both seed oils. Major fatty acids were linoleic acid, oleic acid, palmitic acid, stearic acid; while minor fatty acids were linolenic acid, arachidic acid, palmitoleic acid, and myristic acid. Antioxidant activity of watermelon seed oil determined by DPPH radical scavenging assay indicated that the Kinnaree seed oil contained higher antioxidant activity than the Yellowstone seed oil, with the IC50 values of 4.42 ± 2.37 mg/mL and 4.99 ± 2.14 mg/mL, respectively. The finding from this research could be beneficial to endorse the use of undervalued watermelon seeds as additives for future development of nutraceutical, cosmeceutical and functional food products.

1. INTRODUCTION

Watermelon (*Citrullus lanatus*) seed could be considered as underutilized by-product from watermelon consumption. Extracted oils from watermelon seeds contain various bioactive compounds including essential fatty acids, tocopherols, riboflavin, carotenoids, thiamine, flavonoid and phenolic compounds [1, 2]. And numerous bioactivities of the oils have been reported such as antioxidant, antimicrobial, anti-cancer, anti-inflammatory, anti-diabetic, cardioprotective and hepatoprotective activities [2-6]. The major composition of the watermelon seed oils are poly-unsaturated fatty acids (PUFAs) with linoleic acid as the most abundant fatty acid. Different cultivars of watermelon seeds yield different content of oils which are in the range of 10-35% and also contain distinct fatty acid compositions [7-8].

There are many varieties of watermelon cultivated in Thailand; however, the information about seed oil composition and bioactivities of those varieties are still limited. The seed oil from Kinnaree cultivar was reported to contain eight types of fatty acids, mainly in a group of PUFAs [3]. Five watermelon cultivar, Kinnaree, Torpedo, Yaya, Runrun and King orange have been shown to contain antioxidant and nitric oxide inducing activities [9]. However, there is no information about fatty acid profile and antioxidant activity of the seed oil from the Yellowstone variety. Therefore, this work aims to comparatively evaluate fatty acid composition of seed oils from two different watermelon varieties, Kinnaree and Yellowstone, which contain red flesh and yellow flesh, respectively, using gas chromatography connected with flame ionization detector (GC-FID). Also, free radical inhibitory activity of the extracted oils was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Since there are increasing number of utilizations of watermelon seed oils in cosmetics, pharmaceutics, therapeutics, and food processing [2], this study could provide valuable information about active components and intrinsic antioxidant property of seed oils from Kinnaree and Yellowstone cultivars to use as a basis for various applications.

2. MATERIAL AND METHODS

2.1 Material

Watermelon seeds from Kinnaree and Yellowstone cultivars were provided by Nichchar Charm farm, Chonburi, Thailand. Standard fatty acid methyl esters (FAMEs), standard α-tocopherol, 2-2'-diphenyl-1-picrylhydrazyl (DPPH) and boron trifluoride and were purchased from Sigma-Aldrich Corp. (St. Louis, Mo., U.S.A.). Chromatographic grade solvents (N-hexane, methanol, and heptane) were purchased from Merck KGaA (Darmstadt, Germany).

2.2 Extraction of watermelon seeds

The whole seeds from Kinnaree and Yellowstone watermelon were dried in a hot air oven (Memmert® CTC256, Buchenbach, Germany) at 60 °C until a stable weight was obtained. Dried seeds were ground prior to extraction with hexane. Five grams of fine powder were incubated in 10 mL of hexane for 24 hours. Watermelon residual was separated from the extract by filtering through a 0.45 µm nylon syringe filter (GE Healthcare, Waltham, MA, USA). After that hexane was removed from the extracts in the desiccator connected to vacuum pump. The yield of seed oil could be calculated by equation (1).

$$\%yield = \frac{\text{weight of extracted oil}}{dried\ weight\ of\ watermelon\ seed} \times 100 \quad (1)$$

2.3 *Analysis of fatty acid by GC-FID*

Fatty acid profiles were analyzed by GC-FID following the method from Petchsomrit et al. 2020 [3] with slight modification. Prior to the analysis, fatty acids were esterified to obtain fatty acid methyl esters (FAMEs). A portion of watermelon seed oil (100 μL) was added to eight volumes of 0.5M methanolic NaOH (800 μL). The mixtures were heated at 80 °C for 7min. And 20% methanolic boron trifluoride (900 μL) was added and incubated at 80 °C for 2min. After that, 400 mL heptane was added to the solution and incubated at 100 °C for 1min. Phase separation was carried out to separate FAMEs from the mixture, using 1500 μL saturated NaCl.

FAMEs were analysed using a 6890-gas chromatography equipment with ChemStation software (Agilent Technologies, Palo Alto, Ca., U.S.A.). Stationary phase was a HP-FFAP column (Agilent Technologies, Palo Alto, Ca., U.S.A.) with the column length of 30 m, inner diameter of 0.25mm and film thickness of 0.25 μm. Helium was used as a mobile phase. One microliter of each FAME sample was injected to the system at an injection temperature of 200 °C using spitless mode. Temperature programming was performed for the separation, starting from 150 °C to 220 °C, with a rate of 4 °C/min, followed by constant temperature at 220 °C for 20 min. Flame ionization detector was chosen for FAMEs detection. The FAME standard including caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), linolenic acid (C18:3), behenic acid (C22:0), erucic acid (C22:1), and lignoceric acid (C24:0) methyl esters were used for both qualitative and quantitative analyses. For quantitative measurements, standard curve of each FAME was constructed based on the relationship between concentration of FAMEs and the area under the peak.

2.4 *DPPH radical scavenging activity assay*

Antioxidant activity was evaluated using DPPH radical scavenging activity assay following the method from Molynuex, 2004 (10) with slight modification. Briefly, 50 μL of extracted seed oils in heptane at different concentrations were mixed with 100 μL of 0.2 mM DPPH in methanol in a microplate. The mixtures were incubated at room temperature for 30 min and the absorbance was monitored at 517 nm using a microplate reader (VersaMax, Molecular Devices, Sunnyvale, Ca., U.S.A). Antioxidant activity was calculated as % DPPH inhibition (see Equation (2)).

$$\%\ DPPH\ inhibition = \frac{Absorbance(control) - Absorbance(sample)}{Absorbance(control)} \times 100 \quad (2)$$

3. RESULTS AND DISCUSSION

3.1 *Seed oil Extraction yield*

Seed oils were extracted from two watermelon cultivars: Kinnaree with red flesh and Yellowstone with yellow flesh. Although these two cultivars contain different flesh colors as shown in Figure 1(A) and (B), the shape and size of their seeds were similar. Also, both varieties provided light-yellow colored and scentless oils from hexane extraction. The %yield of seed oil from Kinnaree cultivar was shown to be higher than that of Yellowstone cultivar (Table 1). This result was in

agreement with a previously published report [3], which showed that %yield of seed oil from Kinnaree watermelon was 7.72 ± 4.61%. However, the efficiency of this solvent extraction method at room temperature was lower than using elevated temperature [7-8].

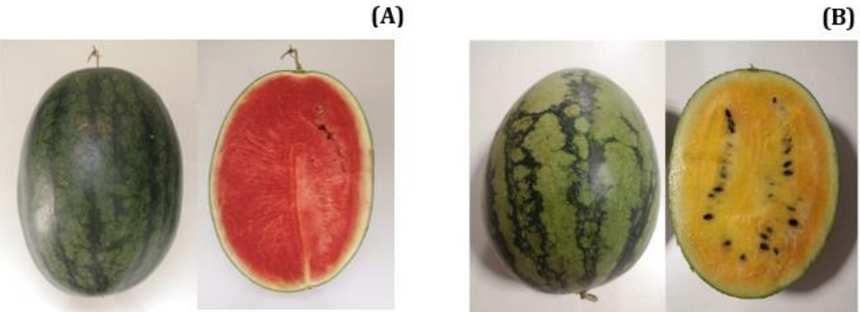

Figure 2. Outer and inner parts of Kinnaree (A) and Yellowstone (B) watermelon

Table 1. Yield of watermelon seed oil from Kinnaree and Yellow Stone cultivars

Cultivar	% yield
Kinnaree	5.14 ± 0.61 %
Yellow Stone	4.24 ± 1.05 %

3.2 *Analysis of FAMEs by GC-FID*

Derivatives of fatty acids from Kinnaree and Yellowstone seed oils were analyzed by GC-FID, in comparison with 14 types of analytical standard FAMEs from commonly found fatty acids in plant oils. The order of elution of FAME standards was shown in Figure 2(A), in accordance with their carbon number and degree of unsaturation, as followed: caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), linolenic acid (C18:3), behenic acid (C22:0), erucic acid (C22:1), and lignoceric acid (C24:0), respectively. The chromatogram revealed that 8 fatty acids were found in both Kinnaree and Yellowstone seed oils. Four of these could be classified as saturated fatty acids, including myristic acid, palmitic acid, stearic acid and arachidic acid. The other four were unsaturated fatty acids, which were palmitoleic acid, oleic acid, linoleic acid, and linolenic acid (Figure 2(B) and Figure 2(C)).

The result from quantitative analysis was shown in Table 2. The % fatty acid composition calculated from proportional peak area of each FAME indicated that the most abundant fatty acid found in both Kinnaree and Yellowstone seed oils was linoleic acid, which was classified as PUFA. Other published reports also revealed that linoleic acid was a major fatty acid found in seed oil from various cultivars of watermelon, including Kinnaree, Mateera, Sugar baby, Q-F-12, D-W-H-21, and Red Circle-1885. (3, 11, 12). A slightly higher proportion of linoleic acid was observed in Yellowstone seed oils (54.81 ± 11.75% of total fatty acid) than Kinnaree seed oil (50.49 ± 12.14% of total fatty acid), accounted for absolute quantity of 267.53 ± 30.88 µg fatty acid/ g watermelon seed and 254.40 ± 65.05 µg fatty acid/ g watermelon seed, respectively (Table 2). The order of % fatty acid composition of seed oils from both species showed similar trend that major FAMEs were from unsaturated fatty acid. From Kinnaree watermelon, the proportion of linoleic acid was greater than oleic acid, palmitic acid, stearic acid, linolenic acid, arachidic acid, palmitoleic acid, and myristic acid,

respectively. Although comparable trend of % fatty acid composition was observed in Yellowstone seed oil, a minor difference was recognized that %composition of palmitic acid was slightly higher than oleic acid (Table 2). This observation revealed that Yellowstone seed oils contained different fatty acid composition, as compared to those from Kinnaree, Mateera, Sugar baby, Q-F-12, D-W-H-21, and Red Circle-1885 varieties [3, 11, 12].

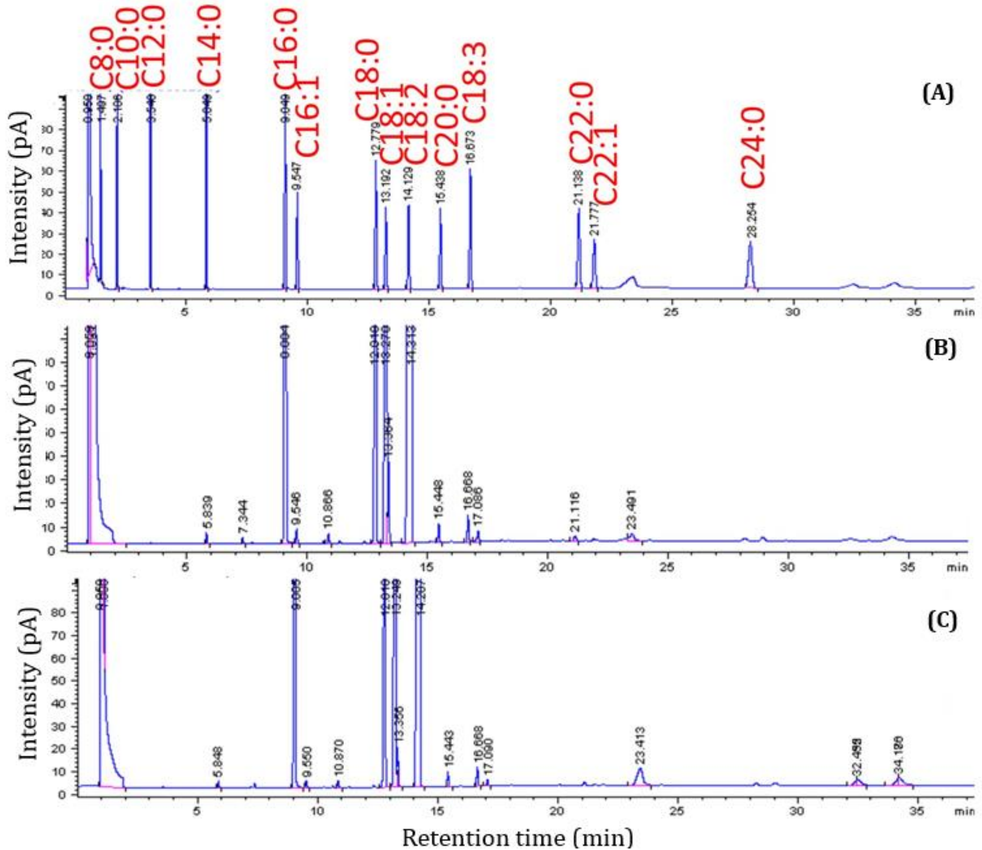

Figure 2. Chromatograms of standard FAMEs (A), FAMEs from Kinnaree watermelon seed oil (B) and FAMEs from Yellowstone watermelon seed oil (C)

Table 1. Fatty acid composition of Kinnaree and Yellowstone watermelon seed

Fatty acid	Abbreviation	% composition		Quantity (µg fatty acid/ g watermelon seed)	
		Kinnaree	Yellowstone	Kinnaree	Yellowstone
Caprylic acid	C8:0	ND*	ND	ND	ND
Capric acid	C10:0	ND	ND	ND	ND
Lauric acid	C12:0	ND	ND	ND	ND
Myristic acid	C14:0	0.06 ± 0.00	0.06 ± 0.01	0.27 ± 0.12	0.20 ± 0.10
Palmitic acid	C16:0	10.81 ± 1.45	11.90 ± 4.02	55.90 ± 19.89	51.67 ± 16.07
Palmitoleic acid	C16:1	0.13 ± 0.02	0.11 ± 0.04	0.70 ± 0.26	0.50 ± 0.17
Stearic acid	C18:0	5.01 ± 1.18	6.12 ± 0.04	29.47 ± 7.71	31.27 ± 2.84
Oleic acid	C18:1	14.81 ± 3.21	11.72 ± 2.51	85.03 ± 22.56	58.03 ± 6.91
Linoleic acid	C18:2	50.49 ± 12.14	54.81 ± 11.75	254.40 ± 65.05	267.53 ± 30.88
Arachidic acid	C20:0	0.15 ± 0.04	0.18 ± 0.04	1.40 ± 0.26	1.43 ± 0.15

Linolenic acid	C18:3	0.23 ± 0.08	0.26 ± 0.04	0.87 ± 0.25	0.93 ± 0.06
Behenic acid	C22:0	ND	ND	ND	ND
Erucic acid	C22:1	ND	ND	ND	ND
Lignoceric acid	C24:0	ND	ND	ND	ND

*ND = not detected

3.3 Determination of antioxidant activity of watermelon seed oil

The antioxidant property of watermelon seed oils was evaluated by DPPH radical scavenging ability. The seed oils from Kinnaree and Yellowstone cultivars showed good antioxidant activity which was directly proportional to the oil concentration (Figure 3(A) and Figure 3(B)). The IC50 values against DPPH radicals of the Kinnaree and Yellowstone seed oils were 4.42 ± 2.37 mg/mL and 4.99 ± 2.14 mg/mL, respectively, indicating that Kinnaree seed oil had higher efficiency to scavenge DPPH radicals. The ability to scavenge free radicals could be resulted from the presence of α-tocopherol, phenolic and flavonoid compounds and some fatty acids found in the oils [2]. As compared to the positive control, both samples showed lower antioxidant activity, since the IC50 value of α-tocopherol was 50.45 ± 1.37 µg/mL (Figure 3(C)).

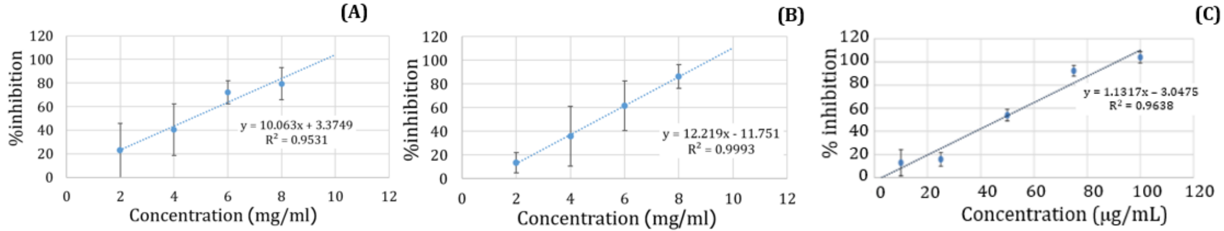

Figure 3. % DPPH inhibition of (A) Kinnaree seed oil, (B) Yellow stone seed oil and (C) α-tocopherol

4. CONCLUSIONS

This work revealed that watermelon seed oils from two cultivars, Kinnaree and Yellowstone, contained eight types of fatty acids: linoleic acid, oleic acid, palmitic acid, stearic acid, linolenic acid, arachidic acid, palmitoleic acid, and myristic acid. Of which, linoleic acid was found to be the most abundant fatty acids found in both varieties. Kinnaree seed oil provided higher extraction yield and better antioxidant activity as compared to Yellowstone seed oil. The result from this study could be advantageous for the future use of seed oils from various watermelon cultivars as functional food commodities and for nutraceutical, pharmaceutical and clinical applications.

5. NOMENCLATURE

DPPH 2,2-diphenyl-1-picrylhydrazyl
FAME Fatty acid methyl ester
FID Flame ionization detector
GC Gas chromatography

6. ACKNOWLEDGEMENTS

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Effect of enzyme treatment on quality of lychee juice

AP-P044

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ABSTRACT

Lychee juice contains phytonutrient such as flavonoids and bioactive compound. Enzymatic treatment plays a pivotal role in the processing of fruit juices. This study aimed to assess the impact of Viscozyme®L (an enzyme treatment) on the quality of lychee juice. Lychee juice underwent separate treatments with Viscozyme®L at varying incubation times (0, 2, 4, and 6 h) and temperatures (50, 55, and 60°C). Various quality parameters, including total soluble solid, color attribute, bioactive compound (total phenolic content and total flavonoid content), and antioxidant activity (evaluated through DPPH and FRAP assays), were analyzed. Results indicated that enzyme treatment significantly enhanced ($p \geq 0.05$) the total phenolic content, total flavonoid content, and antioxidant activity via FRAP and DPPH assays compared to the control group without enzyme treatment. The most favorable outcomes, with the highest total soluble solid (12.3±0.06°Brix), total phenolic content (517.53±2.55 mg GAE/L), total flavonoid content (416.82±0.96 mg QE/L), and antioxidant activity measured by DPPH (714.96±4.02 mg TE/L) and FRAP (562.87±0.67 mg TE/L), were achieved following a 2-h incubation at 55°C. In summary, these findings provide valuable insights for the future production of functional food ingredients.

1. INTRODUCTION

Lychee (*Litchi chinensis* Sonn.) is a significant economic fruit in Thailand and high commercial value in the international market. According to a report from the Office of Agricultural Economics. Thailand is an important produce in the world. About 30% is exported to China, Singapore, Philippines, Malaysia, and Australia, etc. Most of the produce, more than 70%, is used for domestic consumption [1]. During 2022, the amount of produce released to the market is approximately 43,817 tons [2] because lychees are rich in vitamin C, vitamin B. and flavonoids that have antioxidant properties which promote consumer health.

Chakrapad lychees are cultivated primarily for their delightful sweetness, juiciness, and distinctive flavor profile. Despite comprising a significant portion of water (76–91%), lychee fruit contains an array of valuable constituents such as bioactive compounds (phenolics/antioxidants),

essential minerals (phosphorus, iron, calcium), vitamins (thiamine, niacin, riboflavin, vitamin C), sugars (sucrose, glucose, fructose), fats, proteins, and flavor-enhancing elements (limonene, geraniol, neural), collectively rendering it a superlative fruit choice. [3].

In juice processing, enzymes are commonly added during maceration, prior to the pressing of fruits to obtain satisfactory juice yield [4]. Viscozyme®L, a multi-enzyme complex containing cellulase, hemicellulase, arabanase, xylanase, β-glucanase and pectinases, is normally able to catalyze the degradation of cellulose and pectin in cell walls. It is not only help in softening the plant tissue but also lead to the release of cell component that may be recovered with high yield [5] upon enzyme treatment degradation of the pectin leads to a reduction in the water holding capacity of pectin which released more juice into the mixture and consequently increased juice yield [6], leading to an improvement in juice clarity and the release of bioactive compounds trapped in plant cells [7]. These enzymes facilitate the degradation of the cell middle lamella and primary wall, thereby releasing polyphenolic compounds with antioxidant activity located in the cell vacuoles. At the same time, physicochemical properties such as viscosity and the carbohydrate composition of juice are also impacted [8]. Factors in enzymatic treatment include enzyme concentration, substrate concentration, incubation time, temperature, environment exposed and pH [9][10]. This study aimed to assess the impact of Viscozyme®L treatment by varying time (0, 2, 4, and 6 hours) and temperature (50, 55, and 60°C) on the quality (total soluble solid, colour attribute, total phenolic content, total flavonoid content, and antioxidant activity by DPPH and FRAP assays) of lychee juice.

2. MATERIAL AND METHODS

2.1 Chemicals and reagents

Frozen Chakrapad lychees (Chiang Rai, Thailand), Viscozyme®L (Novozymes, Denmark), 2,2-diphenyl-1-picryl-hydrazyl (DPPH) (Sigma, Switzerland), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (Sigma Aldrich, USA), 6- hydroxy-2,5,7,8-tretamethylchroman-2-carboxylic acid (Trolox) (Sigma, Switzerland), aluminium chloride (KemAus, Australia), methanol (Krungthepchemi, Thailand), ferric chloride (Fisher Scientific, UK), Folin-Ciocalteu reagent (Loba, India), gallic acid (Fluka, Spain), glacial acetic acid (ANaPURE, New Zealand), hydrochloric acid 37% (ANaPURE, New Zealand), quercetin (Sigma-Aldrich, Germany), Sodium acetate (CH_3COONa) (KemAus™, Australia), Sodium carbonate (Na_2CO_3) (KemAus™, Australia) and Sodium hydroxide (NaOH) (QRëC, New Zealand) were used in this experiment.

2.2 Preparation of enzyme-treated Lychee pulp sample

The frozen lychees were obtained from Chiang Rai province and thawing was performed in a refrigerator for 24 h at 4°C. Then, the lychee was transferred into blender and the lychee puree were boiled at 90°C for 1 min and then cooled until the temperature dropped to 60°C and packed in an aluminum foil container and being frozen at -20°C. (The thawed lychee puree was kept in a refrigerator for 24 h at 4°C prior to the experiments). To prepare lychee juice, natural pH (4.38) was adjusted to pH 5.5 with acetate buffer pH 5.5 and Viscozyme®L enzyme was used (300AGU/mL) [11], with the optimum temperature and pH for the enzyme activity between 50 to 60°C and pH 4.5 to 6.5, respectively [12]. A total of 49 g of lychee pulp sample were weighed into flask and then treated with 1 mL of 1%v/v Viscozyme®L at temperatures 50, 55 and 60°C for 0 2 4 and 6 h, respectively. The enzymatic treatment was performed by incubating the sample in an incubator shaker.

The treated lychee pulp samples were then immediately heated in a 90°C water bath for 5 min to stop reaction, and centrifuge at 4,500 x g for 10 min. The supernatant (clear juice) was collected and filtered through filter paper Whatman no.1 before the further experiment [13].

2.3 Analytical method

2.3.1 Determination of bioactive compound and antioxidant activity of lychee juice

The total phenolic compound (TPC)

The total phenolic compound (TPC) was measured using the Folin–Ciocalteau method at the absorbance of 765 nm with spectrophotometer (GENE-SYSTEM 20 Visible, Thermo Fisher Scientific, USA). The result was expressed as milligram gallic acid equivalent per liter of sample (mg GAE/L sample) [14].

The total flavonoid content (TFC)

The total flavonoid content (TFC) was determined as described by Daupor et al. [15] with some modification. The sample (1.5 mL) was mixed with 10% aluminum chloride (1.5 mL). The mixture was incubated for 30 min at room temperature (25±1°C). The absorbance value at 430 nm was measured with spectrophotometer. The experiment used quercetin as a standard solution and the result was reported as milligram quercetin equivalent per liter of sample (mg QE/L sample).

DPPH scavenging capacity assay

DPPH scavenging capacity assays were described by Brand-Williams et al. [16] with some modification. The absorbance was performed at the wavelength of 515 nm with spectrophotometer. The experiment used Trolox as a standard solution and the result was reported milligram Trolox equivalent per liter of sample (mg TE/L sample).

$$A_{diff} = A_{initial} - A_{final} \quad (1)$$

Where, A_{diff} was the absorbance difference between FRAP solution and sample, $A_{initial}$ was the absorbance of FRAP and A_{final} was the absorbance of sample. A_{diff} was calculated in the above equation and the result was expressed in mg Trolox/L.

Ferric Reducing Antioxidant Power assay (FRAP assay)

The procedure was performed according to Benzie et al. [17] with some modification. FRAP assay was measured using the extract (150 µL) mixed with 2.85 mL of FRAP reagent. The mixture was incubated in the dark for 30 min. The absorbance value at 593 nm was measured with a spectrophotometer. The experiment used Trolox as a standard solution and the result was reported milligram Trolox equivalent per liter of sample (mg TE/L sample).

$$A_{diff} = A_{final} - A_{initial} \quad (2)$$

Where, A_{diff} was the absorbance difference between FRAP solution and sample, $A_{initial}$ was the absorbance of FRAP and A_{final} was the absorbance of sample. A_{diff} was calculated in the above equation and the result was expressed in mg Trolox/L.

2.3.2 Determination of physicochemical properties of lychee juice

The physicochemical properties of samples (control and enzyme-treated) were analysed as described by Rejab et al. [18]: Total soluble solid was measured using a digital hand refractometer (Hanna Instrument, 96801) in the range of 0-30 °Brix. The colour values were obtained with a chroma meter (Minolta, Model CR-300 series, Japan) by CIE LAB system (L*, a* and b*); where, L* represents the brightness, a* represents the red and green values and b* value represents the yellow and blue.

2.4 Statistical analysis

The experiment design was conducted using a completely randomized design (CRD) with analysis of variance (ANOVA) by SPSS software version 29.0.1.0 (SPSS Inc., Chicago, IL, USA) and means were compared with Turkey's (HSD) multiple comparison test at the 95% of confidence level.

3. RESULTS AND DISCUSSION

3.1 The total phenolic compound (TPC), and total flavonoid content (TFC)

The total phenolic compound result was shown in Figure 1. The total phenolic compound of control and treated with 1%v/v Viscozyme®L sample range from 21.14±1.73 mg GAE/L to 517.53±2.55 mg GAE/L. While the total flavonoid content (Figure 2) values range from 82.38±1.25 mg QE/L to 416.82±0.42 mg QE/L. It was found that TPC and TFC decreased with increased temperature and time, provided the highest total phenolic compound (517.53±2.55 mg GAE/L) and total flavonoid content (416.82±0.42 mg QE/L) at 2 h at 55°C ($P \leq 0.05$) compared with control group without enzyme treatment (327.53±2.10 mg GAE/L and 336.54±0.83 mg QE/L, respectively). According to Nguyen's study on how the quality of mulberry juice is affected by Viscozyme®L treatment, enzyme treatment significantly increased the total phenolic compound by twice when compared to the untreated juice [12]. This may be the result of enzyme degrading pectins in the middle lamella of fruit tissue and the increased extraction of antioxidants from the cellular cytoplasm [19].

3.2 DPPH scavenging capacity assay, and Ferric Reducing Antioxidant Power assay (FRAP assay)

Figure 3 illustrates the antioxidant activity as determined by the DPPH assay, with recorded values from 72.46±5.20 mg TE/L to 714.96±4.02 mg TE/L. Concurrently, Figure 4 displays the FRAP values ranging from 60.64±4.02 mg TE/L to 562.87.82±0.67 mg TE/L. It was observed that both antioxidant activity by DPPH and FRAP assays exhibited a decline with increased temperature and duration of incubation. Notably, enzymatic treatment with a 2-hour incubation at 55°C demonstrated the highest antioxidant activity by DPPH and FRAP assays yielding values of 714.96±4.02 mg TE/L and 562.87±0.67 mg TE/L, respectively ($P \leq 0.05$). In contrast, the control sample without enzyme treatment exhibited values of 270.79±1.91 mg TE/L and 341.53±3.71 mg TE/L for DPPH and FRAP assays, respectively. Enzyme facilitates the degradation of the cell middle lamella and primary wall, thereby releasing polyphenolic compounds with antioxidant activity located in the cell vacuoles. Increasing antioxidant capacities were found when applying enzyme to produce several fruit juices, such as blackcurrant [20] and pomegranate [21].

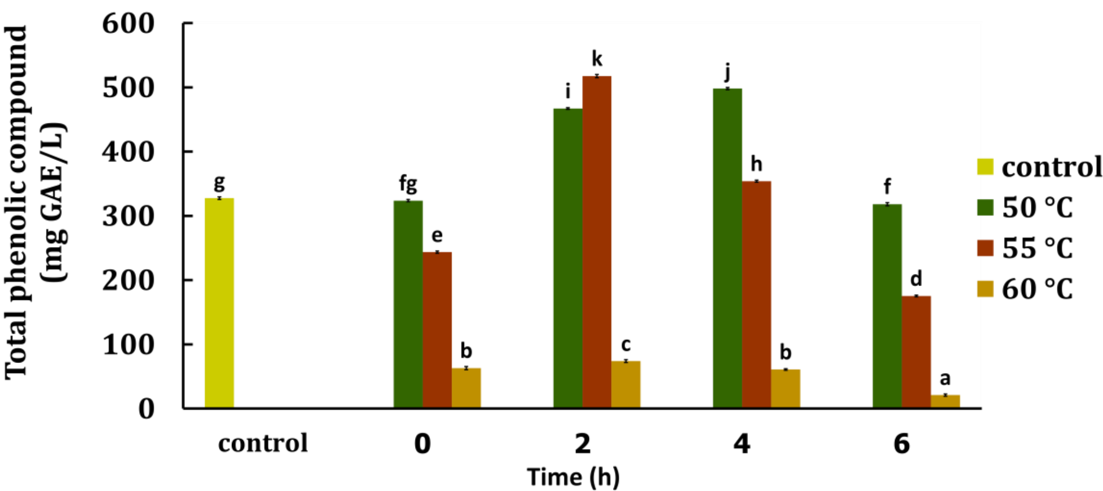

Figure 1. The effects of enzyme treatment by varying incubation time (0, 2, 4, and 6 h) and temperature (50, 55 and 60°C) on total phenolic compound of lychee juice treated with 1 mL of 1%v/v Viscozyme®L compared to control sample without enzyme treatment. The values are mean ± SD (n=3). Means with differences letters are significantly different (P≤0.05).

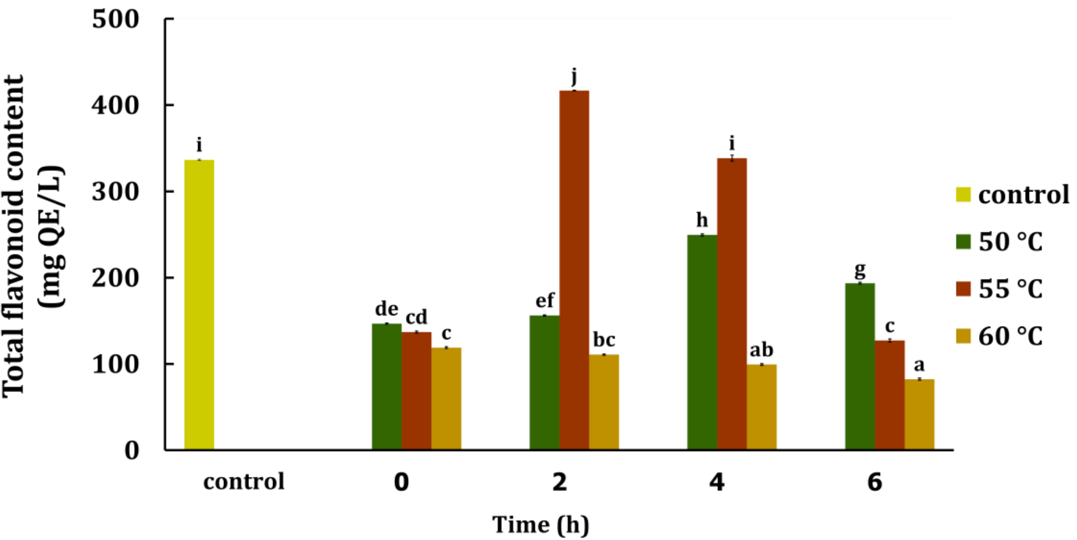

Figure 2. The effects of enzyme treatment by varying incubation time (0, 2, 4, and 6 h) and temperature (50, 55 and 60°C) on total flavonoid content of lychee juice treated with 1 mL of 1%v/v Viscozyme®L compared to control sample without enzyme treatment. The values mean ± SD (n=3). Means with differences letters are significantly different (P≤0.05).

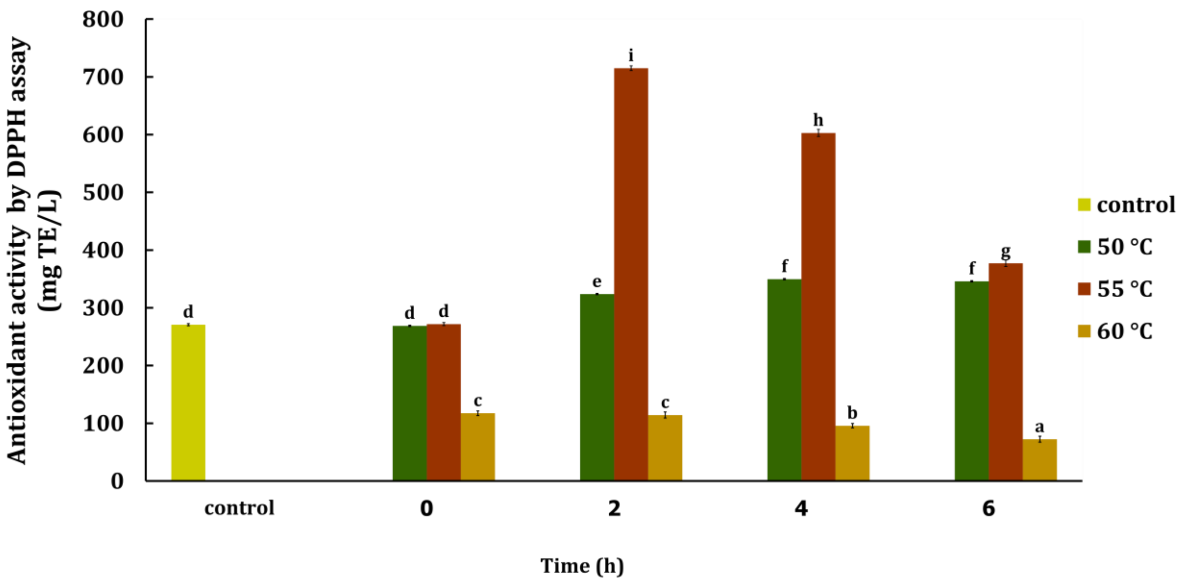

Figure 3. The effects of enzyme treatment by varying incubation time (0, 2, 4, and 6 h) and temperature (50, 55 and 60°C) on antioxidant activity by DPPH assay of lychee juice treated with 1 mL of 1%v/v Viscozyme®L compared to control sample without enzyme treatment. The values are mean ± SD (n=3). Means with differences letters are significantly different (P≤0.05).

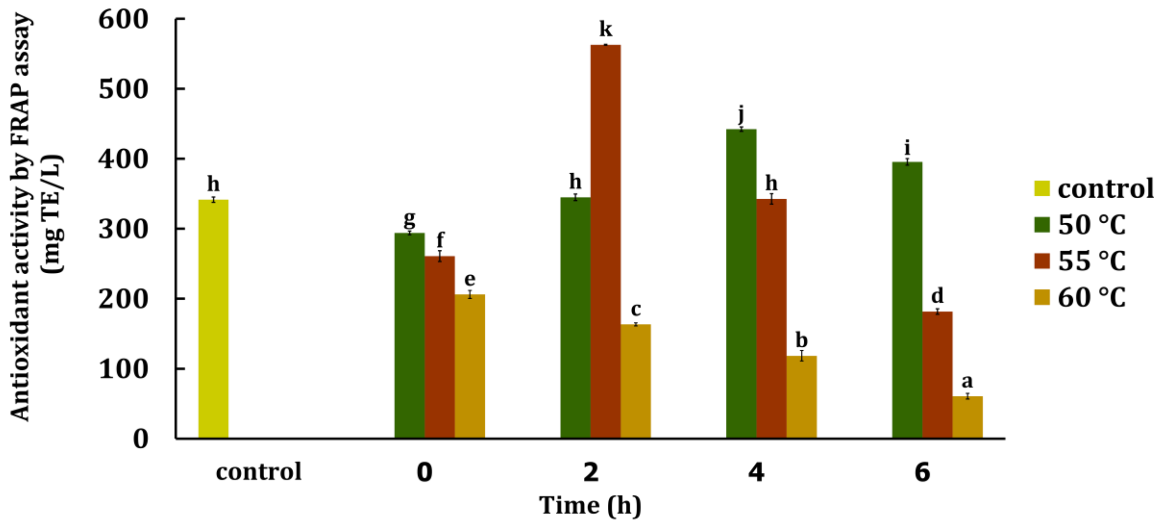

Figure 4. The effects of enzyme treatment by varying incubation time (0, 2, 4, and 6 h) and temperature (50, 55 and 60°C) on antioxidant activity by FRAP assay of lychee juice treated with

1 mL of 1%v/v Viscozyme®L compared to control sample without enzyme treatment. The values are mean ± SD (n=3). Means with differences letters are significantly different (P≤0.05).

3.3 Color and °Brix

Statistically significant differences (P≤0.05) were observed among the samples (both control and enzyme-treated sample) including total soluble solid (°Brix) and color attributes, as detailed in Table 1. The total soluble solid fell within the range of 11.22 to 12.8 °Brix. Regarding color parameters (L*, a*, and b*), values ranged from 28.00 to 28.93, 0.02 to 0.17 and 0.25 to 1.96, respectively. The color of the juice plays a pivotal role in consumer sensory perception and serves as a crucial quality indicator. Hence, any treatment applied to fruit juice must not alter its color integrity [22]. This finding indicated that the L* values of lychee juice samples suggested a darkened hue, likely attributed to heat exposure during enzyme inactivation and enzymatic browning reactions. Conversely, the a* and b* values contributed to a lighter and brighter appearance. Based on these color assessments, the enzyme-treated lychee juice exhibited a light-yellow color. The total soluble solids exhibited a slight deviation, which could be attributed to the rise in soluble sugars resulting from the conversion of insoluble pectin by pectinolytic enzymes, along with the cellulase's activity on cellulose, generating soluble sugars. In a study conducted by Wilkins et al., the utilization of pectinase and cellulase resulted in the highest glucose and total sugar content, consequently contributing to an increased total soluble solid [23].

Table 1. Physical properties of lychee juice treated with enzyme compared to control sample without enzyme treatment

Treatment	Time (h)	Physical properties			
		Total soluble solid (°Brix)	L*	a*	b*
control		12.8±0.21^{g}	28.00±0.05^{a}	0.04±0.02^{a}	0.25±0.01^{a}
50°C	0	11.2±0.12^{a}	28.34±0.04^{bc}	0.05±0.05^{ab}	0.46±0.02^{b}
	2	11.3±0.10^{ab}	28.80±0.03^{e}	0.04±0.02^{a}	0.57±0.02^{de}
	4	11.8±0.06^{de}	28.38±0.02^{cd}	0.04±0.05^{a}	0.55±0.02^{cd}
	6	11.5±0.06^{bc}	28.07±0.06^{a}	0.04±0.02^{a}	0.64±0.02^{ef}
55°C	0	11.9±0.06^{e}	28.44±0.07^{cd}	0.04±0.02^{a}	0.50±0.04^{bc}
	2	12.3±0.06^{f}	28.48±0.07^{d}	0.04±0.06^{a}	0.65±0.03^{f}
	4	12.3±0.06^{f}	28.23±0.03^{b}	0.06±0.01^{abc}	0.67±0.02^{fg}
	6	11.3±0.06^{ab}	28.42±0.02^{cd}	0.06±0.01^{abc}	0.73±0.03^{g}
60°C	0	11.9±0.06^{e}	28.93±0.02^{f}	0.02±0.02^{a}	0.86±0.03^{h}
	2	11.8±0.06^{de}	28.87±0.02^{ef}	0.13±0.02^{bcd}	1.93±0.01^{i}
	4	11.6±0.00^{cd}	28.93±0.03^{f}	0.14±0.02^{cd}	1.94±0.03^{i}
	6	11.8±0.06^{cde}	28.93±0.02^{f}	0.17±0.01^{d}	1.96±0.03^{i}

*Data are presented as mean ± standard deviation (n=3).
* a-i different letter within each column indicates statistically significant differences (P≤0.05).

L*(the brightness value), a*(positive is red, negative is green) and b*(positive is yellow, negative is blue)

4. CONCLUSIONS

The food industry is striving to develop functional products with greater nutritional benefits. The Viscozyme®L impact of enzymatic addition on the quality characteristics of lychee juice was assessed in this study. Our research showed that Viscozyme®L enzyme-treated lychee juice resulted in significant improvement in the total phenolic compound, total flavonoid content, and antioxidant activity (DPPH and FRAP assay), with the highest content and activity at 2 h at 55 °C. Therefore, this study could provide more important knowledge for future enzyme treatment application in the healthy beverage sector.

5. ACKNOWLEDGEMENTS

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Effect of Protein Glutaminase Deamidation on Functional Properties of Pea Protein Isolate

AP-O055

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ABSTRACT

Effect of enzymatic deamidation on functional properties of pea protein isolate (PPI) using protein-glutaminase (PG) was investigated at various enzyme activation times (15 min, 30 min, 1 h, 3 h, 6 h, and 12 h). Secondary structure analysis using circular dichroism and molecular mass distribution using SDS-PAGE suggested that PG deamidation could lead to the conformational change of PPI. The study revealed that deamidated PPI (DPPI) exhibited the improved functional properties compared to the untreated sample. After deamidation for 1 h, the solubility of DPPI was increased significantly at pH 3.0, 5.0, and 7.0 ($p < 0.05$) and it was found that the highest solubility of 36.79%, 46.85%, and 88.57% at pH 3.0, 5.0, and 7.0, respectively were observed. In addition, PG deamidation demonstrated a significant decrease in water-holding capacity (WHC) compared to the untreated sample. For oil-holding capacity (OHC), the results showed no difference from the untreated sample except that DPPI at 12 h exhibited higher OHC. The emulsifying activity index (EAI) was increased for all DPPIs. The emulsifying stability index (ESI) was decreased for most DPPIs, except at 30 minutes, where it remained similar to the untreated sample. Both foaming capacity and foaming stability of DPPI were higher than untreated PPI. These results indicated that modification of protein using PG could be a crucial technique affecting PPI's functional properties, making it suitable for use in beverages, especially for high-protein drink.

1. INTRODUCTION

The consumption of high-protein foods is on the rise, with a growing number of individuals interested in enhancing their protein intake, reducing fat, or using protein as a dietary supplement. However, high-protein foods come with restrictions for specific consumer groups, including health-related limitations, ethical concerns, and religious beliefs that may restrict the consumption of protein from certain sources [1]. Hence, the selection of alternative protein sources to replace animal-based proteins or proteins derived from allergenic plants like soybeans has become a critical factor in the production of high-protein food products [2].

Pea protein (*Pisum sativum*) has become increasingly popular as a plant-based protein source since it is regarded as a safer option for consumers due to its lower allergenicity rate and ease of digestion. The study compared the components of pea protein and soybean protein indicated that, in general, pea protein contained greater quantities of total protein, essential amino acids,

and non-essential amino acids [3-5]. It was found that primary components of pea protein are glutamic acid and aspartic acid [6,7]. However, these benefits are tempered by challenges related to solubility, particularly around the protein's isoelectric point (pH 4 to 6). Proteins at their isoelectric pH exhibit minimal solubility due to a lack of net charge, leading to increased hydrophobic interactions and aggregation, resulting in precipitation [4]. This phenomenon poses significant challenges in utilizing pea protein isolates in mildly acidic beverage formulations, where stability and prevention of sedimentation are crucial. The beverage industry often formulates products under extreme pH conditions to mitigate off-flavors, but mild acidity (pH 4.0 to 5.0) is preferred for sensory reasons. Unfortunately, this pH range aligns with the isoelectric point of many plant proteins, including pea protein isolates, leading to solubility challenges [8]. The resultant droplet flocculation and emulsion destabilization reduce emulsifying properties, potentially causing sedimentation issues during storage and distribution. Addressing these solubility and stability concerns is critical for enhancing the applicability of pea protein isolates in a broader range of food and beverage products.

Protein glutaminase (PG; EC 3.5.1.44) is an enzyme used to modify protein structures through deamidation. PG can accelerate the deamidation reaction, converting amide groups of glutamine residues into carboxyl groups while releasing ammonia. This reaction specifically targets glutamine residues within protein chains, with no reactivity toward free glutamine, resulting in an increase in negative charges on proteins, enhancing electrostatic repulsion between protein molecules [9]. PG deamidation provides various enhancements in protein characteristics, such as solubility and emulsifying capacity [10]. Enzymatic deamidation leads to structural modifications in proteins, influencing their hydrophilic and hydrophobic regions. These alterations affect the protein affinity for both water and oil, ultimately enhancing its emulsifying properties [11]. Several studies, such as those investigating rice protein and soy protein subjected to PG modification, have indicated that PG deamidation has the potential to reduce the allergenic properties of the proteins. These modified proteins tended to induce fewer allergic reactions, making them potentially safer for individuals with allergies or sensitivities. For example, a study on the impact of PG deamidation on rice protein, revealing an increase in the protein structural flexibility, resulting in enhanced solubility and emulsification properties [11]. Similar findings were observed in studies involving soy protein, suggesting that PG deamidation improved both solubility and emulsification properties [12]. The utilization of enzymatic deamidation with PG is a versatile technique within the food industry. It not only alters solubility, but also elevates emulsification capabilities, potentially reducing protein allergenicity in various food applications. As pea protein continues to draw significant interest from both the food industry and consumers, there exists a noticeable lack of knowledge regarding methods to enhance its diverse characteristics. An examination of the pea protein's composition reveals that glutamine is a predominant component, making enzymatic modification with PG a promising approach for improvement. Therefore, the aim of this study was to investigate the impact of enzymatic modification of pea protein by PG on protein's functional properties.

2. MATERIALS AND METHODS

2.1 Materials

Pea protein (PP, NUTRALYS® S85F) was obtained from Roqutte Co., Ltd. (Paris, France). The protein powder composition consisted of 82.68% protein, 8.73% lipid, 5.12% ash, and 3.47% carbohydrate. Protein glutaminase (PG) "AMANO" 500 was supplied by Amano enzyme Inc. (Nagoya,

Japan). All other reagents and chemicals (analytical grade) were purchased from Bio Rad Laboratories, Inc. (Hercules, USA), Sigma-Aldrich (St. Louis, MO, USA), and Thermo Scientific (Pittsburgh, PA, USA).

2.2 Sample preparation

Pea protein isolate (PPI) was prepared by defatting PP using soxhlet extraction with hexane [12]. The obtained PPI contained 91.41% protein content, using an N-to-protein conversion factor of 6.25 with Kjeldahl method. The PPI sample was vacuum-sealed and stored at -18 °C for further experiments and analysis.

2.3 Enzymatic deamidation

Deamidation of PPI by PG was performed using the optimal conditions with some modifications [12,14]. The reactions were conducted in 0.01 M citrate-phosphate-borate buffer at pH 7.0 containing 30 mg/mL PPI and PG which E/S ratio equal to 36 U/g protein. The reactions were incubated at 50 °C for 15 min, 30 min, 1 h, 3 h, 6 h, and 12 h in triplicates and stopped reactions by increasing the temperature to 80 °C for 10 min. A control sample of PPI was treated under the same conditions without PG for 6 h.

2.4 Determination of degree of deamidation (DD) and degree of hydrolysis (DH)

The DD and DH were measured by determination of amount of released ammonia produced from deamidation process using ammonia assay kit (Abcam, Cambridge, UK). The DD was expressed as the percentage of the amount of released ammonia from DPPI compared to the amount of released ammonia from completely hydrolyzed PPI which was treated with 2 N sulfuric acid (H_2SO_4) at 100 °C for 4 h [14]. The DH was calculated as a percentage, determined by ratio of soluble protein in supernatant after precipitate by 0.2 N trichloroacetic acid (TCA) and the soluble protein in complete hydrolyzed sample. The soluble protein in supernatant was determined by DC (detergent compatible) protein assay kit (Bio-Rad Laboratories Inc., Hercules, CA) and measured the absorbance at 630 nm. The concentration of soluble protein was quantified by bovine serum albumin (BSA) standard curve with R^2 » 0.99 [14].

2.5 Sample preparation for functional properties determination

Upscaled PG deamidation was performed in 0.01 M citrate-phosphate-borate buffer at pH 7.0, containing 100 mg/mL of PPI and PG (36 U/g protein). The mixtures were incubated at different times (15 min, 30 min, 1h, 3h, 6h, and 12h) and inactivated PG by heating to 80 °C for 10 min. DPPI samples were dialyzed, freeze-dried, vacuum-sealed, and stored at -18 °C for further analysis.

2.6 Determination of protein secondary structure by circular dichroism (CD)

The secondary structure of protein samples was determined by the CD [11]. One mg of each sample was dispersed in 1 mL of 0.1 M phosphate buffer at pH 7.0. The CD spectra was measured at 190-250 nm using JASCO spectropolarimeter (Model J-815, Tokyo, Japan). The percentage of secondary structure was obtained from DichroWeb using CONTIN-LL software [12,16].

2.7 Determination of molecular mass distribution by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was conducted by preparing loading sample, 20 mL of each sample (1 mg/mL in deionized water). The samples were mixed with 6 mL NUPAGEâ LDS sample buffer (Life Technologies Cor., Carlsbad, CA) and 2 mL sample reducing agent. The mixtures were incubated at 70 °C for 10 min then briefly spun for 30 s. A protein standard (PageRuler™ Plus Prestained Protein Ladder) and each sample were loaded to precast polyacrylamide gel (NUPAGEâ Novexâ 4-12% Bis-Tris gel) with NUPAGEâ MES running buffer. The loaded gel was run at 220 V for 30 min.

The gel was washed with deionized water for 5 min (3 rounds) before staining with simply blue safe stain (Life Technologies Cor., Carlsbad, CA) for 1 h, and destained in deionized water overnight. The molecular mass and band intensity of samples were determined using gel documentation and analysis software [11,14].

2.8 Determination of functional properties of the protein

Protein solubility (PS) at pH 3.0, 5.0, and 7.0 was determined using the published method with some changes [11]. One mg of freeze-dried protein sample was dissolved in 1.5 mL of 0.1 M phosphate-acetate buffer and left to fully dissolved overnight at room temperature. After that, each sample was vortexed and centrifuged at 3000 rpm, 10 °C for 10 min (Kubota model 5310, Japan). The protein content was measured by DC protein assay and %PS was calculated as:

$$\%\mathbf{PS} = \frac{\textbf{Protein in supernatant}}{\textbf{Total protein}} \times \mathbf{100} \quad (1)$$

Water holding capacity (WHC) and oil holding capacity (OHC) were determined according to the publish method with some modifications [14]. In each sample, 0.1 g of freeze-dried sample was dispersed in 1 mL deionized water (for WHC) or palm oil (for OHC). The mixture was vortexed for 30 s and left to stand at room temperature for 30 min before being centrifuged at 13,600 rpm, 25 °C for 10 minutes (Kubota model 5310, Japan). The WHC and OHC were expressed in grams of water or oil retained per gram of protein. Determination of foaming capacity (FC) and foaming stability (FS) were performed using published method with some modifications [17]. A 0.1 g of freeze-dried sample was dispersed in 20 mL 0.1 M phosphate buffer at pH 7.0 and homogenized at 19,000 rpm for 1 min to form the foam. The FC and FS were calculated as percentage using the equation (2) and (3). The V_{F0} is volume of foam immediately after homogenization, V_{FT} is volume of foam at interval time, and V_L is volume of protein mixture before foaming.

$$\%\mathbf{FC} = \frac{\mathbf{V_{F0} - V_L}}{\mathbf{V_L}} \quad (2)$$

$$\%\mathbf{FS} = \frac{\mathbf{V_{FT}}}{\mathbf{V_{F0}}} \times \mathbf{100} \quad (3)$$

Emulsifying activity index (EAI) and emulsifying stability index (ESI) were measured following the method of Pearce and Kinsella with some changes [18]. A 150 mg of sample was dispersed in 15 mL of 0.1 M phosphate buffer at pH 7.0, then homogenized with 5 mL palm oil at 22,000 rpm for 1 min. A 50 mL of aliquot was diluted with 4,950 mL of 0.1% SDS before measuring the absorbance at 500 nm. The EAI and ESI were calculated following equations (4) and (5).

$$\text{EAI (m}^2\text{/g)} = \frac{2 \times 2.303 \times \text{A}_0 \times \text{dilution factor}}{\text{C} \times \emptyset \times 10000} \quad (4)$$

$$\text{ESI (min)} = \frac{\text{A}_0}{\Delta\text{A}} \times t \quad (5)$$

Where A_0 is the absorbance of diluted emulsion immediately after homogenization, c is weight of protein per unit (g/mL) in the aqueous phase before emulsion formation, Æ is oil volume fraction of emulsion, DA is the change of absorbance between 0 and 10 minutes, and t is the time interval (10 minutes).

3. RESULTS AND DISCUSSION

3.1 PPI deamidation by PG

The deamidation of PPI by PG was conducted under optimal conditions aimed at achieving a high DD and a low DH. The impact of incubation time on %DD and %DH is depicted in Figure 1. It was found that %DD rapidly increased to around 60% within 1 h and gradually reached 70% by 12 h. This rapid increase in %DD at the beginning may be due to the activity of the PG to the glutamine residues in the PPI (as a substrate) at initial phase of deamidation process. Yamagushi et al. [9] found that specificity of PG on the protein substrate can affect the rate of the deamidation reaction. However, the gradual increase in %DD after 1 h may be caused by a lack of substrate, which can lead to the reaction reaching a plateau [19]. These results align with studies on soy and wheat gluten [12,19]. In addition, the %DH rose to 4% within 15 min and continued to increase until reaching 6% at the 12 h. These results could be due to PG activity, which can increase the number of negative charges on protein molecule, heightening electrostatic repulsion within the protein molecules. This resulted in protein unfolding, and small peptide fragments could be released [12]. In addition, the enzyme used in this study was a commercial product, which might retain some protease activities, potentially leading to an increase in %DH [14]. Based on these results, both %DD and %DH increased rapidly within first 15 min. However, even %DD was increased at higher level, the %DH still be in a lower range. This would be due to the fact that PG can increase the deamidation within the protein chains, but not cleave the peptide bonds in the proteins [14].

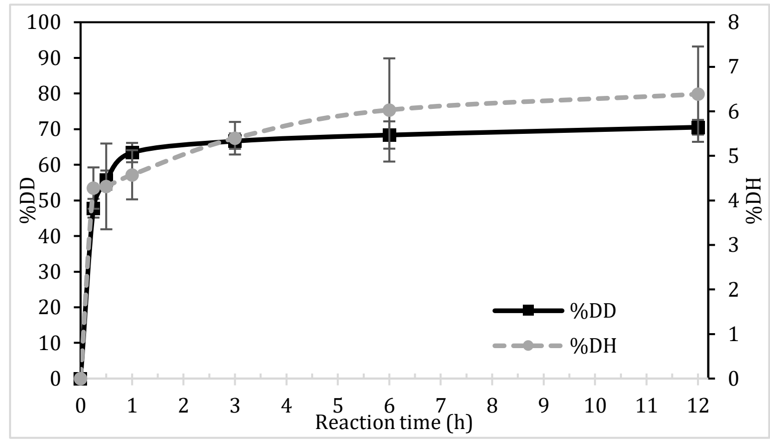

Figure 1. Degree of deamidation (DD, %) and degree of hydrolysis (DH, %) of pea protein isolate (PPI) after deamidation by protein-glutaminase (PG) as a function of time

3.2 Determination of protein secondary structure

Secondary structure of untreated-PPI, control-PPI and DPPI samples were evaluated using the far UV spectra (190-250 nm). From the CD spectra, percentage of secondary structure of each sample was calculated and shown in Table 1. The result suggested that after PG deamidation, compared to untreated-PPI and control-PPI samples, the amount of a-helix and b-turn were increased. Previous studied on wheat gluten and oat protein suggested that after deamidation process, the glutamine residues were converted to glutamic acid and then increased the negative charge and caused an increment of electrostatic repulsion among the b-sheet of wheat gluten, then the loop structure could be forme

This loop formation could imply the more flexible of a-helix and b-turn [11,21]. In addition, the number of b-sheet and random coils of DPPI samples were decreased when compared to untreated-PPI and control-PPI. Previous studies have shown that cavitation, shear stress, and turbulence which may occur during incubation process could affect both covalent and non-covalent bonds of proteins. This influence could be resulted in depolymerization and rearrangement of the secondary structure, transforming b-sheets and random coils into a-helix [22]. The increased flexibility of α-helix and β-turn structures, along with the transformation of β-sheets and random coils into α-helices, could have implications for the functional properties of DPPI in various applications, such as food and pharmaceutical industries. These structural changes suggest that the deamidation of PPI by PG leads to modifications of protein structures, which can potentially broaden its functional applications.

Table 1. Secondary structure derived from CD spectra of untreated-pea protein isolate (PPI), control-PPI, deamidated PPI (DPPI) at various times

Treatment	Secondary structure (%)				
	α-helix	β-sheet	β-turn	unordered	sum
Untreated	9.7	32.5	15.6	42.3	100.0
Control	10.4	29.1	18.5	42.1	100.0
15 min	39.1	12.4	24.3	24.3	100.0
30 min	24.7	10.4	27.9	37.0	100.0
1h	27.4	10.5	28.5	33.6	100.0
3h	34.0	4.9	38.8	22.4	100.0
6h	34.8	5.2	33.8	26.2	100.0
12h	24.2	5.6	31.1	39.1	100.0

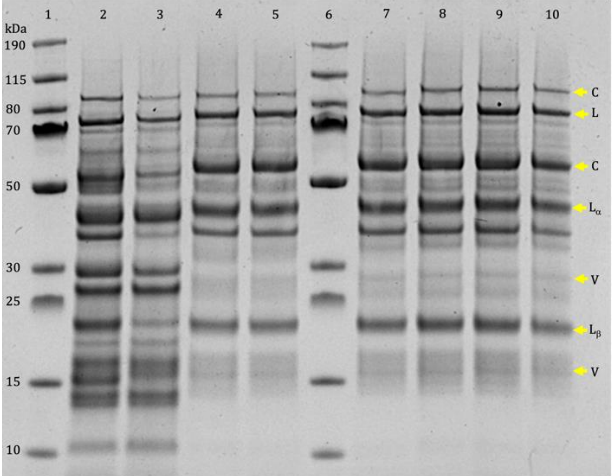

Figure 2. SDS-PAGE patterns of marker (1,6), untreated-PPI (2), control-PPI (3), DPPI for 15 min (4), 30 min (5), 1 h (7), 3 h (8), 6 h (9), and 12 h (10). C is convicilin, L is legumin, L_a is acid legumin, L_b is basic legumin, and V is vicilin.

3.3 Molecular mass distribution by SDS-PAGE

The molecular mass distribution of the samples was analyzed using SDS-PAGE and shown in Figure 2. Lanes 1 and 6 are markers, while lanes 2, 3, 4, 5, 7, 8, 9, and 10 are untreated-PPI, control-PPI, DPPI at 15 min, 30 min, 1 h, 3 h, 6 h, and 12 h, respectively. Bands were identified based on previous studies by Jiang et al. [23] and Cheng et al. [24]. The approximate molecular mass of each band was 67.45 and 47.27 kDa for convicilin (C), 64.11 kDa for legumin (L), 35.75 kDa for α-legumin (L_{α}), 24.42 and 11.99 for vicilin (V) and 17.55 kDa for β-legumin (L_{β}). The results revealed that after deamidation, the intensity of C and V bands in DPPI samples exhibited similarity but differed significantly from untreated PPI. Specifically, the intensity of C bands increased, while V bands decreased. This effect could be ascribed to the combined impact of heat treatment and protein hydrolysis, where the proteins could be unfolded from the heating process. In addition, the hydrolysis led to the release of shorter-chain peptides and proteins, resulting in an increase in %DH [12]. Additionally, pea protein contains a high amount of glutamine residues, thus it can be converted to glutamic acid by PG deamidation and cause conformational changes in the protein.

3.4 Protein solubility

Protein solubility of all samples at different pH is shown in Figure 3. DPPI samples exhibited higher solubility than untreated PPI and control samples at the same pH, highlighting the impact of deamidation on protein functionality. Both untreated and control samples showed the lowest solubility at pH 5.0, possibly due to the isoelectric point (pI) of pea protein being around 4.5 [25,26]. The observed shift in pI of DPPI after deamidation, caused by the increase in negative charges, is consistent with the increase in solubility at pH 5.0 compared to pH 3.0. The process of deamidation, catalyzed by PG's activity and facilitated by heating, leads to the exposure of buried hydrophobic residues and the introduction of negative charges within the protein structure [10]. These modifications likely disrupt the native protein interactions, resulting in increased protein solubility. After deamidation for 1 h, the solubility of DPPI significantly increased at all pH levels ($p < 0.05$), with the highest solubility observed at 36.79%, 46.85%, and 88.57% at pH 3.0, 5.0, and 7.0, respectively. This increase in solubility highlights the potential of deamidation to improve the functional properties of pea protein, aligning with previous studies on wheat gluten, oat, and soy proteins, which have shown increased solubility following deamidation [12,21]. These findings contribute to the understanding of the functional properties of deamidated pea protein and its potential as a valuable ingredient in food formulations.

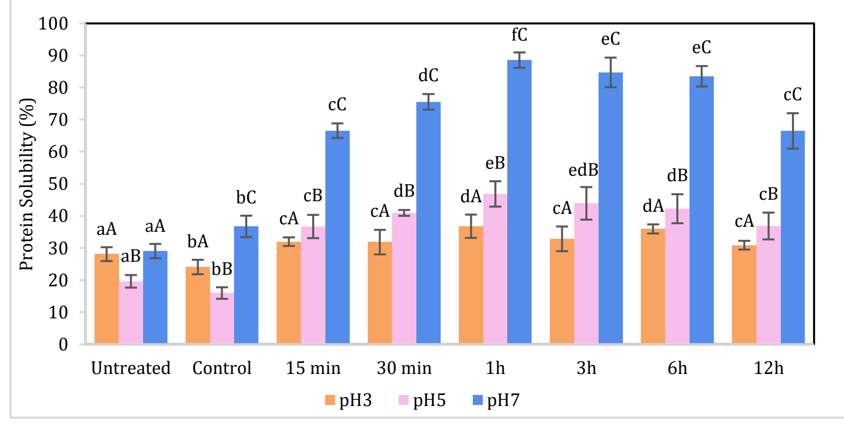

Figure 3. Protein solubility of PPI and DPPI at various pH values. Same lowercase letters for same pH indicate no significant difference ($p > 0.05$, n = 3); same uppercase letters for same treatments across different values indicate no significant differences ($p > 0.05$, n = 3).

3.5 Water- and oil-holding capacity

Table 2 illustrates the impact of deamidation on water- (WHC) and oil-holding capacity (OHC). The results indicated that WHC values of control-PPI and all DPPI samples were significantly decreased compared to untreated-PPI. It could be due to the effect of PG deamidation and heat treatment during enzyme inactivation. Fuhrmeister and Meuser [28] found that protein isolate from wrinkled pea subjected to heat treatment exhibited lower WHC values compared to the protein without heat treatment. The decrease in WHC could be attributed to alterations in protein structure induced by heat, affecting the protein's ability to retain water [29]. The OHC values showed no significant difference among treatments, except for the control-PPI and DPPI after 12 h. Both PPI and DPPI contain a relatively low amount of fat, which likely contributes to OHC primarily through physical entrapment mechanisms. According to previous studies, the bulk density of the protein was not affected by the deamidation reaction, which results in changes in physiochemical properties rather than changes in the protein surface area. This indicated that the deamidation process, while influencing certain properties of the protein, did not lead to substantial structural changes that could affect OHC [30,31].

3.6 Emulsifying properties

Emulsifying activity index (EAI) and emulsifying stability index (ESI) were assessed in phosphate buffer at pH 7.0 and the results were presented in Table 2. The increase in EAI across all DPPI samples indicated that deamidation could enhance the protein ability to stabilize emulsions. These results were in agreements with the results found in soy and evening primrose proteins [12,32]. This improvement is likely due to structural changes induced by the PG deamidation. These changes could increase protein solubility and surface activity, improving interactions at the oil-water interface [12,32]. In contrast, the decrease in ESI was found in most DPPI samples, suggesting a reduction in emulsion stability. This decrease could be attributed to altered protein-protein interactions and the changes in protein structure. Joshi et al. [33] noted that when subjected to high temperature, emulsions stabilized by lentil protein isolate had very large average oil droplet sizes, indicating the formation of large aggregates. The exposure of hydrophobic groups due to high-temperature heat treatment could promote greater protein-protein interactions, leading to the formation of large protein aggregates. In addition, emulsion stability could be greatly influenced by the size and conformation of proteins or protein aggregates. For a stable emulsion, proteins were rapidly migrated to and realigned at the oil-water interface. Tang et al. [34] found that the ESI of pea protein hydrolysis using transglutaminase (TG) was also decreased. It was explained that TG could catalyze the formation of new peptide bonds, thus increasing the overall molecular mass of the proteins. However, the lack of significant difference in ESI for DPPI treated for 30 min suggested that the duration of deamidation was critical. A 30-min deamidation period may not enough to induce the changes in protein structure to significantly affect emulsion stability.

Table 2. Water-holding capacity (WHC), oil-holding capacity (OHC), emulsifying activity index (EAI), emulsifying stability index (ESI), and foaming capacity (FC) of untreated-PPI, control-PPI, and DPPI

Treatment	WHC (g water/g protein)	OHC (g oil/g protein)	EAI (m^2/g protein)	ESI (min)	%FC
Untreated	$5.75^{a} \pm 0.36$	$1.53^{a} \pm 0.30$	$4.56^{a} \pm 0.52$	$18.96^{c} \pm 0.51$	$64.50^{a} \pm 0.71$
Control	$4.63^{b} \pm 0.32$	$1.95^{b} \pm 0.10$	$3.04^{a} \pm 0.26$	$15.63^{ab} \pm 0.27$	$96.00^{b} \pm 1.41$
15 min	$3.58^{c} \pm 0.34$	$1.55^{a} \pm 0.08$	$10.68^{b} \pm 1.27$	$13.66^{a} \pm 1.20$	$97.00^{b} \pm 4.24$
30 min	$3.51^{c} \pm 0.21$	$1.76^{ab} \pm 0.15$	$11.71^{bc} \pm 1.75$	$16.89^{bc} \pm 1.86$	$101.50^{b} \pm 7.78$
1h	$3.75^{c} \pm 0.49$	$1.75^{ab} \pm 0.13$	$14.28^{de} \pm 1.16$	$14.24^{ab} \pm 0.93$	$104.50^{b} \pm 4.95$
3h	$3.37^{c} \pm 0.16$	$1.71^{ab} \pm 0.07$	$15.93^{e} \pm 1.74$	$13.67^{a} \pm 1.00$	$82.00^{c} \pm 4.24$

6h	$3.82^{c} \pm 0.48$	$1.72^{ab} \pm 0.12$	$14.94^{de} \pm 2.45$	$15.99^{ab} \pm 3.87$	$73.00^{ac} \pm 2.83$
12h	$2.24^{d} \pm 0.03$	$1.98^{b} \pm 0.11$	$13.35^{cd} \pm 0.50$	$14.17^{ab} \pm 2.07$	$71.50^{ac} \pm 4.95$

*Within columns, values with different subscripts indicate significant differences at $p<0.05$.

3.7 Foaming properties

Foaming capacity (FC) and foaming stability (FS) were assessed in pH 7.0 phosphate buffer and results are shown in Table 2 and Figure 4, respectively. After deamidation, DPPI samples showed higher FC compared to untreated sample, with the highest FC was observed in DPPI treated for 1 h (104.50%). The increase in FC after deamidation can be attributed to the increase in solubility and %DH, leading to an increase in soluble protein [35]. Additionally, Tang et al. [34] noted that enzyme treatments could improve protein ability to trap air bubbles and form foam. Protein hydrolysis decreased the protein molecular mass, likely enhancing their movement to the interface and increasing their flexibility to reorganize at the interface. Enzyme treatment also altered the amphiphilicnature of the protein surface. However, the FC significantly decreased after 1 h, this was possibly due to protein aggregation or non-covalent linkages within the protein molecules. In addition, FS of all treatments were decreased over time. It was found that a higher %DD could result in a higher FS. The decrease in FS could be attributed to the decrease in protein-protein interaction,

which may be caused by the heating step used for enzyme inactivation. This heating step can alter the secondary and tertiary structure of proteins, increasing the net charge of the protein and leading to excessive repulsion between proteins, hence decreasing their interactions [1920].

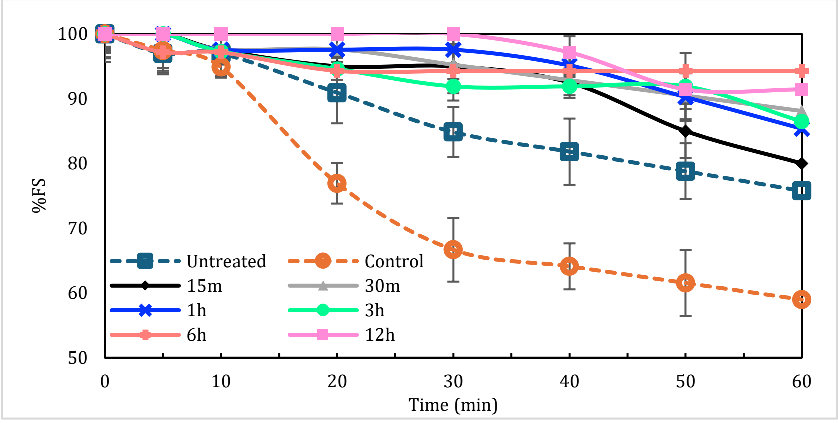

Figure 4. Foaming stability (FS) of untreated-PPI, control-PPI, and deamidated pea protein isolate

4. CONCLUSIONS

Pea protein deamidation using PG, specifically an incubation time of 1 h, resulted in acceptable %DD and %DH for desirable outcome. A high %DD improved protein solubility, while a low %DH prevented an increase in bitterness. This process also improved the functional properties of PPI including solubility, foaming properties, and emulsifying properties. DPPI produced under these conditions showed enhanced solubility at different pH levels compared to untreated-PPI. The improved solubility could make DPPI a promising ingredient for various food and industrial applications requiring high solubility. Additionally, DPPI exhibited improved foaming properties, beneficial for stable foams in food applications like meringues or aerated desserts. Furthermore, DPPI showed enhanced emulsifying properties, indicated by an increase in the EAI. This suggested that DPPI may be used as an effective emulsifier in products requiring stable emulsions. Given all these improved properties, DPPI can be effectively applied in beverages, high-protein drinks, aerated food products, and emulsified products, making it a versatile ingredient for a wide range of applications.

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Free-radical scavenging abilities of protein hydrolysate derived from Cardamom rhizomes

AP-P064

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Keywords

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Bioactive peptide
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ABSTRACT

Living organisms naturally produce reactive oxygen species (ROS) as part of their physiological processes. These ROS are usually regulated by antioxidant defense mechanisms to maintain a stable redox balance. However, excessive ROS production during oxidative stress can lead to cellular damage and various diseases. Plant-derived antioxidant peptides have shown promise in combating free radicals and neutralizing reactive intermediates, offering potential protection against oxidative stress-related conditions. In this study, the enzymatic hydrolysis method using alcalase was employed to hydrolyze proteins extracted from cardamom rhizomes. The antioxidant properties of the resulting crude protein hydrolysate were evaluated using various assays, including the DPPH, ABTS, and nitric oxide assays. The findings demonstrated that the crude protein hydrolysate derived from cardamom rhizomes exhibited strong antioxidant and free radical scavenging activities, particularly evident in the DPPH, ABTS, and nitric oxide assays. Under the conditions of a 0.5 E: S ratio, the cardamom rhizome-derived protein hydrolysate exhibited the highest antioxidant activity in the ABTS, DPPH, and nitric oxide assays. In the ABTS assay, the IC_{50} value was measured at 24.38 µg/mL, while in the DPPH assay, it was 135.20 µg/mL. Additionally, for the nitric oxide assay, the IC_{50} value was found to be 87 µg/mL. These results highlight the potential of cardamom rhizome-derived peptides as potent antioxidants with significant free radical scavenging properties, suggesting their potential application in combating diseases related to oxidative stress.

1. INTRODUCTION

Oxidative stress arises from an imbalance between the production of reactive species, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), and the body's antioxidant defenses. These reactive species, containing oxygen or nitrogen, are generated as byproducts of normal cellular metabolism, particularly during processes like mitochondrial respiration and enzymatic reactions. While reactive species serve essential functions in cell signaling and immune defense, excessive production can lead to oxidative damage to cellular components such as DNA, proteins, and lipids [1]. Oxidative stress is implicated in various pathological conditions, including neurodegenerative diseases, cardiovascular diseases, cancer, and aging [2,3]. Factors such as environmental pollutants, radiation, smoking, and certain drugs can exacerbate oxidative stress by increasing the production of reactive species or impairing antioxidant defences [4]. Understanding the mechanisms underlying oxidative stress and its impact on cellular function is critical for the development of therapeutic strategies aimed at mitigating its harmful effects and promoting overall health and well-being.

Protein hydrolysate is the process by which proteins are broken down into peptides. This breakdown can occur through either chemical or enzymatic processes. Enzymatic hydrolysis is the most common method to generate bioactive peptides [5]. They offer high specificity, ensure a mild reaction, and contribute to product safety [6]. Bioactive peptides are organic compounds consisting of short sequences of amino acids linked by peptide bonds. Typically ranging from 2 to 20 amino acids in length, they are derived from proteins found in various sources such as plants, animals, and microorganisms [7]. These peptides generally exhibit a molecular weight of less than 6 kDa [8] and are released through the enzymatic breakdown of proteins. They possess health- promoting properties, including antioxidant activity, anti-inflammation, immunomodulation, and enhancement of the immune system [9]. The characteristic properties of antioxidant peptides, including their amino acid composition, sequence, structure, and molecular weight, contribute to their ability to scavenge free radicals, reduce oxidative stress, and exert health-promoting effects in the body [10].

Cardamom, in Thailand known as "krawan," is a versatile spice and medicinal plant that belongs to the ginger family, Zingiberaceae. It is typically cultivated in dense crowns of high trees with lush foliage and abundant leaf litter. This aromatic herb thrives primarily in tropical regions around the globe, notably in countries like Thailand and Kampuchea [11]. The main bioactive compounds found in cardamom include terpenes and phenolic compounds, which possess potent antioxidant properties and play a crucial role in protecting against chronic diseases and oxidative stress [12]. Additionally, these extracts have been shown to augment antioxidant activity and bolster the immune system in murine models of atherosclerosis [13].

Currently, there's a noticeable absence of documented reports on bioactive peptides from cardamom that demonstrate antioxidant activity. This knowledge has sparked a compelling interest in exploring cardamom for the discovery of novel peptides with potential biological activities. The exploration of crude protein hydrolysates from cardamom will have applications as natural antioxidants in functional foods, dietary supplements, or pharmaceutical formulations, providing consumers with a convenient and natural way to support their health. Additionally, these peptides could contribute to the development of innovative food products with enhanced nutritional profiles and improved shelf-life.

2. MATERIAL AND METHODS

2.1 Materials and chemicals

Cardamom was obtained from Chanthaburi province, Thailand. Alcalase®, 2,2'-Azino-bis (3-ethybenzothiazoline-6-sulfonic acid) (ABTS), Bovine-serum albumin (BSA), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), (N-(1-naphthyl) ethylenediamine dihydrochloride (NED or Griess reagent), Thiobar-bituric acid (TBA) were supplied by Sigma-Aldrich Chemicals Co. (USA). All of chemicals were analytical grade.

2.2 Preparation of hydrolysis from cardamom rhizomes

Cardamom rhizomes were mixed with 20 mM phosphate buffer saline at pH 7.2 and were stirred overnight at 4°C. Precipitation of proteins was carried out by using 80% saturation Ammonium sulfate salt. The hydrolysis reaction was carried out with Alcalase enzyme using various concentrations of 0.0, 0.5, 1.0, and 2.0 (v/v) enzyme/substrate ratios. The hydrolysis reaction was conducted for 4 hours at 55°C in a shaking incubator at 150 rpm and was terminated by heating the mixture at 90°C for 20 min. The supernatant was collected and stored at 4°C until required for analysis.

2.3 Measurement of protein content

The protein contents of the cardamom protein hydrolysate were determined using the Bradford assay [14]. Various concentrations of Bovine Serum Albumin (BSA) were used as a protein standard for the construction of the calibration curve. The absorbance was measured at 595 nm using a microplate reader.

2.4 Determination of DPPH assay

The antioxidant activity of cardamom protein hydrolysate was determined following the method reported by [15] with slight modifications. 100 µL of the sample was mixed with 400 µL of the DPPH solution, then incubated in the dark for 10 minutes at room temperature. The absorbance was measured at 517 nm using a microplate reader. The results were reported as the IC_{50} of the sample (µg/mL).

2.5 Determination of ABTS assay

The antioxidant activity of cardamom protein hydrolysate was determined following the method reported by [16] with slight modifications. 10 µL of the sample was mixed with 600 µL of the ABTS solution, then incubated in the dark for 10 minutes at room temperature. The absorbance was measured at 734 nm using a microplate reader. The results were reported as the IC_{50} of the sample (µg/mL).

2.6 Determination of NO assay

The antioxidant activity of cardamom protein hydrolysate was determined following the method reported by [17] with slight modifications. 50 µL of the sample was combined with 50 µL of 10 mM SNP and incubated in the light for 2.5 hours. Subsequently, 100 µL of 0.33% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 100 µL of 0.1% (w/v) NED were added. The absorbance was measured at 540 nm using a microplate reader. The results were reported as the IC_{50} of the sample (µg/mL).

2.7 Calculation of IC_{50} value

The percentage of radical scavenging was calculated as follow:

$$\% \text{ Inhibition} = \frac{(\text{Abs control-Abs blank})\text{-}(\text{Abs sample-Abs background})}{(\text{Abs control-Abs blank})} \times 100$$

Where Abs control is the absorbance of control (no sample), Abs sample is the absorbance of the protein hydrolysates, Abs background is the absorbance of the sample, and Abs blank is the

absorbance of deionized water. The IC_{50} values were calculated. The determination of IC_{50} values were done using GraphPad Prism Version 4.00.

2.8 Statistical analysis

The experiment was conducted using a completely random block design (CRD) with three replications. Analysis of variance (ANOVA) was performed using SPSS software version 29.0.1.0. Comparison of mean was executed using Duncan's Multiple Range Test (DMRT) to determine of difference between the means <0.01.

3. RESULTS AND DISCUSSION

3.1 Amino acid composition of cardamom protein hydrolysate

To determine the amino acid composition of cardamom protein hydrolysate, an analysis was conducted following the AOAC 994.12 methods. The results outlining the amino acid composition of cardamom protein hydrolysate are provided in Table 1.

Table1. Amino acid composition in cardamom protein hydrolysate

Amino acid	content (mg/g protein)
Aspartic acid	0.8
Glutamic acid	0.9
Glycine	0.4
Histidine	<0.1
Isoleucine	0.3
Leucine	0.6
Lysine	0.5
Alanine	0.6
Arginine	0.6
Methionine	0.2
Phenylalanine	0.3
Proline	0.3
Serine	0.4
Threonine	0.3
Tryptophan	0.2
Tyrosine	0.2
Valine	0.4

The results indicate that the protein hydrolysate extracted from cardamom rhizomes is abundant in amino acids such as glutamic acid, followed by aspartic acid (Asp). Additionally, hydrophobic amino acids make up 3.3 mg/g protein of the total composition. This composition suggests that peptides derived from plants, such as those found in cardamom rhizomes, may possess potent antioxidant effects, owing to the distinctive composition, sequence, size, and characteristics of their amino acid side chains [18].

The presence of hydrophobic amino acids is pivotal for the antioxidant properties of the protein hydrolysate, as they contribute to various mechanisms such as proton donation, electron donation, and inhibition of lipid peroxidation [19]. Moreover, aromatic amino acids like tyrosine (Tyr), tryptophan (Trp), and phenylalanine (Phe), as well as histidine (His), cysteine (Cys), and methionine (Met) residues, also play a role in bestowing antioxidative properties to the peptides [20]. Therefore, individual amino acids significantly contribute to the antioxidant capacity of the peptide fraction, with the antioxidant activities of bioactive peptides arising from their ability to scavenge radicals [21].

3.2 DPPH radical scavenging activity

The DPPH radical scavenging assay is commonly used to evaluate the effectiveness of free radical scavengers due to the simplicity of the reaction. Ascorbic acid was used as a positive control in this study. The IC_{50} value for ascorbic acid was determined to be 16.80 µg/mL. Table 2 presents a comparison of the DPPH radical scavenging activity of three samples extracted from different enzyme-to-substrate (E: S) ratios.

Table2. DPPH radical scavenging activity of cardamom protein hydrolysate.

E:S ratio (v/v)	IC_{50} of DPPH (µg/mL)
0.5	$135.20^{b} \pm 4.45$
1.0	$150.63^{c} \pm 2.96$
2.0	$144.23^{d} \pm 5.99$
Ascorbic acid	$16.80^{a} \pm 0.28$

*Data are expressed in the mean ± standard deviation (n=3)
Different letters indicate that values are significantly different ($p \leq 0.01$)

The results were calculated to obtain the IC_{50} value, representing the concentration needed to neutralize or scavenge 50% of the DPPH free radicals present in the assay system. A lower IC_{50} value suggests that the substance is more effective at scavenging free radicals and has stronger antioxidant activity [22]. The results show a significant difference among samples from E: S ratios of 0.5, 1.0, and 2.0 ($p \leq 0.01$). It was found that the IC_{50} value at the 0.5 E: S ratio was the lowest, at 135.20 ± 4.45 µg/mL, indicating great antioxidant activity compared to the other conditions, where the IC_{50} values at 1.0 and 2.0 E: S ratios were 150.63 ± 2.96 and 144.23 ± 5.99 µg/mL, respectively.

3.3 ABTS radical scavenging activity

The ABTS radical scavenging assay evaluates the comparative capacity of antioxidants to neutralize ABTS radicals produced in water [23]. This assay is often employed to assess the total antioxidant capacity (TAC) of natural products, such as crude extracts [24], in comparison to a standard such as ascorbic acid, used as a positive control. There is an IC_{50} value for Ascorbic acid of 4.66 µg/mL. The findings demonstrate antioxidant properties based on IC_{50} values, which represent the concentration required to scavenge radicals by 50%, as shown in Table3.

Table3. ABTS radical scavenging activity of cardamom protein hydrolysate.

E:S ratio (v/v)	IC_{50} of ABTS (µg/mL)
0.5	$24.38^{b} \pm 1.31$
1.0	$25.07^{b} \pm 1.14$
2.0	$47.81^{c} \pm 2.58$
Ascorbic acid	$4.66^{a} \pm 0.27$

*Data are expressed in the mean ± standard deviation (n=3)
Different letters indicate that values are significantly different ($p \leq 0.01$)

A lower IC_{50} value suggests that the substance is more effective at scavenging free radicals and has stronger antioxidant activity. The results indicate significant differences between the 0.5 and 1.0 E: S ratios compared to the 2.0 E: S ratio ($p \leq 0.01$). Specifically, at the 0.5 E: S ratio, the lowest IC_{50} values were observed 24.38 ± 1.31 µg/mL, indicating the strongest ABTS radical scavenging activity, followed by the 1.0 E: S ratio at 25.07 ± 1.14 µg/mL, and the 2.0 E: S ratio with 47.81 ± 12.81 µg/mL, respectively.

3.4 Nitric oxide radical scavenging activity

The nitric oxide assay is employed to quantify the levels of nitric oxide (NO) in a sample. Nitric oxide undergoes conversion into nitrite ions (NO^{2-}) when exposed to oxygen. These nitrite ions subsequently interact with sulfanilamide and N-1-naphthylethylenediamine dihydrochloride (NED), leading to the formation of a colored azo compound [25]. Ascorbic acid is utilized as a positive control in this assay, with an IC_{50} value recorded at 4.66 μg/mL. Table 4 presents a comparative analysis of the nitric oxide radical scavenging activity among three samples obtained from varying enzyme-to-substrate (E: S) ratios.

Table4. Nitric oxide radical scavenging activity of cardamom protein hydrolysate.

E:S ratio (v/v)	IC_{50} of NO (μg/mL)
0.5	87.00^{a} ± 4.15
1.0	94.15^{b} ± 12.81
2.0	103.13^{c} ± 16.91
Ascorbic acid	407.10^{d} ± 2.77

*Data are expressed in the mean ± standard deviation (n=3)
Different letters indicate that values are significantly different ($p \leq 0.01$)

The results indicate that all crude protein hydrolysates from cardamom rhizomes exhibited significant antioxidant activity, with notable differences observed at a p-value of ≤ 0.01. Among the various experimental conditions tested, the lowest IC_{50} value was recorded at the 0.5 E: S ratio, measuring 87.00 μg/mL. This suggests that the 0.5 E: S ratio demonstrated a superior radical scavenging ability compared to the other conditions. Specifically, the IC_{50} values for the 1.0 E: S ratio and the 2.0 E: S ratio were measured at 94.15 μg/mL and 103.13 μg/mL, respectively.

The 0.5 E: S ratio is considered optimal for achieving the highest antioxidant activity in the hydrolysis process. This ratio achieves an optimal balance between the concentration of enzyme (E) and the availability of substrate (S), ensuring maximal efficiency in breaking down the substrate into bioactive peptides [26,27]. With the enzyme concentration at an adequate level and substrate availability optimized, the hydrolysis process is enhanced, leading to the generation of amino acids and peptides that act as electron and hydrogen donors or possess radical trapping ability [28]. Furthermore, the composition of peptides produced during hydrolysis is likely to be highly favorable for antioxidant activity. The peptides generated at this ratio may contain sequences that exhibit potent antioxidant effects, contributing significantly to the observed antioxidant activity [29]. These findings underscore the importance of optimizing the enzymatic hydrolysis process, particularly the enzyme-to-substrate ratio, to maximize the antioxidant potential of cardamom rhizome-derived protein hydrolysates.

4. CONCLUSIONS

The study aimed to extract protein hydrolysate with antioxidant properties from cardamom rhizomes. Through various assays, including DPPH, ABTS, and nitric oxide assays, the crude protein hydrolysate derived from cardamom rhizomes demonstrated significant antioxidant activity. This activity was particularly evident within a 99% confidence interval when the extraction process was conducted at a 0.5 enzyme-to-substrate (E: S) ratio, under specific conditions, including a temperature of 55 degrees Celsius, a pH of 7, and a duration of 4 hours. These findings suggest that the protein hydrolysate obtained from cardamom rhizomes shows potential as a source of bioactive peptides with antioxidant properties.

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The double burden of malnutrition in peri-urban and rural area of Satun, Thailand.

AP-P069

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ABSTRACT
Due to rapid dietary and lifestyle changes, Thai population may be at risk of the “double burden” of under- and over-nutrition. Some studies found interactions between micronutrient deficiencies and overnutrition. This study therefore aimed at assessing the extent of double burden of malnutrition in school children and women in the south of Thailand. The subjects were 7-14 y children (n=92) and 18-50 y, non-pregnant or lactating women (n=217) living in a peri-urban or rural area of Satun province. For the children height-for-age, weight-for-age and body mass index (BMI) z-scores and for the women height z-scores, BMI, waist circumference (WC) and blood pressure were assessed. In the children serum zinc, serum retinol, hemoglobin were analyzed. 18.9% and 26.7% of children were stunted and underweight respectively, while 60% and 63%, respectively, of women had BMI≥ 25 and central obesity. Zinc deficiency and low serum retinol were common in children for both setting. The prevalence of stunted women being obese was significantly higher than of the non-stunted. The studied population clearly shows signs of a double burden of under- and overnutrition in the same community. Additionally, stunted obesity was found in the women, indicating a greater risk for malnourished people to develop chronic disease later in life. Nutrition education could be one of the major issues to improve awareness of the population.

1. INTRODUCTION

Thailand is undergoing nutrition transition like in many developing countries with changing their eating habit to more refine and high energy dense food instead of traditional food which contains more complex carbohydrate, fruit, and vegetable (1). In addition to the sedentary lifestyle and increase in physical inactivity this lead to reduce daily energy expenditure. Nutrition related and non-communicable diseases is now widespread and increasing their impact in Thailand (2) as many campaigns from the government trying to push the awareness to people. Since this will lead to high health care costs in the near future.

Undernutrition has the adverse effects on physical and mental development as well as immune defense especially for child and reproductive women. This is mainly a threat for children in low- and middle-income countries. Hunger is the main contributor of undernutrition which the number in developing countries has been improved since 1990 by falling 39% in 2014.

Even though, the level of hunger in the world is still serious with 805 million people affected (3).

Despite undernutrition continue to be a public health problem in many parts of the world, the rate of obesity was increasing more rapidly than the stunning rate was reported in some countries i.e. China (4). In women, undernutrition seems to be replaced by overnutrition (5); although at the same time child undernutrition persists (3). A comparison between different countries showed that the prevalence for overweight and undernutrition in one household is highest for low to middle-income countries (15.5 %) and lowest for the countries at both ends (low: 3.7 %, high: 5.4 %) of the GNI per capita scale (6). In the south of Thailand, ethnicity, religion and cultural are differ from other parts of the country as well as their socioeconomic status. This study aimed at assessing the extent of double burden of malnutrition in children and women who lived in the same community in southern Thailand where majority of population were Muslim.

2. MATERIAL AND METHODS

2.1 Study site and subjects

The study was carried out in Satun province located in the south of Thailand. There were two different study sites; one was in a peri-urban area at the border of the city Satun and the other in a rural part on the coast and about 15 km. outside of the city. In both areas the research was done with school children aged 7- 14 y (n=92) as well as with non-pregnant or -lactating women in the age of 18 - 50 y (n=217). The subjects came from the same population, whereas the number for each was different. Calculation of the number of samples was not performed because of this study was the screening process for the feasibility of the study site of the previous publication (7). This study was conducted during July-August 2009 and the study protocol was approved by the ethics committees of the ETH Zurich, Switzerland.

2.2 Data collection

2.2.1 General information of women

In the main part of the data collection a short questionnaire was conducted with 217 women. It contained questions about name, age, education, the number of people in their household, the number of children born alive, total household income, occupation of the women, general health status.

2.2.2 Anthropometric measurements

The weight of all participants was measured barefoot and wearing light clothes on a digital weight scale to the nearest 0.1 kilogram. Subjects had to stand unassisted, in the center of the weight scale and look straight ahead. Height was also measured to the nearest 0.1 centimeter barefoot, with heels, buttocks and shoulders in contact with the wall and looking straight ahead. For the children the z-scores for “weight for age”, “height for age”and “BMI for age” were calculated with the computer program Epi Info version 3.5.1(based on the CDC recommendations,2000). For the women BMI was calculated as weight (kg) / height^2 (m). The WHO recommends BMI cutoff points for Asian population (the definition of underweight, overweight and obesity in adults) were applied (8). The women’s height was also transformed into height z-scores. The cut-off for stunting was taken below a standard deviation of -2 as proposed by the WHO. Waist circumference was measured for all the women with the standard method (9) and the cutoffs for central obesity was >80 cm for women (10).

2.2.3 Blood pressure

Blood pressure was recorded in women with an automatic measuring tool (OMRON, M6, upper arm, blood pressure monitor). The term hypertension was used if either the SBP was 140 mmHg or above and/or the DBP was 90 mmHg or above (11).

2.2.4 Blood sampling and analysis

Blood sample of children were taken for measuring hemoglobin (Hb), serum ferritin (SF), serum zinc (SZn) and serum retinol (SR). The details of the procedure were explained previously (7)

2.3 Statistical analysis

Statistical analysis was done with SPSS software version 16.0 for Windows. All the data was tested for normal distribution. Parameters with no normal distribution were either logarithmically or square-root transformed and further used like this. To compare the data of the two groups of living area (peri-urban vs. rural), 'Independent samples t-test' was used and the level of significance was set at a p-value below 0.05. Chi-square test was used for the prevalence of anthropometrics and biochemical data.

3. RESULTS AND DISCUSSION

Data of anthropometric and biochemical measurements of children were showed in table 1. There were no significant differences of those data between study sites except BMI for age Z- score (BAZ), serum retinol (SR) and hemoglobin (Hb) which were found to be higher in peri-urban setting. Of 92 children, undernutrition remained the important problem and prevalence of such conditions were high in both study sites as indicated by weight for age Z-score (WAZ) of -0.97, height for age Z-score (HAZ) of -1.0 and BAZ of -0.5. The mean of prevalence of stunting, underweight and thinness for both study site were 18.9%, 26.7% and 16.7% respectively which the latter was significantly higher in rural (27%) than peri-urban area (9.4%) (Table 2). These were higher than data of latest health survey in 2019 which 3.2%, 5.5% of school children were stunted and thinness respectively (1). However, prevalence of overweight was 8.9% with no stunted obese was detected. Zinc deficiency was highly prevalent in this area and it was confirmed in the different communities of who living in the same province (7). Followed by low vitamin A status which ratio of effected children were significantly higher in rural (45.7%) than the peri urban area (20.4%) while vitamin A deficiency (VAD) only found in rural part (2.9%) and children showed moderate to low of iron status. VAD has been affected to young and school children in southern Thailand especially in the province where Muslim are majority which is the leading cause of dead due to measle and it was found that related to low intake of animal food sources, green vegetables and yellow fruits (12)

Table 3 showed the socioeconomic, anthropometric and blood pressure of 217 women with the average of age of was 36.3 y. The household size and number of children per women in rural area were significantly higher than in peri-urban area when other parameters were not significantly difference between the 2 setting. However, year of education of women (6.5 y) was rather low as well as salary of household (6,000 THB). Obesity was the major problem for this population group as the mean BMI and waist circumference (WC) of both study site were 26.4 (kg/m^2) and 84.9 cm. respectively. The markers of blood pressure (both systolic and diastolic) were in normal range. Thus, for the women subjects, they have been affected by overnutrition rather than undernutrition.

Table 1. Anthropometric and biochemical data of schoolchildren divided by living area

Parameter	Peri-urban setting (n=55)	Rural Setting (n=37)
Age (y)[1]	9.5 ± 1.8	9.7± 1.8
% male	49	43
Weight (kg)[2]	27.5 ± 10.16	25.9 ± 8.1
Weight for age Z-score[1]	-0.74 ± 1.36	-1.28 ± 1.26
Height (m)[1]	1.3 ± 0.12	1.3 ± 0.11
Height for age Z-score[1]	-0.88 ± 1.15	-1.55 ± 0.81
BMI (kg/m^2)[3]	15.82(13.04-27.78)	15.08(12.29-25.76)
BMI for age Z-score[1]	-0.27 ± 1.1	-0.93 ± 1.4*
Serum ferritin (µg/L)[2]	41.79 ± 24.26	32.5 ± 20.9
Serum zinc (µmol/L)[1]	10.3 ± 2.2	10.8 ± 1.5
Serum retinol (µmol/L)[1]	1.23 ± 0.3	1.08 ± 0.3*
Hb (g/L)[1]	128.5 ± 8	125.0 ± 7.7*

*BMI: body mass index; Hb: hemoglobin
[1]Mean ± SD
[2]Geometric mean ± SD
[3]Median (range)
* Significantly different from peri-urban setting (independent sample's t-test; $p< 0.05$)

Table 2. Prevalence of malnutrition based on anthropometric parameters and micronutrient status in children divided by living area

Parameter		Peri-urban setting (n=55)	Rural setting (n=37)
		%	%
stunting	height for age Z-score < -2	18.9	18.9
underweight	weight for age Z-score < -2	20.8	35.1
thinness	BMI for age Z-score < -2	9.4	27*
overweight	BMI for age Z-score > +1	7.5	10.8
obesity	BMI for age Z-score > +2	3.8	0
ID (SF<15mg/L)		1.9	8.6
Zinc deficiency		49.1	37.1
VAD (< 0.70 µmol/l)		0	2.9
Low VA status(< 1.05 µmol/l)		20.4	45.7*
Anemia (Hb<115 or 120g/L)		3.6	13.5

*ID: iron deficiency; SF: serum ferritin; VAD: vitamin A deficiency; Hb: hemoglobin
*Significantly different from peri-urban setting (chi-square test; $p< 0.05$

Relatively high of stunting rate was detected (17.1%) and this was significantly more severe in rural (23.0%) than peri-urban area (11.6%). Similarly to the prevalence of overweight significantly larger in rural area. Almost 60% and 63% of women were obese and had central obesity respectively (mean of both study site). Central obesity is an excess accumulation of fat in the abdominal area, particularly due to excess visceral fat. Central obesity is associated with higher risk of diseases such as cardiovascular diseases, neurological diseases, diabetes, hypertension, fatty liver, gallstones, gout, osteoarthritis, sleep apnea, skin disorder (13). Over the past 2 decades, prevalence of obesity and central obesity of Thai people (>15 y) has been increased (2) and our population had higher prevalences compared to the lates National survey (1) which 46.6%, 50.4% of women were obese and had central obesity respectively. Moreover, prevalence for overweight and obesity were higher in stunted women with the significantly differed only found between stunted obese and non-stunted obese (table 4). Previous studies showed that early life stunting is a risk factor for the development of obesity in adult through a physiological adaptation mechanism referred to as 'catch-up' or 'post-starvation obesity' explained by a series of important long-lasting changes such as lower energy expenditure, higher susceptibility to the effects of high-fat diets, lower fat oxidation, and impaired regulation of food intake (14-16). This was in line of our study which was found higher prevalence of stunting and overweight in the rural area.

Table 3. Socioeconomic, anthropometric data, blood pressure and prevalence of malnutrition of women divided by living area

Parameter	Peri-urban setting (n=104)	Rural Setting (n= 113)
Age[1]	35.4 ± 9.3	37.1 ± 8.0
No. of people in HH[1]	4.6 ± 1.5	5.0 ± 1.8*
Salary of HH per month (THB) [2]	6,000 (2,000-30,000)	6,000 (1,500-40,000)
No. of children per women[1]	2.3 ± 1.3	3.0 ± 1.8*
Years of education per women[1]	6.9 ±3.6	6.1 ± 3.8
Weight (kg) [1]	60.4 ± 12.6	62.2 ± 10.8
Height (m) [1]	1.53 ± 0.06	1.52 ± 0.05
BMI (kg/m^2) [1]	25.8 ± 5.5	27.0 ± 4.7
Waist circumference (cm) [1]	84.4 ± 11.5	85.4 ± 7.4
Systolic BP (mmHg) [2]	115.0 (89.5-178.0)	118.0 (84.0-188.5)
Diastolic BP (mmHg) [2]	70.5 (48.0-106.0)	67.5 (48.5-103.5)
Underweight (BMI < 18.5; %)	7.7	2.7
Stunting (Height z-score <-2; %)	11.6	23.0*
Overweight (BMI ≥ 23.00; %)	65.4	80.5*
Obesity (BMI ≥ 25.00; %)	53.9	63.7
Waist circumference (> 80 cm; %)	59.6	66.4
Hypertension (SBP ≥ 140&/or DBP ≥ 90; %)	10.7	12.4

[1] Mean ±SD (all such values)
[2] Median (range) (all such values)
* Significantly different from peri-urban setting (independent sample's t-test; $p< 0.05$)

Table 4. Prevalence and number of total, overweight and obese stunted and non-stunted women

	Total	**Overweight**	**Obesity**
Stunted women	17.1% (37) [1]	67.6% (25)	40.5% (15)*
Non-stunted women	82.9% (180)	57.2% (103)	23.9% (43)

[1] prevalence (number of subjects) (all such values)
*significant difference between stunted obesity and non-stunted obesity

However, the exact reasons of double burden of malnutrition are not yet clear. The main contribution of such condition was introduction of cheap, energy- dense foods in transition countries cause high energy intake with low nutrient density such as protein and micronutrient (17).

4. CONCLUSIONS

In our study population signs of a double burden of undernutrition/micronutrient deficiency and obesity were found in the same community. On one hand, around 20-30% of the children are stunted and underweight in addition to suffering from micronutrient deficiencies (zinc and vitamin A). On the other hand, nearly 60% of women were obese, stunted women were significantly more often obese than the non-stunted women.

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Exploring the Functional and Structural Properties of Four Types of Pulse Flour

AP-O080

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ABSTRACT

This study aimed to investigate the physicochemical and functional properties, as well as structural properties, of four types of pulse flour: black bean, red bean, mung bean, and white bean. The results revealed significant differences in proximate analysis and color (L*, a*, and b*) ($p \leq 0.05$). Analysis of pasting properties indicated that black bean flour exhibited the highest pasting temperature (85°C) and peak viscosity (1167 cPs). Conversely, white bean flour demonstrated a high breakdown value (163 cPs), with mung bean flour showcasing the highest setback (511 cPs) at a statistically significant level ($p \leq 0.05$). Exploring thermal properties, black bean flour displayed values of To (77.81°C), Tp (83.250°C), and Tc (88.27°C), which did not significantly differ from those of red bean ($p>0.05$). Moreover, white bean flour exhibited the highest enthalpy value of 3.05 J/g ($p \leq 0.05$) among the types analyzed. In terms of amino acid content, mung bean was found to contain higher amounts of each type of essential amino acid such as Threonine, Phenylalanine, and Isoleucine compared to other pulse varieties. Additionally, it was found that pulse flour was a beta-sheet structure, and the crystalline structure was the main structure. This highlights the unique properties inherent in each type of pulse, suggesting diverse potential for future food applications.

1. INTRODUCTION

Pulses, considered one of the world's most widely consumed economic crops, hold significant importance due to their affordability and rich nutritional composition [1]. Their versatility in culinary applications and high nutritional value have made them a staple in various cuisines globally. Additionally, pulses serve as a prominent source of plant-based nutrition in Thailand, where they are recognized for their health benefits, particularly their high protein content [2]. Also common pea starch improves water retention and stability [3]. This includes water and oil absorption (VAC and OAC respectively), foaming, emulsification, texture, gel, and viscosity. These characteristics can was applied to food. This nutritional profile makes pulses an essential component of balanced diets, especially for individuals following vegetarian or plant-based diets.

Moreover, the distinct properties of various pulse types underscored their nutritional significance. For example, red beans were noted for their strong trypsin inhibitory effect, which could vary based on factors like variety and cultivation conditions [4]. Conversely, black beans were lauded for their rich anthocyanin and folate content, which aided in cardiovascular health by minimizing the risk of artery blockages. Mung beans were distinguished by their thiamine levels and low glycemic index, making them a favorable option for diabetics [5]. Additionally, white beans contained α-amylase, a crucial enzyme in carbohydrate metabolism. Analysis the properties of these four bean types revealed that they were low in fat while still being nutritionally valuable.

In the context of vegetarian diets, pulses play a crucial role by providing essential nutrients such as protein, zinc, calcium, and magnesium [6]. This makes them an invaluable source of nutrition for individuals who abstain from consuming meat. Additionally, the affordability and nutritional density of pulses make them an attractive option for health-conscious consumers seeking nutrient-rich food choices. However, it's essential to recognize that the chemical composition and physical characteristics of pulses can vary significantly depending on factors such as genetics, growing conditions, and processing methods [7]. These variations can influence not only the nutritional content but also the sensory attributes and functional properties of products.

The objective of this research is to comprehensively study the physico-chemical, functional, and structural properties of four key types of pulses: mung bean, red bean, black bean, and white bean. By understanding these properties, this knowledge can facilitate the utilization of pulses in a wider range of food applications.

2. MATERIAL AND METHODS

2.1 Raw materials

Four varieties of pulses: white bean, mung bean, red bean, and black bean were supplies by SCP Foods Co., Ltd., located in Sisaket, Thailand, All pulses were dried in a hot air oven until their moisture content reached below 12%. Subsequently, the pulses were milled to achieve a particle size for passing through an 80 mesh sieve, resulting in whole flour. The flour was then carefully packaged in laminated aluminium foil bags and stored at room temperature for further analysis.

2.2 Physico-chemical properties

2.2.1. Color

Color parameters of four types of pulses varieties and their flours were measured using Chroma meter (Color Flex Model 45/0, USA), These parameters included L* (darkness/lightness), a* (greenness/redness), and b* (blueness/yellowness).

2.2.2. Proximate analysis

Moisture, ash, fat, fiber, protein and carbohydrate content were determined according to AOAC (2000) [8] .

2.2.3. Amino acids profile

Amino acids were analysed using the Microtephra Automatic Amino Acid Analyzer (AAA L-800, Hitachi, Japan) following the method of AOAC (2016) [9].

2.3 Functional properties

2.3.1. Pasting properties

Pasting properties were determined using a Rapid Visco- Analyzer (RVA 4500, Perten Instruments, Sweden). Parameters including pasting temperature, peak viscosity, breakdown, final viscosity, trough viscosity and setback (calculated as peak viscosity - trough viscosity) were recorded [10].

2.3.2. Thermal properties

Thermal properties were assessed using a Differential Scanning Calorimeter (DSC, Mettler Toledo, DSC1/400W, USA) under a nitrogen atmosphere. Subsequently, various thermal properties of the flour, including onset temperature (To), peak temperature (Tp), end temperature (Te), and enthalpy value (ΔH), were determined according to Sang et al (2010) [11].

2.4 Structural properties

Structural properties was measured using Fourier Transform Infrared Spectroscopy (FT-IR) Sample pulses flour underwent drying and stored with a desiccant prior to FT-IR analysis. FT-IR spectrum recording was conducted using a Golden-Gate single reflector ATR on an FTS700 FT-IR spectrophotometer equipped with a DTGS detector (DIGI-LAB, Randolph, MA). Spectra were recorded in absorbance mode across the mid-infrared region, ranging from 4000 to 400 cm^{-1}, with a resolution of 4 cm^{-1}. Data were collected over 32 scans [12].

2.5 Statistical Analysis

Analytical measurements and all data were presented by mean values ± standard deviation. Each parameter was analysed by analysis of variance (ANOVA). Differences between treatments at the 95% ($p \leq 0.05$) were indicated as significant.

3. RESULTS AND DISCUSSION

3.1 Physico-chemical properties

3.1.1 Color

The color value of all pulses flours were analyzed using a chroma meter (Table 1). The results were presented in L*, a*, and b* values. Among the pulse flour samples, white bean flour demonstrated the highest yellow coloration, followed by mung bean flour, which exhibited a blend of white and yellow hues due to its b* value associated with yellowness. The color of pigmented in pulses flours was influenced by phenolic compounds, such as anthocyanins (purple), proanthocyanins (red), and carotenoids (yellow) [13]. Red bean flour displayed a reddish-yellow hue, while black bean flour exhibited the lowest brightness value. The distinct coloration of each pulse flour may be attributed to various key compounds. The divergence in coloration among pulse flour types displayed their unique compositions. Red bean and black bean flour contained anthocyanins, imparting their characteristic red hues, while mung bean and white bean flour contained chlorophyll and carotenoids, contributing to green and yellow coloration, respectively. In summary, each type of pulse exhibited distinct coloration attributable to the specific components present in that particular type.

Table 1 Color of four types of pulse flour

Sample	L*	a*	b*
Mung bean	88.56±0.01^{a}	-1.90±0.06^{d}	18.90±0.03^{b}
Red bean	82.15±0.02^{c}	0.29±0.03^{b}	9.58±0.06^{d}
White bean	82.44±0.01^{b}	0.28±0.04^{a}	28.44±0.07^{a}
Black bean	81.41±0.02^{d}	0.31±0.01^{c}	10.13±0.04^{c}

*Mean ± SD, each value in the table is the mean of five replication (color; n = 5).

$^{a-d}$ Values with different letters in the same column are significant differences ($p \leq 0.05$).

Figure 1. Pulses flours (A) Red bean, (B) White bean, (C) Black bean, (D) Mung bean

3.1.2 Proximate analysis

The proximate analysis of various pulse flours was shown in Table 2, including moisture, ash, fat, protein, fiber, and carbohydrate content. The moisture content ranged from 9-10%, ash 2-3%, fat 0-2%, protein 19-25%, fiber 3-17%, and carbohydrate 51-69%. Regarding protein content, which ranged from 19.48 to 25.97%, mung bean flour exhibited the highest protein content at 25.97%, followed by black bean flour at 21.32%. It was found that both mung beans and black beans had high protein content, similar to that of peanuts, which was approximately 25% [14]. Conversely, no statistically significant difference (p>0.05) was observed between white bean and red bean flour, consistent with findings from previous studies [15-17]. Rich in protein, especially in lysine, pulses served as crucial components for cereal protein, making protein intake significant in vegetarian diets. In terms of fiber content, which ranged from 3.62% to 17.46%, mung bean flour boasted the highest fiber content at 17.46%. It was found that pulses flours in this study had higher fiber content than soybeans, (2.35%) [18], with no statistically significant difference (p>0.05) observed between white bean and red bean flour. These findings underscored the importance of pulses, vegetables, and grains as essential dietary fiber sources crucial for regulating metabolic processes, as outlined [16]. For fat content, ash content, and carbohydrate content, each type of pulse flour exhibited significantly different chemical compositions (p≤0.05), influenced by pulse type, growth conditions, and storage conditions. Pulses were notable for their high nutritional value, particularly in carbohydrates and protein, rendering them valuable sources of energy and cost-effective protein with low fat content [19].

Table 2 Chemical composition of four types of pulse flour

Proximate analysis	Mung bean	Red bean	White bean	Black bean
Moisture ^{ns} (%)	10.15±0.82	10.79±0.44	9.97±0.25	9.91±0.07
Protein (%)	25.97±0.06^{a}	19.63±0.15^{c}	19.48±0.20^{c}	21.32±0.20^{b}
Fiber (%)	17.46±0.76^{a}	6.19±0.33^{b}	6.42±0.45^{b}	3.62±0.20^{c}
Fat (%)	0.91±0.07^{c}	1.62±0.07^{b}	2.92±0.02^{a}	1.66±0.33^{b}
Ash (%)	4.00±0.37^{a}	4.06±0.04^{a}	2.98±0.21^{b}	3.62±0.20^{a}
Carbohydrate (%)	51.96±0.34^{b}	69.08±0.61^{a}	68.37±0.81^{a}	69.71±0.48^{a}

*Mean ± SD, each value in the table is the mean of three replication (n = 3) .

^{ns} Non significant (p>0.05)

$^{a-c}$ Values with different letters in the same row are significant differences ($p \leq 0.05$).

%Carbohydrate =100–(Moisture+Protein+Crude fat+Crude fiber+Ash)

3.1.3 Amino acid profile

The Amino acid profile of various types of pulses flour was shown in Table 3, revealing significant differences in both essential and non-essential amino acid content ($p \leq 0.05$). Essential amino acids found in pulses flour included Threonine (15-19 g/ 100 g protein), Lysine (0.7-1.2 g/ 100 g protein), and Phenylalanine (0.7-1.2 g/ 100 g protein), while non-essential amino acids present in significant amounts included glycine (10-21 g/ 100 g protein), glutamic acid (2-3 g/ 100 g protein), and arginine (0.4-1.4 g/ 100 g protein). Each type of pulses exhibited distinct amino acid profiles. Mung bean flour displayed the highest threonine content (19.92 grams per 100 grams of protein), followed by red bean (16.56 g/ 100 g protein). Threonine was identified as a crucial nutritional modulator impacting nutrient metabolism. Studies had shown that threonine supplementation could enhance fat metabolism in the liver and mitigate lipid metabolism disorders by regulating lipogenesis signalling pathways and thermophilic gene expression [20] and the amount was higher than in soybeans (1.33 grams per 100 grams of protein) [21]. Additionally, two types of glutamic acid and arginine were found in pulses and were thought to be important for brain development, acting to protect the nervous system in children [22]. White beans were found to have the highest lysine content (1.21 g/ 100 g protein), while black bean flour exhibited elevated levels of leucine (1.80 g/ 100 g protein) and valine (0.52 g/ 100 g protein) and was generally known as a good source of Lysine but deficient in Cystein and Methionine. In conclusion, each type of pulses exhibited a unique amino acid profile, highlighting their diverse nutritional characteristics.

Table 3. Amino acid profile of four types of pulse flour

Types of amino acids	Amino acid content (g/100g protein)			
	Mung bean	Red bean	White bean	Black bean
Essential amino acids				
Histidine	0.954±0.06^{a}	0.40±0.034^{c}	0.35±0.01^{d}	0.42±0.01^{b}
Isoleucine	1.44±0.04^{a}	0.34±0.03^{d}	0.36±0.01^{c}	0.39±0.03^{b}
Leucine	0.76±0.09^{d}	1.52±0.23^{c}	1.52±0.18^{b}	1.80±0.06^{a}
Valine	0.30±0.27^{d}	0.51±0.05^{b}	0.39±0.01^{c}	0.53±0.04^{a}
Phenylalanine	1.54±0.28^{a}	0.84±0.14^{c}	0.12±0.00^{d}	1.00±0.06^{b}
Methionine	0.40±0.18^{a}	0.14±0.03^{c}	0.08±0.01^{d}	0.18±0.01^{b}
Lysine	1.03±0.46^{b}	1.02±0.23^{c}	1.21±0.02^{a}	0.74±0.06^{d}
Threonine	19.93±3.70^{a}	16.56±3.23^{b}	15.84±0.68^{d}	15.52±6.92^{c}
Tryptophan	ND	ND	ND	ND
Arginine	0.40±0.01^{d}	1.06±0.32^{c}	1.21±0.07^{b}	1.42±0.03^{a}
Non-essential amino acids				
Glycine	15.86±0.74^{b}	10.24±0.03^{d}	21.73±0.07^{a}	10.59±0.37^{c}
Serine	0.75±0.04^{c}	0.90±0.85^{b}	1.31±0.15^{a}	0.30±0.03^{d}
Tyrosin	0.90±0.03^{a}	0.19±0.00^{d}	0.81±0.01^{b}	0.32±0.00^{c}
Cystein	0.80±0.20^{d}	0.83±0.06^{b}	0.82±0.01^{c}	0.87±0.06^{a}
Glutamic acid	3.59±0.06^{a}	2.41±0.69^{b}	1.87±0.79^{c}	1.79±0.02^{d}
Glutamine	ND	ND	ND	ND
Alanine	ND	ND	ND	ND
Asparagine	ND	ND	ND	ND
Aspartic acid	ND	ND	ND	ND

*Mean ± SD, each value in the table is the mean of three replication (n = 3).

ND: Non detect

a-d Values with different letters in the same row are significant differences ($p \leq 0.05$).

3.2.2. Thermal properties

The thermal properties of various pulses flours using Differential Scanning Calorimetry (DSC), as detailed in Table 5, showed significant distinctions. Black bean flour exhibited the highest onset temperature (To) at 77.82 °C, alongside recording the highest peak temperature (Tp), which was statistically comparable to that of red bean ($p > 0.05$). Moreover, black bean flour also demonstrated the highest end temperature (Te). Conversely, white bean flour displayed a greater enthalpy value compared to other pulses types, likely due to its elevated fat content of 2.9%. This elevated fat content impeded dough or starch swelling, necessitating higher gelatinization temperatures. The energy required for gelatinization was amplified due to the disruption of starch double helix order by the delivered energy, primarily attributable to non-starch components such as proteins, fats, and fibers, which obstructed gelatinization. The formation of complex compounds, including amylose-lipid and amylose-protein complexes, further elevated gelatinization temperatures by fortifying starch structure. Although black bean flour and red bean flour exhibited no significant difference in fat content ($p > 0.05$), black bean flour yielded a higher enthalpy value than red bean, likely attributed to its elevated fiber content, which hindered water absorption and thereby elevated enthalpy. The information about pulse flour indicated the temperature at which proteins unfolded and coagulated. This influenced the texture and other characteristics.

Table 5 Thermal properties of four types of pulse flour

Thermal properties	Mung bean	Red bean	White bean	Black bean
T_o (°C)	$73.21±1.20^{c}$	$77.32±0.27^{a}$	$71.23±0.13^{d}$	$77.82±0.30^{a}$
T_p (°C)	$80.25±0.82^{b}$	$83.34±0.23^{a}$	$77.75±0.11^{c}$	$83.25±0.59^{a}$
T_e (°C)	$86.43±1.43^{ab}$	$88.08±0.04^{a}$	$85.05±0.98^{b}$	$88.27±0.69^{a}$
ΔH (J/g)	$1.82±0.35^{bc}$	$1.56±0.20^{c}$	$3.06±0.09^{a}$	$2.23±0.21^{b}$

*Mean ± SD, each value in the table is the mean of three replication (n = 3).

$^{a-d}$ Values with different letters in the same row are significant differences ($p \leq 0.05$).

3.3 Structure properties

The structural properties of amide I distribution in various pulses flours were shown in Figures 2, where it was observed that the amide I band (1590-1720 cm-1) encompassed three primary structural elements: β-sheet (1610–1640 cm-1), α-helix (1650–1658 cm-1), and β-turn (1660 - 1670 cm-1) [27]. The β-sheet predominated as the principal component in each pulses type, which aligned with prior findings [28-30]. The protein structure across all pulses varieties primarily comprised beta structures, notably β-sheet and β-turn, with the former characterized by intermolecular hydrogen bonding and exhibiting lower flexibility compared to the latter, which represented a more loose and flexible helical structure. Turning to the investigation of starch distribution within the absorption range, distinct categories were discernible: Crystalline (994-995 cm-1), amorphous starch (1022-1023 cm-1), and ordered starch (1044-1045 cm-1)[31]. It was evident that the structure of pulses flour leaned towards being semi-crystalline, endowing it with strength and flexibility. This crystallinity was associated with the prevalence of β-sheet structures in pulses flour, contributing to its high crystallinity. Consequently, it became apparent that the structural composition of each type of pulses flour exhibited similarities, with protein constituting the primary characteristic. This information was essential for understanding how processing and cooking affected the protein structure of pulses and, consequently, their digestibility and functionality in food formulations.

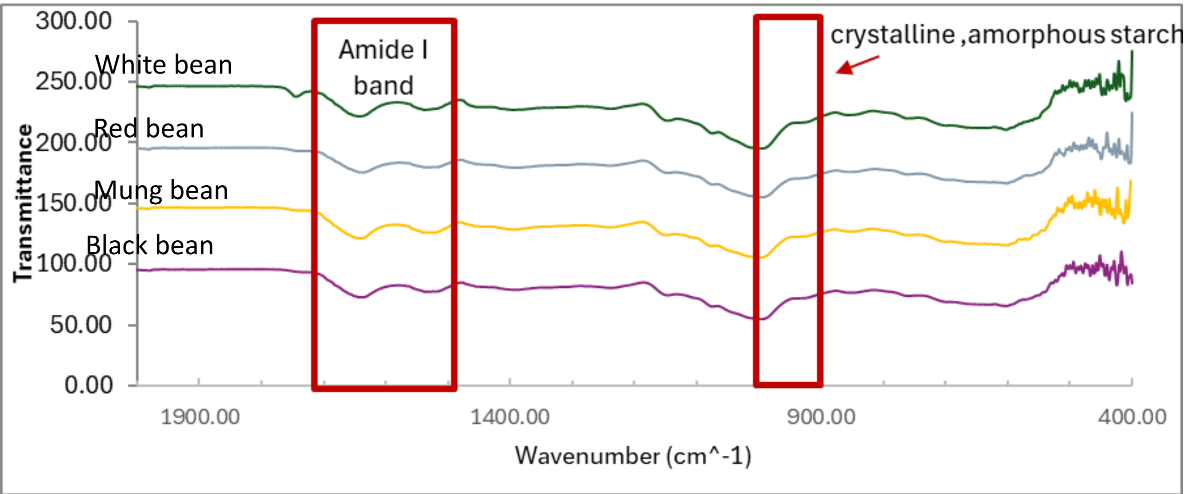

Figure 2. FT-IR spectra of pulses flours

4. CONCLUSIONS

Through an analysis of the physico-chemical, functional, and structural properties of four varieties of pulse flours—mung bean, black bean, red bean, and white bean—it was observed that mung bean flour exhibited significantly high levels of protein (25.97%), fat (0.91%), fiber (17.46%), and carbohydrates (51.96%). These varying chemical compositions influenced the functional properties of the flours distinctively. Additionally, it was noted that peak viscosity was exceptionally high for white bean at 1167.00 cP, correlating with the enthalpy value that registered the highest energy value (3.06 J/g), surpassing the other bean types. Furthermore, mung bean was found to have a higher concentration of essential amino acids compared to the other pulses. The structural characteristics revealed that pronounced presence of β-sheet structures (1610–1640 cm-1) within the pulses. Consequently, it was evident that each type of pulse flour possessed unique nutritional profiles, with mung bean flour notable for its enhanced nutritional content and superior amino acid profile. This underscored the potential of pulse flour, across all varieties studied, as a valuable resource for developing health foods and highlighted its potential for future innovations in food science and nutrition.

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Effect of Banana Ripening Stages and Drum Drying on Physico-Chemical and Bioactive Compounds of Banana Flakes

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ABSTRACT

Kluai Namwa is a native banana commonly grown and consume in all regions of Thailand. This research was conducted to study the effect of ripening stages of Kluai Namwa fruits for banana flour production and to study the production of crispy banana flake from banana flour. Banana flour was produced by varying three degree of ripeness stages (1st, 2nd, and 3rd stages). For production of banana flakes, drum dryer was used with 0.30 mm roller gap and 3.5 rpm roller speed and drum surface temperatures was set at 120 and 130°C. The results indicated that Kluai Namwa banana flour at the 3rd stage of ripening had highest acid and reducing sugar content compared to other stages. Banana flour produced at 1st stage of banana ripening had low total phenolic content, antioxidant activity, and β-carotene content ($p < 0.05$). The ripening stages of banana and drum dryer temperature affected the properties of banana flakes. The results showed that banana flake produced from flour at the 3rd ripening stage with drum dry temperature set at 120-130 °C contained high amount of total phenolic compounds and antioxidant activities. Water absorption and water solubility of flake increased with increasing drum dried temperature. The increasing of ripening stages of the flour decreased bulk density and milk absorption value of banana flakes.

1. INTRODUCTION

Bananas (*Musa* spp.) is one of the most available agricultural products in Thailand. Bananas are a potential source of nutrients such as carbohydrates, bioactive compounds, and antioxidants. The bioactive compounds mainly found in bananas including polyphenolic compounds and carotenoids such as α-carotene, β-carotene, and β-cryptoxanthin [1]. There are many commercial banana cultivars in Thailand including ‘Kluai Hom Thong’ (Musa AAA group), ‘Kluai Khai’ (Musa AA group) and ‘Kluai Namwa’ (Musa ABB group). Kluai Namwa are climacteric fruits and harvested at the unripe stage. As a climacteric fruit, ripening process of banana is related to physicochemical properties such as bioactive compounds, starch, sugar, potassium and phosphorus [2].

Banana ripening consists of a change in colour from green to yellow starting from the center of the banana fruit and then extending to its tips and is associated with softening and change in surface characteristics. The chemical composition and bioactive compounds of banana fruit also change during ripening stage. Total acidity content of 'Kluai Leb Mue nang' banana at mature green stage was lower than ripe and overripe stages and the total antioxidants and total phenol content of banana at ripe stage was significantly higher than that of mature green and overripe stage [3]. Netlak et al., (2023) [1] reported that β-carotene content of banana varies depending on harvesting time and ripening stages.

Banana flour can be produced from unripe banana pulp. Green bananas are an excellent source of carbohydrates protein, lipids, minerals and vitamins [4,5]. The green banana flour contains up to 61.3-76.5 g/100 g starch on dry basis [6]. Banana flour is beneficial for health due to its high content of resistant starch which generates a low blood glucose response since it is not digested, but fermented in the colon by the bacterial microflora [7][8]. Banana flour can be utilized as a food ingredient to improve properties of food products.

Flakes are ready to eat food products which low water content and crunchy texture. Banana flour can be used for nutrient addition to the flakes. Commercial production of flakes uses whole grains or fruit, which is cooked and drum-dried to produce thin flakes or manufactured using twin screw extruder. Drum drying is an economical method for drying food products and especially effective for drying highly viscous liquid. Drum drying techniques are used to dry fruit peel such as dragon fruit and pomegranate [9,10]. Caparino et al. (2012) [11] reported the production of mango powders by drum drying at drum surface temperature of 152 °C using mango puree at 95-100 % of ripening stage. Germer et al. (2017) [12] using mango pulp for production of mango flakes by drum drying varying temperature at 119-151°C and residence time (9-41 s). Drum-dried flakes are used as an ingredient for baby food, instant cereal drinks and instant soups. Parameters such as surface temperature, holding time, and solid content that affect degree of gelatinization, water absorption index, water solubility index and pasting property of low amylose rice are important factors in drum drying process [13]. During drum drying, the Maillard browning reaction and starch gelatinization occur. Increases in water absorption index and water solubility were observed in drum dried sweet potato flour due to starch gelatinization and degradation [14]. Many studies revealed changes in bioactive compounds during the drying process. Decreasing total phenolic content, total anthocyanin content, and antioxidant activities were observed in red rice after extrusion cooking [15].

Drum drying affects the properties of flakes; however, no information exists concerning how ripening stage of banana flour and drum drying parameters impact on the quality of banana flakes. Thus, here, the banana flour at three stages of ripening and drying temperatures were investigated to determine their effects on the chemical, physical and bioactive compounds of banana flakes as a potential functional ingredient for food products.

2. MATERIAL AND METHODS

Bananas (*Musa* spp.) c.v. Kluai Namwa Nuan Chan were obtained from Sukhothai province, Thailand. The ripening stages of this fruit were established according to Von Loesecke, (1950) [16] scale, which defined seven ripening stages. Bananas fruit at 1st stage of ripening indicating green, 2nd stage indicating green with trace amount of yellow, and 3rd stage indicating more green than yellow were used in this experiment.

2.1 The chemical and physical properties of banana fruit

Moisture content of banana was measured according to AOAC method AOAC (2000) [17]. The pH of banana pulp was measured according to Moongngerm et al., (2014) [18]. The pulp suspension (8% (w/v)) was stirred for 5 min, allowed to stand for 30 min, filtered, then pH of the filtrate was measured by using a pH meter. Total acidity was measured by titration method Sadler et al., (2010) [19]. Reducing sugar content was measured using the Fehling test. Color of banana pulp was

measured by Hunter Lab Colorimeter (Model 45/0-S, Hunter Associates Laboratory Inc., VA, USA). Color values were recorded as L^* (lightness), a^* (redness), and b^* (yellowness).

2.2 Banana flour production

Banana fruit was washed, peeled, sliced to 0.2 cm thickness, and soaked in 0.1% (w/v) sodium metabisulfite solution for 30 min. The sample was dried at 50 °C for 6 h, ground into flour, and pass through 100 mesh sieve size. Banana flour was kept in aluminium foil bag and stored at 4 °C until further analysis.

2.3 The chemical, physical properties, and bioactive compound of banana flour

Moisture content of banana was measured according to AOAC method [17]. Reducing sugar content was measured using the Fehling test.

Banana flour (5 g) were ground and extracted using 50 mL of 85% aqueous methanol, with continuous stirring for 30 min at room temperature, according to the method of Sompong et al., (2011) [15]. The methanol extracts were filtrated through filter paper (Whatman No.5) and stored until required for use. Total phenolic content (TPC) was determined by the Folin-Ciocalteu procedure documented by Singleton et al., (1999) [20]. Extracted samples (300 µL) were added into test tubes, followed by 1.5 mL of 10% Folin-Ciocalteu reagent and 1.2 mL of sodium carbonate (7.5% w/v). The tubes were allowed to stand for 2 hr before measuring absorbance at 765 nm using a spectrophotometer (Thermo Fisher Scientific Inc., USA). Total phenolic content was calculated as milligrams gallic acid equivalents (GAE) per g dry weight of sample.

The DPPH assay was carried out with slight modification [21]. Briefly, 100 mmol/L of DPPH radical solution was prepared in methanol. Appropriately diluted crude extracts or standards (100 mL) were added to 1.5 mL DPPH solution. After incubating for 30 min in the dark, the absorbance was measured at 517 nm. Results were expressed as Trolox equivalents in milligrams per g dry weight.

β-carotene content was determined according to Carvalho et al., (2014) [22]. Three grams of the sample were transferred to a mortar containing a small amount of hyflosupercel (3 g) and ground with 50 mL of cold acetone and then filtered through a Buchner funnel with filter paper Whatman no 1. The residue was rinsed with acetone and the extract and petroleum ether was added to the extract and rinsed with distilled water. Carotenoids were collected from the extract and then passed through a glass funnel containing anhydrous sodium sulfate to remove the water. The quantification of the extract was carried out by spectrophotometry at 450 nm. petroleum ether was used as a blank sample.

Color of banana flours were measured by Hunter Lab Colorimeter (Model 45/0-S, Hunter Associates Laboratory Inc., VA, USA). Color values were recorded as L^* (lightness), a^* (redness), and b^* (yellowness) and the browning index (BI) was calculated according to Kurek et al., 2016 [23] as follows:

$$\text{BI (Browning index)} = [100(x - 0.31)] / 0.172$$

$$\text{where: } x = (a^* + 1.75L^*) / (5.645L^* + a^* - 3.012b^*)$$

2.4 Production of banana flake

Drum dry banana flour was prepared by passing banana flour slurry (50% solid) to a pilot-scale double-roller drum dryer (JM-T, Jonh-millder) with 0.3 mm roller gap and 3.5 rpm roller speed to produce flakes. Drum surface temperatures was set at 120 and 130 °C. The temperature of drum dryer surface was monitored by digital thermometer. The products were dried at 60 °C for 1 hr and pass through 4 mesh sieve screens. Samples were kept in aluminium foil bag and stored at 4 °C until required for use.

2.5 The chemical and bioactive compounds of banana flakes

The moisture of banana flake was analyzed according to AOAC methods [17]. Total phenolic content, antioxidant activities by DPPH assay, and β-carotene content were determined as described above.

2.6 *Physical properties of banana flakes*

The hardness of banana flakes was analyzed using Instron testing machine (model 4411 S/N H 2082). Water absorption index and water solubility index was analyzed according to Anderson et al., (1969) [24]. Bulk density was measured according to Cheewapramong et al., (2002) [25]. Banana flakes were placed in a measuring cylinder (100 mL) and weighed. Bulk density was expressed as wt of sample/unit volume (g/mL). Milk absorption was measured according to Luckett and Wang (2012) [26]. Eight grams of rice flakes were placed in 60 mL of 0% fat milk at 8°C for 15 s, then the flakes were removed from the milk and drained on a 2.8-mm stainless steel mesh screen for 10 s. Percentage of milk absorption was calculated by dividing the absorbed milk weight by the weight of the original flakes. Color of banana flakes were measured and browning index were calculated as described above.

2.7 *Statistical analysis*

Analysis of variance (ANOVA) was performed using statistical software (Version 17.0, SPSS Inc., USA). Differences among means were compared using Duncan's multiple range test ($\alpha = 0.05$). The experiments were conducted in triplicate.

3. RESULTS AND DISCUSSION

The chemical composition and bioactive compounds of banana are shown in Table 1. The results indicated that banana at the 3rd ripening stage showed significant higher moisture content, total acidity, reducing sugar, and β-carotene compared to those from 1st and 2nd ripening stages. pH of banana fruit tended to decrease as ripening stage increased. As the banana ripened, the acid content mainly malic acid, citric acid, and oxalic acid increased and then decreased after fifth day of storage [27]. Sinanoglou et al., (2023) [28] stated that the malic acid increased during ripening, while oxalic acid decreased caused an overall increasing in titratable acidity and banana flesh moisture content and total soluble solid value during ripening increased were due to the starch hydrolysis into soluble sugars and the moisture migration from the peel to the flesh.

The color parameters of banana peel are shown in Table 1. The L^* indicated the lightness and b^* indicated yellowness showed significant increased ($p<0.05$) with increasing ripening stage. As the banana became ripe, the color of the peel began to change from green to yellow which increased b^* value.

Table 1. Chemical composition and bioactive compounds of banana fruit at different ripening stage

Parameter	Ripening stages		
	Stage 1	Stage 2	Stage 3
Moisture content (%)	64.06±0.13^{c}	66.49±0.06^{b}	67.76±0.25^{a}
pH	7.07±0.01^{a}	6.31±0.01^{b}	6.03±0.01^{c}
Total acidity (%)	0.12±0.01^{c}	0.16±0.01^{b}	0.25±0.02^{a}
Reducing sugar (%)	2.33±0.01^{c}	2.91±0.06^{b}	4.53±0.03^{a}
L^*	57.99±0.52^{c}	64.60±0.32^{b}	66.38±0.76^{a}
a^*	-15.79±0.98^{b}	-13.95±0.86^{a}	-14.16±0.11^{a}
b^*	31.67±0.18^{c}	33.73±0.88^{b}	38.24±0.86^{a}

*Means with the same letter within rows are not significantly different ($p>0.05$)

Chemical compositions, bioactive compounds, and color of banana flour are summarized in Table 2. The reducing sugar of banana flour increased with increasing the ripening stage ($p<0.05$) concurred with the results of fresh banana. Total phenolic content of banana flour at first ripening stage was significantly lower than banana flour produced from banana pulp at 2nd and 3rd ripening stages. Antioxidant activities reflect relative difference in the ability of antioxidant compounds in the extract. DPPH assay measures the ability of the extract to donate hydrogen to the radical. The result indicated that DPPH scavenging activities of banana flour produced from 2nd and 3rd stage of ripening

gave the highest amount of DPPH activities ($p<0.05$), indicating high radical scavenging properties. Results followed the same trend of total phenolic content. Kumar et al., (2019) [29] stated that banana contained high level of polyphenolic compounds including carotenoids, depamine, serotonin, gallocatechin, epicathechin, cathechin. β-carotene is one group of carotenoids found in banana fruits. β-carotene content of banana flour from 3rd stage of ripening contained the highest values compared to the other stages. Netlak et al., (2023) [1] reported that carotenoid mainly found in A genome banana is α carotene, β-carotene, and lutein. β-carotene and lutein are accumulated throughout fruit development and ripening.

Banana flour at the 2rd stage had a highest L^* value compared to flour from 1st stage and 3nd stages. The yellowness (b^*) of banana flour at 3rd stage of ripening was significantly higher than those of 1st stage and 2nd stages, and the value tended to increase as the ripening stage increased. Browning index of banana flour increased with increasing ripening stage of banana fruit. Browning index may also be affected by non-enzymatic browning reactions that can occur during drying of banana. Banana flour produced from banana at 3rd stage of ripening contained high reducing sugars and amino acids that can undergo the Maillard reaction when heated [30].

Table 2. Chemical compositions, bioactive compounds, and color of banana flour at different ripening stage

Parameter	Ripening stages		
	Stage 1	**Stage 2**	**Stage 3**
Moisture content (%) (ns)	2.52±0.75	2.47±0.26	2.22±0.21
Reducing sugar (%)	2.15±0.02^{c}	5.85±0.11^{b}	11.75±0.99^{a}
Total phenolic content (mg GAE/g DW)	0.16±0.01^{b}	1.30±0.05^{a}	1.30±0.18^{a}
DPPH$^{\cdot}$ (mg TE/g DW)	0.13±0.00^{b}	0.65±0.00^{a}	0.66±0.00^{a}
β-carotene (μg/g) DW	3.17±0.04^{c}	3.35±0.05^{b}	3.65±0.05^{a}
L^*	81.51±0.01^{b}	81.82±0.01^{a}	81.11±0.01^{c}
a^*	0.37±0.01^{b}	0.41±0.01^{a}	0.14±0.01^{c}
b^*	9.78±0.01^{c}	10.01±0.01^{b}	10.78±0.01^{a}
Browning index	12.67±0.01^{c}	12.97±0.01^{b}	13.88±0.02^{a}

*Means with the same letter within rows are not significantly different ($p>0.05$)

Table 3. Moisture content, total phenolic content, antioxidant activity measured by DPPH and β-carotene of banana flake at three level of ripening stage with drum dry temperature set at 120-130 °C

Ripening stages	Drum Temp. (°C)	Moisture content (%)	Total phenolic content (mg GAE/g DW)	DPPH$^{\cdot}$ (mg TE/g DW)	β-carotene (μg/g DW)
1	120	5.63±0.10^{a}	0.06±0.00^{e}	0.07±0.00^{e}	-
	130	5.77±0.19^{a}	0.07±0.00^{e}	0.08±0.00^{d}	-
2	120	4.44±0.40^{b}	0.11±0.03^{d}	0.11±0.00^{c}	-
	130	3.62±0.32^{c}	0.15±0.01^{e}	0.12±0.00^{b}	-
3	120	3.59±0.11^{c}	0.21±0.01^{e}	0.13±0.00^{a}	-
	130	3.33±0.15^{c}	0.30±0.01^{d}	0.13±0.00^{a}	-

*Means with the same letter within columns are not significantly different ($p>0.05$)

The ripening stages of banana and drum dryer temperature affected the properties of banana flakes. Moisture content of banana flakes were in the ranges of 3.33-5.77%. Banana flake produced from flour at 3rd ripening stage with drum dried temperature set at 130 °C contained highest amount of total phenolic compounds ($p<0.05$). As drying temperature increased from 120 °C to 130 °C, the total phenolic content tended to increase for banana flour produced at 2nd and 3rd ripening stage. Increase of total phenolic content in the flakes as drying temperature increased resulted from the release of phenolic compounds from thermal degradation of cell walls and subcellular components [31].

The antioxidant capacity determined by DPPH assay displayed a similar trend to total phenolic content. Increase in antioxidant activities after heat treatment was probably due to the release of phenolic compounds by breaking down the cellular constituents and formation of new compounds with enhanced antioxidant activities [32,33].

β-carotene was not found in all banana flakes. A significant loss of β-carotene in banana flake during drum drying process is caused by isomerization of carotenoids which are easily isomerized by heat, acid, and light [34].

Table 4. Color and browning index of banana flake at three level of ripening stage with drum dry temperature set at 120-130 °C

Ripening stages	Drum Temp. (°C)	*L**	*a**	*b**	Browning index
1	120	62.75±0.04[a]	1.45±0.03[f]	12.16±0.02[e]	22.53±0.03[e]
	130	61.97±0.01[c]	2.15±0.03[d]	12.39±0.03[d]	24.11±0.02[d]
2	120	62.52±0.04[b]	2.24±0.01[c]	12.48±0.02[c]	24.17±0.05[cd]
	130	60.55±0.02[d]	1.51±0.04[e]	13.20±0.02[a]	25.61±0.08[b]
3	120	61.65±0.03[c]	2.29±0.02[a]	12.19±0.02[e]	24.26±0.04[c]
	130	59.65±0.04[e]	2.47±0.01[b]	13.09±0.03[b]	26.78±0.07[a]

*Means with the same letter within columns are not significantly different ($p>0.05$)

The color of banana flake produced from flour at different ripening stages with drum dry temperature set at 120-130 °C is shown in Table 4. The *L** values of banana flakes at all ripening stages produced by drum drying at temperature of 130 °C was significantly lower than the flake dried at 120 °C. The *b** value and browning index of the flake at three level of ripening stages dried at 130 °C was higher those dried at 120 °C indicating the Maillard browning reaction at high temperature.

The physical properties of banana flakes are shown in Table 5. Hardness value indicates the crispness of the banana flake products. Hardness of banana flake tended to increase with increasing drying temperature from 120 °C to 130°C. Bulk density measures the mass of particles per unit volume. Products with high bulk density normally require more force to break [27]. The results indicated that increasing in ripening stage of banana flour lower bulk density of flakes.

Water absorption is an indicator of the ability of flakes to absorb water and relates to the availability of hydrophilic groups for binding water molecules. Results indicated that water absorption of banana flakes varied depending on banana ripening stage and temperature applied. WAI gave the high value when drum temperature was set at 130 °C. Water solubility index (WSI) relates to the extent of starch degradation. Banana flakes dried at 130 °C had high WS value compared to those dried at 120 °C. The increase in water solubility of flakes at high temperature was attributed to greater starch gelatinization and degradation during the drying process [13]. Majzoobi et al., (2011) [35] stated that the destruction of starch granules and degradation of starch molecules during drum drying process increased WSI value. Milk absorption was significantly lowest in banana flake produced at 3 rd stage of ripening at all drum dried temperature.

Table 5. Hardness, bulk density, water absorption, water solubility, and milk absorption of banana flakes at at three level of ripening stage with drum dry temperature set at 120-130 °C

Ripening stages	Drum Temp. (°C)	Hardness (kgF)	Bulk density (g/ml)	Water absorption (%)	Water solubility (%)	Milk absorption (%)
1	120	421.07±10.27^{d}	0.17±0.00^{a}	10.52±0.74^{c}	5.44±0.78^{d}	444.26±6.07^{b}
	130	469.83±4.94^{a}	0.17±0.00^{ab}	11.25±0.20^{bc}	6.32±0.69^{d}	463.64±7.41^{a}
2	120	453.90±1.82^{b}	0.16±0.00^{bc}	10.77±0.25^{c}	11.43±0.47^{b}	431.58±9.14^{b}
	130	463.17±3.10^{ab}	0.16±0.00^{cd}	11.88±0.22^{ab}	10.09±0.64^{c}	424.29±11.51^{b}
3	120	383.30±2.78^{e}	0.15±0.00^{d}	11.38±0.76^{abc}	16.53±0.55^{a}	381.98±11.62^{c}
	130	444.30±4.20^{c}	0.15±0.00^{d}	12.22±0.18^{a}	15.67±0.45^{a}	394.27±16.19^{c}

*Means with the same letter within columns are not significantly different (p>0.05)

4. CONCLUSIONS

The research was undertaken to study the impact of drum drying temperature and ripening stage of banana flour on the properties of banana flakes. Banana flour produced from banana at 1st stage of ripening contained highest reducing sugar and the lowest amounts of bioactive compounds including total phenolic content, antioxidant activity, and β-carotene content. The increase in bioactive compounds of flakes including total phenolic content, antioxidant activities were observed when increase ripening stage of banana flour. High drum surface temperature increased water solubility and water absorption of the flakes. Milk absorption and bulk density of the flakes decreased as ripening stage increased. Results indicated that drum dried banana flakes can be used as food ingredients.

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Effect of medium chain triglyceride (MCT) oil and highly branched cyclic dextrin (HBCD) energy gel on glycemic response and insulin sensitivity in healthy subjects

AP-P102

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ABSTRACT

This study evaluated the glycemic efficacy and insulin sensitivity of energy gel for exercise with highly branched cyclic dextrin (HBCD) alone versus energy gel with HBCD plus medium chain triglyceride (MCT) oil. The energy gel characteristics including physicochemical properties, proximate composition, and sensory evaluation were analyzed. Energy gel containing HBCD plus MCT oil had the highest overall acceptability score, ranging from like moderately to like very much. A cross-over study design at 0-180 minutes was used to assess glucose solution, MCT+HBCD, HBCD alone, and the glucose control gel formulation. Highest peak glucose change from baseline after 15 minutes was observed in HBCD and statistically significantly different from the control energy gel, indicating a faster gastric emptying rate. HBCD plus MCT oil gave medium glycemic index value, with no significant differences recorded among the energy gel formulation and insulin response. The energy gel formulation containing HBCD plus MCT oil showed promise as an alternative for individuals seeking sustained energy during exercise.

1. INTRODUCTION

Carbohydrate and fat are the two main sources of energy during endurance exercise [1,2]. Carbohydrate is the dominant energy source, which is digested and stored in the liver and muscles as glycogen [3]. Because of the limitations of carbohydrate storage, prolonged exercise can cause hypoglycemia, fatigue, and exhaustion with reduced performance. Numerous studies have shown the benefits of pre, during, and post exercise carbohydrate supplementation [4-8]. Ergogenic aid products as energy gels are effective and can conveniently provide a quick source of energy during endurance sports. Energy gels offer a concentrated source of energy, making it easier to replace lost energy stores whilst performing exercise or during a sports match. However, high concentrations of carbohydrates can result in stomach up-sets and a sports gel product must not cause any GI disorders [9]. Therefore, alternative source of carbohydrate should be developed as ergogenic energy gels.

Highly branched cyclic dextrin (HBCD) has been proposed as a source of carbohydrate in sports. This new type of dextrin is produced from waxy corn starch by the cyclization reaction of branching enzyme (EC 2.4.1.18) [10,11]. HBCD molecules contain short linear chains of α-(1,4) linked glucose units with branching via α-(1,6) glycosidic bonds. HBCD is highly soluble in water.

The aqueous solution is very stable with low viscosity and contributes little osmotic pressure, meaning that it is absorbed very fast and has a relatively low propensity of retrogradation [12,13]. These properties reduce the possibility of gastrointestinal problems along with intensive exercises including running and cycling [14].

Carbohydrates and fat are the main body fuels both at rest and during exercise. Fat is stored in adipose tissue in the form of triacylglycerol, which comprises three fatty acids attached to a molecule of glycerol [15]. The hydrolysis of triacylglycerol results in the release of free fatty acids and glycerol into the blood circulation to deliver fuel to muscles and tissues [1,15,16]. Fat and medium chain triglycerides (MCTs) composed of a glycerol backbone and 6-12 carbon atoms in three fatty acids have been studied as energy sources in sports. MCTs are more rapidly digested and absorbed into the bloodstream than long chain triglycerides (LCTs), which provide more energy than fat storage in the body [17-19]. In previous studies on humans, the addition of MCT to carbohydrate beverages increased gastric emptying compared with an equicaloric carbohydrate beverage [20]. MCTs have also been reported to increase fat oxidation and energy expenditure (EE) in humans [21,22] by increasing mitochondrial biogenesis and metabolism [19]. Therefore, MCTs could be used as an alternative fuel source for exercising muscles.

This study formulated ergogenic aid products as an HBCD energy gel and an HBCD plus MCT oil energy gel and compared their efficacies on postprandial 0-180 minute blood glucose and insulin in healthy subjects. Few studies have been conducted on HBCD and glycemic response; therefore, the glycemic indices of the developed energy gels were determined to optimize sports nutrition and manage blood sugar levels.

2. MATERIAL AND METHODS

2.1 Development of energy gels from HBCD and MCT oil

2.1.1 Energy gels from coconut water preparation

Three energy gel formulations were prepared based on HBCD, MCT oil, and MCT+HBCD with glucose as the control. The ingredients of each formulation are present-ed in Table 1. Each mixture was heated to 85-90°C for 5 minutes, and the coconut flavour was then added. Then, 85 g of the mixture was hot filled in a stand-up pouch size 14x9x3 cm and sterilized in a water spray retort (KM-P95SS, KM Grand Pack Co., Ltd., Thailand) at 116°C for 19 minutes.

Table 1. Energy gel formulations.

Ingredient (%)	MCT+HBCD	HBCD	Control
HBCD	26.0	26.0	-
Glucose powder	-	-	26.0
Fructose syrup	7.5	7.5	7.5
MCT oil	2.0	-	-
Carrageenan	0.1	0.1	0.1
Coconut water	64.0	64.0	64.0
Water	-	2.0	2.0
Coconut flavour	0.4	0.4	0.4

2.1.2 Determination of physicochemical properties

The pH value was measured using a pH meter (Starter 310, Ohaus, USA). Total soluble solids (TSS) were determined using a digital refractometer (Milwaukee MA871, Milwaukee Instruments Co., Ltd., Romania). The color properties were measured using a Hunter Lab Colorimeter (ColorFlexEZ, Hunter Associates Laboratory, Inc., Reston, VA, USA). The color parameters included lightness (L*), redness (a*), and yellowness (b*). The equipment was standardized with a white plate

color standard, with the mean value of three measurements taken for the L*, a*, and b* values. The osmolality was determined by freezing-point depression (Advanced Wide-Range Osmometer 3W2, Advanced Instrument Co., Ltd., MA, USA).

2.1.3 Determination of proximate composition

Moisture content, ash, protein, and fat were determined using standard methods de-scribed by the Association of Official Analytical Chemists, AOAC (2016). Carbohydrate content was determined by calculation using a different method, with energy value calculated by multiplying the protein content by 4, carbohydrate content by 4, and fat content by 9.

2.1.4 Sensory evaluation

The sensory evaluation protocol was approved by the Mahidol University Central Institutional Review Board (MU-CIRB), with COA number 2020/118.0109. The sensory evaluation was performed at the Institute of Nutrition, Mahidol University, Thailand. Sensory characteristics such as appearance, texture, flavor, color, sweetness, and overall acceptability were evaluated by 50 healthy untrained panelists aged between 18 and 60, who normally consumed energy gel products, using a 9-point hedonic scale (1 = dislike extremely and 9 = like extremely). All samples were coded with randomly selected 3-digit numbers, with presentation order randomized between panelists. Drinking water was provided between samples to cleanse the palate before testing the next sample.

2.2. Effect of HBCD and MCT oil energy gels on postprandial glycemic and insulin response in healthy subjects

2.2.1 Ethical approval

The study protocol was approved by the Mahidol University Central Institutional Review Board (MU-CIRB) with COA. No. MU-CIRB 2020/180.0511. Informed consent was obtained from each participant before data collection. All privacy rights were maintained.

2.2.2 Subjects and study design

This randomized cross-over clinical trial included 10 healthy subjects (5 males and 5 females) aged between 20 and 35. Inclusion criteria included blood glucose < 5.6 mmol/L and HbA1c <5.7%. Subjects who used any medications/dietary supplements/insulin injections to lower plasma glucose, had any other disease or condition that could impact adherence to the measurements, had any allergies to gel composition products or smoked or drank alcohol were excluded.

2.2.3 Test products

Three energy gel formulations were tested including MCT+HBCD, HBCD, and control energy gels with glucose solution as the reference. All energy gels had similar visual appearances, physical properties, and packaging, with each energy gel labeled using a three-digit number to conceal the identity.

2.2.4 Study protocol

On the screening day, the participants were asked not to consume any food or bever-age during the previous 10 hours. The screening process included an interview concerning medical history, a physical examination, and laboratory blood collection tests. After overnight fasting, venous blood samples were collected during the examination and used as the baseline. The subjects were asked to consume 155.60 g of HBCD + MCT oil energy gel, 155.20 g of HBCD energy gel, 160.10 g of the control energy gel, and 150 ml of glucose solution within 10 minutes. Each test food contained 32 g of available carbohydrates within the recommended range of 25-50 g [23]. After eating the test food, venous blood samples were collected at 0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes to deter-mine the plasma glucose level. Each intervention was separated by 7-day wash-out periods.

2.3 Statistical analysis

All data were obtained in triplicate, presented as mean ± SD, and subjected to analysis of variance (ANOVA). A significant difference between means was determined by Duncan's Multiple Range Test (DMRT). For the clinical trial, baseline characteristics were summarized as mean ± SD. The average blood increment areas under the curve and at each time point of glucose and insulin levels among the gel formulations were analyzed by one-way ANOVA and repeated measurement ANOVA, respectively.

SPSS version 19.0 for Windows (SPSS Inc. Chicago, IL, USA) was used for statistical analysis. A p-value < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Physicochemical properties of energy gels

The physicochemical properties of energy gels are presented in Table 2. The pH values of the energy gels, which were classified as low-acid food, varied between 5.66 and 5.84. Total soluble solids of all energy gel formulations ranged from 31.50 to 34.00 °Brix. The lightness (L*), redness (a*), and yellowness (b*) values were significantly different among formulations. Energy gel with glucose showed the highest a* and b* values and lowest L* value among the three formulations, with a reddish-brown color, as seen in Figure 1. By contrast, the energy gel with HBCD+MCT oil and HBCD had higher L*values than the control, resulting in a brighter yellow-brown color. Osmolality values of MCT+HBCD and HBCD energy gels were significantly lower ($p < 0.05$) than the energy gel with glucose (884 ± 5.00 and 817 ± 3.00 vs 2,220 ± 3.00, respectively).

Table 2. Characteristics of energy gels using coconut water based on MCT+ HBCD and HBCD with glucose as the control

1. Energy gel product	MCT+HBCD	HBCD	Control
pH	5.84 ± 0.01^{a}	5.84 ± 0.01^{a}	5.66 ± 0.01^{b}
Total soluble solids (°Brix)	34.00 ± 0.02^{a}	33.67 ± 0.29^{b}	31.50 ± 0.00^{c}
Color properties			
- Lightness (L*)	19.50 ± 0.06^{b}	21.87 ± 0.45^{a}	17.00 ± 0.10^{c}
- Redness (a*)	2.87 ± 0.06^{b}	1.53 ± 0.06^{c}	3.13 ± 0.12^{a}
- Yellowness (b*)	13.07 ± 0.06^{c}	14.47 ± 0.55^{b}	18.40 ± 0.10^{a}
Viscosity (Pa.s)	0.4963 ± 0.02^{a}	0.4649 ± 0.01^{a}	0.0494 ± 0.01^{b}
Osmolality (mOsm/kg H_2O)	884 ± 5.00^{c}	817 ± 3.00^{b}	2,220 ± 3.00^{a}

*Mean ± SD values in the same row followed by different letters are significantly different ($p < 0.05$).

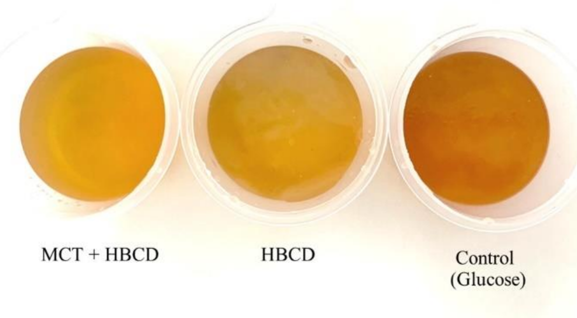

Figure 1. Appearances of the three developed energy gels MCT+HBCD, HBCD alone, and the control

3.2 Nutrition compositions of energy gels

Proximate energy gel compositions are shown in Table 3. The MCT+HBCD energy gel provided the highest total energy at 135.57 kcal per 100 g, while the control energy gel based on glucose had the highest total sugar per 100 g. The carbohydrate and protein contents of energy gels were not significantly different, with fat only detected in the MCT+HBCD energy gel formulation.

Table 3. Proximate compositions of the three developed energy gels MCT+HBCD, HBCD and the control

20. Properties per 100 g	MCT+HBCD	HBCD	Control
Total energy (kcal)	135.57±0.25[a]	129.24±0.48[b]	125.26±0.06[c]
*Carbohydrate (g)	32.15±0.07[a]	32.22±0.12[a]	31.24±0.02[a]
- Total sugar (g)	9.41±0.26[c]	12.25±0.11[b]	28.19±0.14[a]
Protein (g)	0.07±0.01[a]	0.09±0.02[a]	0.08±0.01[a]
Fat (g)	0.75±0.01	ND	ND
Moisture (g)	66.48±0.07[c]	67.16±0.12[b]	68.06±0.02[a]
Ash (g)	0.55±0.02[b]	0.53±0.01[b]	0.62±0.01[a]

*Data are expressed as mean±SD. Different letters in the same row are significantly different ($p < 0.05$). *Calculated by difference, includes the dietary fibre fraction. ND; Not Detected.

3.3 Sensory evaluation

The sensory attribute scores for each energy gel formulation are shown in Table 4. Results indicated that MCT+HBCD had the highest score among the three formulations for all attributes. The overall acceptability scores of the MCT+HBCD and HBCD energy gels were 7.83 and 7.25, respectively which were not significantly different. By contrast, the control had the significantly ($p<0.05$) lowest overall acceptability score.

Table 4. Sensory evaluation of energy gels from coconut water based on MCT+HBCD, HBCD, and the control

37. Sensory attribute score*	MCT+HBCD	HBCD	Control
Appearance	8.02 ± 0.52[a]	7.59 ± 0.31[a]	6.42 ± 0.20[b]
Color	7.44 ± 0.31[a]	7.32 ± 0.11[a]	6.02 ± 0.40[b]
Flavor	7.65 ± 0.19[a]	7.60 ± 0.28[a]	6.98 ± 0.34[ab]
Taste	8.11 ± 0.45[a]	7.42 ± 0.17[b]	7.01 ± 0.39[b]
Texture	7.97 ± 0.22[a]	8.03 ± 0.35[a]	6.81 ± 0.43[b]
Overall acceptability	7.83 ± 0.26[a]	7.25 ± 0.34[a]	6.76 ± 0.41[b]

*The sensory test was performed using a 9-point hedonic scale (1; extremely dislike, 5; neither like nor dislike, 9; extremely like). Mean ± SD values in the same row followed by different let-ters are significantly different ($p < 0.05$).

3.4 Glycemic and insulinemic response

3.4.1 Baseline characteristics of the participants

Ten participants completed this randomized cross-over controlled trial, with baseline data shown in Table 5. Mean age ± SD was 26 ± 3 years, with body mass index (BMI) 22.9±3.9 kg/m2.

Biochemical assessments included HbA1C, with fasting glucose in the normal range as an inclusion criterion.

Table 5. Baseline characteristics of the study participants.

Parameter	Mean ± SD
Age (years)	26±3
Systolic blood pressure (mmHg)	114±10
Diastolic blood pressure (mmHg)	72±6
Body mass index (BMI; kg/m^2)	22.9±3.9
Blood chemistry	
HbA1C (%)	5.1±0.4
Blood glucose (mmol/L)	4.65±0.22
Triglycerides (mg/dL)	74.41±31.40
Total cholesterol (mg/dL)	197.08±27.67
Cholesterol-high density lipoprotein (HDL) (mg/dL)	73.08±18.74
Cholesterol-low density lipoprotein (LDL) (mg/dL)	108.75±25.60
Kidney function	
Blood urea nitrogen (BUN) (mg/dL)	11.67±2.46
Creatinine (mg/dL)	0.87±0.20
Liver functions	
Aspartate aminotransferase (AST) (Unit/L)	20.25±3.72
Alanine transaminase (ALT) (Unit/L)	14.00±2.80
Alkaline phosphatase (ALP) (Unit/L)	62.75±21.69

3.4.2 Postprandial glucose and insulin response

The peak and incremental area under the curve (iAUC) of blood glucose and insulin were determined 180 minutes after consumption of the reference glucose solution and the three energy gels (MCT+HCBD, HCBD, and the glucose control gel). For the postprandial glucose study, the glycemic indices (GIs) of the three energy gel products were calculated after 120 minutes, as shown in Table 6. The GI values in the human study were lowest in the MCT+HBCD formula, with HBCD giving the highest GI value.

Table 6. Glycemic indices of the three energy gel products.

90. Energy gel product	Glycemic index		
	Mean	SEM	Classification
MCT+HBCD	68.8	9.6	Medium
HBCD	86.9	11.2	High
Control	83.5	10.0	High

*SEM; standard error of mean

Peak blood glucose and insulin change from the baseline were observed 180 minutes after consumption of the three energy gels. The MCT+HCBD and HBCD energy gels had the highest peaks at 30 minutes, whereas the peak of the control glucose solution gel occurred at 45 minutes. Differences in blood glucose levels at each time point among the three gel formulas were analyzed by repeated measurement ANOVA. At 15 minutes, the change in blood glucose in HBCD was significantly higher than the control at 1.135 ±0.438 vs 0.689±0.439 nmol/L, p value = 0.004. No differences were observed between the formulas at the other time points (data not shown). Blood glucose level changes were observed up to 150 minutes in the HBCD energy gel, whereas the MCT+HBCD and the control energy gels showed changes up to 120 minutes. The MCT+HBCD

treatment had the lowest incremental area under the curve (iAUC) (Figure 2A). Similar to the glycemic response, the insulin peak of MCT+HCBD gave the highest value at 30 minutes, whereas the other formulas presented highest peaks at 45 minutes. No differences were recorded among time points for iAUC insulin and gel formulations (Figure 2B).

A.

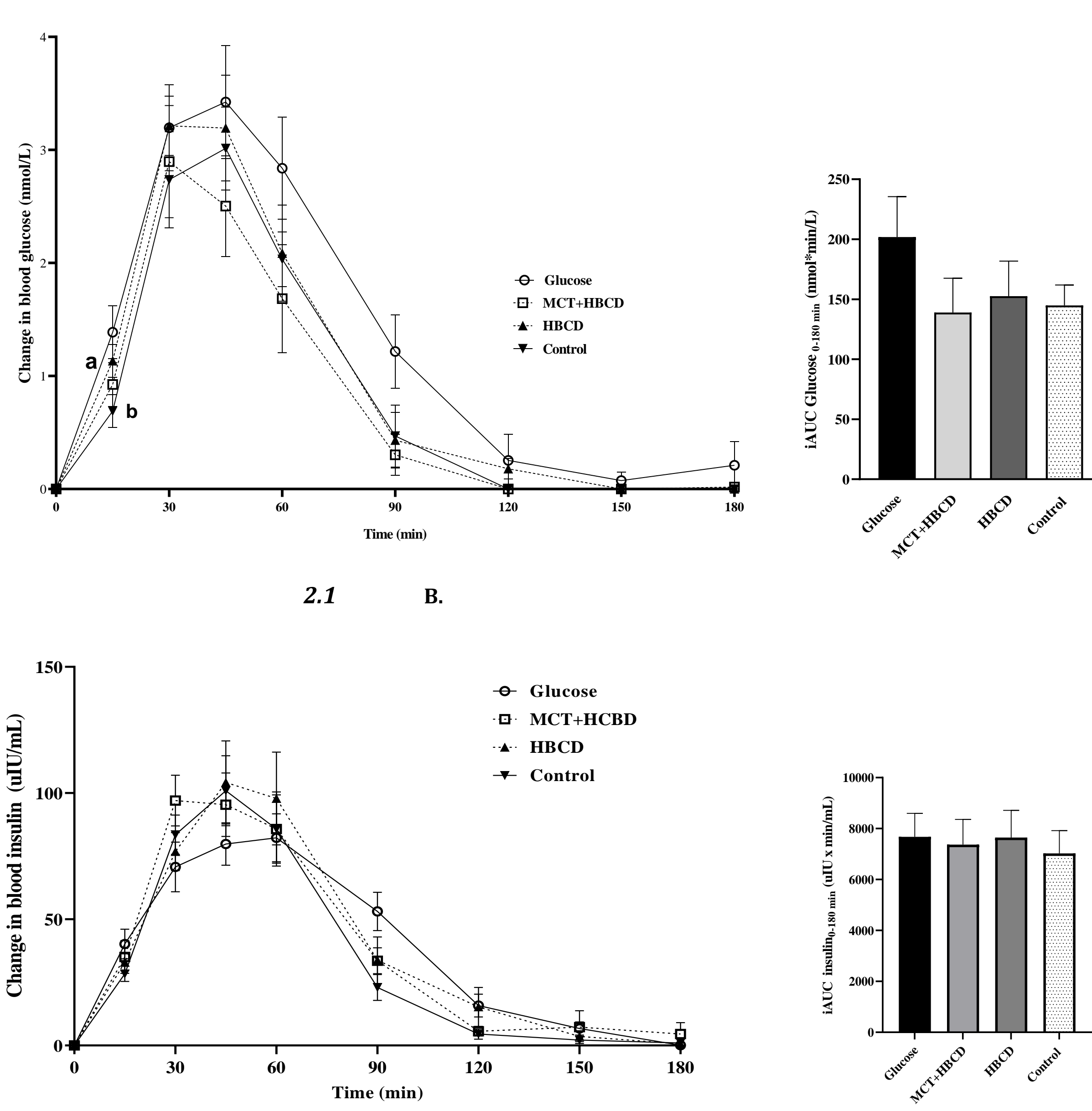

Figure 2. Peak changes from baseline and incremental area under the curve (iAUC) of blood glucose (A) and insulin (B) at 0-180 minutes after gel consumption. Data are expressed as the mean and standard error of the mean (SEM). The significantly difference between HBCD and control gel was observed between difference latter ($p < 0.05$)

Potential implications for exercise and health were assessed after consuming medium chain triglyceride (MCT) oil and HBCD in energy gel products. Few studies have reported on the effect of combining MCT oil and HBCD on glycemic response and glycemic index values. Energy gel developed for exercise should have suitable physicochemical properties with consumer acceptability, lower glycemic index, and faster gastric emptying time. The energy gel with HBCD plus

MCT oil had the highest overall acceptability score, which ranged between like moderately and like very much. Sterilization in a water spray retort inactivated the pathogenic microorganisms, especially Clostridium botulinum, because of the low acid food properties (pH greater than 4.6) [24]. The energy gel structure of HBCD consists of long chains of glucose units. These can be broken down into smaller parts, thereby increasing the dissolved solids and total soluble solids. Energy gel with added glucose had reduced brightness and a reddish-brown color due to sterilization caused by thermolysis and polymerization of the sugar molecules, presenting as brown pigments and caramelization from the non-enzymatic browning of energy gel in glucose [25,26]. Osmolality is a necessary property that allows energy gel to remain in the GI tract during exercise. Energy gel with HBCD had lower osmolality than the glucose control because of the higher molecular weight (average 160,000 and 180.16 g/mol, respectively) [13,27,28]. The highest total sugar content in the control energy gel gave the highest osmolality (2,200 mOsm/kg H2O), possibly causing gastric discomfort when consumed during exercise. By contrast, the osmolality values of energy gels containing HBCD plus MCT oil and HBCD formulations were 884 and 817 mOsm/kg H_2O, respectively. These lower values may help to prevent gastric discomfort and benefit athletes using these products during races. The viscosities of energy gels with HBCD plus MCT oil and HBCD alone were 10 times higher than the control with glucose. HBCD is composed of glucose molecules with cyclic cluster structures, while glucose is a single molecule. The viscosity of food has been reported to impact the sensation of fullness and satiety [29,30] but these sensations were not reported by subjects in this study. Therefore, the physicochemical attributes of MCT+HBCD with low osmolality and high viscosity gave the lowest GI properties when compared with the other sports gels.

Three formulas of energy gels containing MCT + HBCD, HBCD alone, and the glucose control were determined for glycemic and insulinemic responses compared with the glucose solution. The lowest glycemic index (GI) value as medium GI classification was observed in MCT+HBCD, whereas the high GI classification of the HBCD formula was simi-lar to the glucose control energy gel. Previous studies reporting the effects of glycemic index and exercise performance presented inconsistent results. Lower GI food may confer an advantage when consumed before endurance exercises [31]. Low GI food consumption showed lower plasma lactate during exercise, suggesting that a low GI meal generated lower levels of glycolysis, increased fat oxidation, and decreased carbohydrate oxidation [6,7,32]. In young male subjects, the ingestion of moderate GI foods before exercise gave higher glucose availability and improved cycling exercise performance [8]. Conversely, Jamurtas and colleague (2011) [5]. found no significant changes in exercise performance after consuming low and high glycemic index food. High GI carbohydrate foods might also enhance post-exercise refueling of muscle glycogen [33,34], suggesting the benefit of HBCD ingesting both during and after exercise.

Four grams of MCT oil were suggested as the optimal intake based on the results of this study. MCT oil contributes to the moderate glycemic index value of the energy gel product. Fat interferes with glucose absorption and the presence of fat in a meal lowers the food glycemic index [35]. MCT oil-based products have been reported as advantageous in weight management [36]. Many discussions have addressed the effectiveness of MCT oil on exercise performance. MCT oil supplementation increases fatty acid levels in the body, stimulates fat oxidation and reduces carbohydrate oxidation, resulting in greater glycogen storage in the muscles [21,37].

At 180 minutes post-consumption, a glycemic and insulinemic response study showed no significant differences in the incremental area under the curve among sample groups undergoing energy gel tests. HBCD had a faster gastric emptying rate than the glucose control energy gels when comparing significant changes in blood glucose peaks at 15 minutes. This benefit might help to prevent some issues such as bloating and gas in the stomach, thereby promoting effective digestion and absorption [38,39], and maximizing fuel oxidation and glycogen storage during exercise [40]. HBCD energy gel also sustained glucose in the blood for up to 150 minutes, with 120 minutes recorded for the glucose control energy gel.

Most sports drink products available on the market have high glycemic index values [41]. This is of concern for the general public and people suffering from diabetes who are interested in consuming these products. Results in this study showed that the gel supplement containing MCT oil, with a moderate glycemic index, offered an alternative for general consumers who may not engage in daily physical activity, while still addressing energy provision and enhancing exercise performance for athletes. Study limitations included the small sample size. Future studies investigating the sports performance aspect of this product should use athletes as subjects to better elucidate long-term effects on energy metabolism. In summary, highly branched cyclic dextrin offers a favorable alternative to carbohydrates, which have higher glycemic indices and faster gastric emptying rates, as an energy supplement both during and after exercise. The combined MCT oil and HBCD energy gel had a medium glycemic index value and was suitable both for daily consumption and also for pre-exercise.

4. CONCLUSIONS

This study elucidated the comparative effects of exercise energy gels formulated with highly branched cyclic dextrin (HBCD) alone versus those enriched with a combination of HBCD and medium chain triglyceride (MCT) oil. Analyses of the physicochemical proper-ties, proximate composition, and sensory attributes provided a comprehensive under-standing of the energy gels. This study successfully developed an energy gel containing HBCD plus MCT oil, which had high sensory acceptability ranging between like moderately and like very much. The results of a randomized cross-over clinical trial involving 10 healthy subjects revealed postprandial blood glucose responses and insulin sensitivity patterns associated with each gel variant 180 minutes after intake. HBCD offers advantages in terms of solubility, viscosity building, and lower osmolality that could be possible to reduced gastric emptying rate.

5. ACKNOWLEDGEMENTS

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Effect of Processing Treatment on Lipoxygenase Activity and Quality of Mung Bean Beverage

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ABSTRACT

Plant-based beverages offer a promising alternative product, however, the beany flavor attributed to lipid-degrading enzymes limits their consumption. This work aimed to investigate the effects of processing treatments namely soaking in 0.5% $NaHCO_3$ (NA); soaking in tap water and steaming (ST); soaking in 0.5% $NaHCO_3$ and steaming (NA+ST); soaking in tap water and steaming with pandan leaves (ST+PL); and soaking in 0.5% $NaHCO_3$ and steaming with pandan leaves (NA+ST+PL), on lipoxygenase (LOX) activity, color, total phenolic content (TPC), and antioxidant activity measured by DPPH and ABTS assays of the mung bean beverage. Results showed that streaming treatments (ST, NA+ST, ST+PL, and NA+ST+PL) were effective in inhibiting mung bean LOX accounting for 32.84-44.78% and 69.76-95.70% for natural and oat milk flavors, respectively, compared to control samples. Meanwhile, NA treatment or NA combination with heat (NA+ST and NA+ST+PL) could not inactivate or enhance the inactivation of the mung bean LOX. Steaming treatments for both flavors contained significantly lower L^* and higher b^* values than the controls ($p \leq 0.05$), leading to a yellow-green color of steamed products. Although TPC and antioxidant activity were reduced for both flavors, pandan leaves addition during steaming has the potential to enhance the phenolic level and antioxidant properties. This study demonstrated that thermal application significantly removed beany flavor and improved bioactive content. Thus, NA+ST+PL is useful in developing mung bean beverages as a healthy plant-based product.

1. INTRODUCTION

Plant-based proteins are rapidly increasing in popularity around the world because of their nutritional and functional benefits [1]. There has been a growing demand for plant-based beverage alternatives, particularly among lactose intolerant, milk protein allergic, vegetarians, vegans, and those seeking healthier diets [2]. Mung beans (*Vigna radiata* L.), one of the most important legume crops, are rich in protein (20–32%), essential amino acids (lysine), insoluble dietary fiber, vitamins (B, C, E, K, thiamine, riboflavin, niacin, and pantothenic acid), and minerals (Ca, Fe, Mg, Mn, P, K, and Zn), as well as high amounts of bioactive components such as tannin, phenolic compounds (flavones, flavonoids, isoflavones), and phytosterol [1,3,4]. Studies have demonstrated that mung beans contain antioxidant, anti-inflammatory, antitumor, and anti-diabetic properties [3,5]. There has been increased interest in mung bean proteins as a source of bioactive peptides due to their potential in human health and the food industry for antioxidant, anti-obesity, cholesterol-lowering, enhancing mineral bioavailability, and preventing cancer. Plant-based foods, edible films, and active substance carriers have all been developed using mung bean proteins and peptides [6]. However, due to its

unpleasant bean flavor and the lack of information about processing characteristics, mung beans consumption and industrial production were limited [3].

The beany flavor has been reported to be caused mainly by hexanal, (E)-2-hexenal, hexanol, 1-octene-3-ol, and (E, E)-2,4-decadienal, which directly affect the sensory properties of mung bean products [7]. These are mainly attributed to the action of endogenous lipid-degrading enzymes such as lipoxygenase (LOX). LOX catalyzes the oxidation of polyunsaturated fatty acids, leading to the formation of hydroperoxides that are further degraded into volatile compounds, especially hexanal [2]. Various processing methods, novel processing such as superheated steam, extrusion [8], non-thermal processes such as microwave heating [9], and ohmic heating [10], and heat processes including baking [11] and blanching [12] have been applied to inactivate lipid-degrading enzyme activity. On the other hand, heat stability is critical to the functionality of plant-based beverage products. Recently, fermentation was used to reduce the undesirable flavors of beans [3,7]. Additionally, chemical methods were also used to inhibit the enzymatic reaction, which was related to the chelating and pH properties of the substances [13]. Sodium bicarbonate ($NaHCO_3$) was used to increase the pH of the soaking solution, contributing to the inactivation of the LOX. This had a significant effect on beany flavor reduction in soymilk [14].

According to the benefits of the thermal process, the most commonly used and effective technique to reduce the beany flavor components, a practical chemical method, and lack of data study on mung bean beverage enzyme inhibition [15]. Therefore, this study aimed to investigate the effect of chemical pre-treatment and thermal processing on LOX activity, color, total phenolic content (TPC), and antioxidant activity measured by DPPH and ABTS assays in the development of mung bean beverages. As a result of this study, valuable information and guidance could be provided regarding the removal of beany flavor from mung bean beverage products by conventional processing methods.

2. MATERIAL AND METHODS

2.1 Material

Folin-Ciocalteu's reagent was purchased from LOBA Chemie Ltd. (Maharashtra, India). Dye reagent concentrate (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used. Linoleic acid, Tween 20, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzthiaz-oline-6-sulfonic acid) (ABTS), gallic acid, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Bovine serum albumin (BSA) was obtained from Sisco Research Laboratories (Maharashtra, India). Other chemicals used were analytical grade. Several ingredients were purchased at the local market, including mung beans (*Vigna radiata* L., Raitip brand), sodium bicarbonate (McGarrett brand), pasteurized oat milk (Good Mate brand), sugar (Mitr Phol brand), and fresh pandan leaves.

2.2 Treatments

Processing of natural flavor mung bean beverage as a control sample involves six main steps as follows: cleaning the mung bean, soaking in tap water for 4 h, grinding with water in a ratio of 1:6 (mung bean: water) for 30 seconds, filtering the slurry by double layers of white cloth, heating the liquid part at 80-85°C for 5 minutes and addition of sugar, hot filling into the glass bottle and stored at 4-10°C [2,14]. For the processing of oat milk flavor as a control sample, a combination of water and oat milk in a ratio of 1:1 was used in the grinding step. Briefly, there are 5 processing treatments: soaking in 0.5% sodium bicarbonate ($NaHCO_3$) solution for 4 hours (NA); soaking in tap water for 4 hours and steaming (98-100˚C) for 10 minutes before grinding (ST); soaking in 0.5% $NaHCO_3$ for 4 hours and steaming for 10 minutes (NA+ST); soaking in tap water for 4 hours and steaming for 10 minutes with addition of pandan leaves during steaming (ST+PL); and soaking in 0.5% $NaHCO_3$ for 4 hours and steaming for 10 minutes with addition of pandan leaves during steaming (NA+ST+PL). The fresh pandan leaves cut into 5 cm were used as 40% of the mung bean weight to improve flavor

due to the 2-Acetyl-1-pyrroline (2AP) aroma compound in the plant [16]. After steaming, it was removed before grinding.

To prepare the samples for analysis, the 20 mL samples were centrifuged for 30 minutes at 4°C at 5,000 rpm. The supernatant was used to determine protein content, LOX activity, total phenolic content (TPC), and antioxidant activity.

2.3 Protein concentration determination

The protein concentration was determined using the dye-binding method [17]. The contents of sample (25 µL) and Bradford reagent (1.2 mL) were mixed and left for 15 minutes before reading the absorbance at 595 nm. A standard curve was constructed using bovine serum albumin.

2.4 Lipoxygenase activity determination

LOX assay was a modified spectrophotometric method as described in [18]. The substrate solution was freshly prepared by mixing 20 µL of linoleic acid, 20 µL of Tween-20, and 1.25 mL of deionized water. The solution was clarified by adding 125 µL of 1 N NaOH and adjusting to 25 mL with 0.1 M phosphate buffer pH 6.0. The substrate solution of 1 mL was used in the assay and the reaction was started by adding 10 µL of crude enzyme extract. The formation of hydroperoxides was monitored spectrophotometrically every 30 seconds for 5 minutes (UV-1900i, Shimadzu, Tokyo, Japan) as an increase in absorbance at 234 nm due to the presence of a conjugated hydroperoxy diene moiety. One unit of LOX activity was defined as an increase in absorbance of 0.001 at 234 nm per minute per mg of protein under assay conditions.

2.5 Color measurement

The color was measured using a HunterLab MiniScan EZ colorimeter (Hunter Associates Laboratory, Reston, VA, USA) under D-65 diffuse illumination. The lightness (L^*), redness (a^*), and yellowness (b^*) were measured and converted into Chroma (C^*) and hue angle ($h°$) values using the following equation:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (1)$$

$$h^{\circ} = \arctan\frac{b^*}{a^*} \quad (2)$$

2.6 Total phenolic content and antioxidant activity analysis

The TPC was determined according to the method described by [19] with minor changes. A volume of 0.3 mL of the sample was mixed with 0.75 mL of 20% Folin–Ciocalteu reagent and allowed to incubate for 3 minutes. Subsequently, 0.3 mL of 3.75% (w/v) Na_2CO_3 solution was added. The absorbance was read at 765 nm after the reaction was performed in the dark for 40 minutes. The results were expressed as milligrams of gallic acid equivalents per milliliter of sample (mg GAE/mL).

The 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay was carried out using the method described by [20]. The radical was prepared by mixing 24 mg of DPPH with 100 mL of ethanol and left in the dark for 12–16 hours. The solution was adjusted to an absorbance of 0.80±0.02 at 515 nm and left for 1 hour before use. The 1.2 mL DPPH working solution was mixed with 40 µL of the sample (ethanol was used as a blank). After incubation in the dark for 30 minutes, the absorbance was measured at 515 nm. The antioxidant capacity was calculated using the standard Trolox curve, and the results are expressed as micromoles of Trolox equivalents per milliliter of sample (µmol TE/mL).

The 2,2'-azino-bis(3-ethylbenzthiaz-oline-6-sulfonic acid) (ABTS) assay was slightly modified from that described by [21]. The ABTS radical cation ($ABTS^{\bullet+}$) was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate at a ratio of 2:1, and the mixture was left in the dark for 12–16 hours. The solution was diluted to obtain an absorbance of 0.70±0.02 at 734 nm with phosphate-buffered saline (1xPBS) and left for 1 hour before use. The reaction of the extract (40 µL) with 1 mL of the working solution was read after being incubated in the dark for 6 minutes (PBS was used as the blank). The results are expressed as µmol TE/m

2.7 ***Statistical analysis***

The experiment was conducted in a completely randomized design (CRD). Results are reported as means ± standard deviations. The differences between means were examined by analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) using SPSS software version 29 (IBM Corp., Armonk, NY, USA), and differences were considered significant at $\alpha = 0.05$. All experiments were conducted in triplicate. Paired-sample t-test ($p \leq 0.05$) was performed to evaluate the statistical significance of the differences between the product flavor means.

3. RESULTS AND DISCUSSION

3.1 *Effect of processing treatment on protein concentration*

As shown in Table 1, the protein concentration of the control and NA treatment was significantly higher than those of ST, NA+ST, ST+PL, and NA+ST+PL treatments for both product flavors ($p \leq 0.05$), accounting for 7.93-8.23 and 9.25-9.38 mg BSA/mL for natural and oat milk flavors, respectively. Meanwhile, it ranged from 6.02-6.48 and 7.54-8.21 mg BSA/mL in the treatment of ST, NA+ST, ST+PL, and NA+ST+PL for natural and oat milk flavors, respectively. The lower content could be protein loss due to steaming during the process applied to ST, NA+ST, ST+PL, and NA+ST+PL. There was an 18.28-24.09% and 11.24-18.49% protein loss in natural and oat milk flavors, respectively, compared to the control sample. According to reports, almond milk's stability decreases with increasing temperature. A temperature of 55 to 75°C initiated denaturation and partial aggregation, while a temperature of 85 to 95°C induced more extensive denaturation [22]. Results showed that ST combined with NA or PL in both flavors retained higher protein than ST or NA+ST+PL treatments. As a result, NA or PL could be used to preserve proteins during heat treatment. Additionally, it was found that the oat milk flavor contained higher protein than the natural ($p \leq 0.05$). This could be because oats provide high protein content (15–20 g/100 g) and have all essential amino acids, especially rich in lysine [23]. It was reported as 2 g/200 mL protein content on the nutrition label of the oat milk product used in this study. Accordingly, heat treatment and ingredient selection have a significant effect on the protein content of the mung bean beverage.

Table 1. Protein concentration of the mung bean beverage.

Treatment	Protein content (mg BSA/mL)		Protein change (%)**	
	Natural flavor	Oat milk flavor	Natural flavor	Oat milk flavor
Control	7.93 ± 0.20aB	9.25 ± 0.11aA		
NA	8.23 ± 0.08aB	9.38 ± 0.22aA	+ 12.61	+ 1.41
ST	6.02 ± 0.55bB	7.54 ± 0.12cA	- 24.09	- 18.49
NA+ST	6.48 ± 0.57bB	8.21 ± 0.36bA	- 18.28	- 11.24
ST+PL	6.36 ± 0.45bB	7.99 ± 0.21bcA	- 19.80	- 13.62
NA+ST+PL	6.24 ± 0.22bA	7.61 ± 0.34cA	- 21.31	- 17.73

* The data are presented as the means ± standard deviations (n = 3). The different small letters indicate significant differences in the means among the treatments in the same flavor, whereas capital letters indicate significant differences in the means between the product flavors in the same treatment ($p \leq 0.05$). ** Calculation based on comparison with control samples (+ = increase, – = decrease).

3.2 *Effect of processing treatment on lipoxygenase activity*

LOX activity of the control samples exhibited 23,177.60±102.21 and 14,063.06±2,909.25 units/mg protein for natural flavor and oat milk flavors, respectively (Figure 1). The enzyme activity of the natural flavor was higher than that of the oat milk flavor for all treatments, which could result from the initial amount of the enzyme in the product. The result shows that soaking in 0.5% $NaHCO_3$

(NA) could not inactivate the LOX activity. This is probably due to the limited activity of the enzyme and substrate due to soaking whole-grain mung beans. This was possible to reduce NA absorption to grains due to pericarp and less surface area, then loss of ability to inhibit the enzyme [14]. However, the streaming process (ST, NA+ST, ST+PL, and NA+ST+PL treatments) was effective in inhibiting LOX activity accounting for 32.84-44.78% and 69.76-95.70% for natural and oat milk flavors, respectively, compared to those of the control samples. In addition, pre-treatment with $NaHCO_3$ soaking could not enhance the inhibition of LOX activity, when comparing ST, NA+ST, and NA+ST+PL treatments. In addition, results showed that the addition of pandan leaves combined with steaming did not enhance to reduce LOX activity when compared between ST and ST+PL treatment or NA+ST and NA+ST+PL treatments. This could be explained that although phenolic compounds promised to inhibit lipoxygenase enzyme [24] the compounds could be destroyed by heat during steaming. Based on the results of this study, steaming for 10 minutes is an effective method for inhibiting LOX activity in mung bean beverages. Various heat treatments are used in the food industry, primarily to inactivate bacteria and inactivate enzymes that can cause detrimental effects. The denaturation of the enzyme at high temperatures (breaks hydrogen and ionic bonds) leads to a change in the secondary and tertiary structures of the enzymes, resulting in a loss of LOX activity [25]. This finding agrees with the results reported by [2], who found that microwave and conventional thermal processing were sufficient to inactivate the trypsin inhibitor and LOX activities in soymilk.

3.3 Color characteristics

Color parameters of mung bean beverage samples are shown in Table 2. Results showed that the oat milk flavor exhibited higher levels of L^*, b^*, and C^* than the natural flavor ($p \leq 0.05$). According to the product of natural flavor, steaming treatments (ST, NA+ST, ST+PL, and NA+ST+PL) contained significantly lower L^* and h° values than the control ($p \leq 0.05$), while providing higher levels of a^* and b^* than the control ($p \leq 0.05$). The pattern of L^* and b^* values in oat milk flavor was similar to the natural flavor, although the levels of a^*, C^*, and h° of all treatments fluctuated compared to the control. Figure 2 shows the mung bean beverage product. In comparison with the control, steaming treatments produced a dark green color in the natural flavor product. Meanwhile, the oat milk flavor exhibited a bright green color and all steaming treatments produced a similar green color as the control. These could result from oat milk in the product. This result is consistent with the color of the mung bean paste product treated by thermal process [26]. The decreasing L^* value after steaming may be a result of enzymatic browning induced by peroxidase and polyphenol oxidase, which are inhibited at 80°C [27]. Even so, this study was conducted at temperatures greater than 80°C. The probability of enzymatic browning is therefore low. Moreover, the decreasing L^* value may reflect non-enzymatic browning, corresponding to the reaction between sugar and free amino acids catalyzed by heat, which is responsible for the loss of L^*. Meanwhile, the increase in the b^* value is probably because of the heat degradation of other components besides chlorophyll, such as pheophytin [28]. Steaming dissolved chlorophyll ions and replaced their central Mg^{2+} ions with H^+ ions to produce yellow-green pheophytin [29], resulting in a gradual increase in the b^* value. It was found that pretreatment by soaking in 0.5% $NaHCO_3$ (NA treatment) caused a significant loss of L^* for both flavors when compared to the control ($p \leq 0.05$), and combination treatment (NA+ST or NA+ST+PL) induced a dramatic loss of L^*, especially in the natural flavor. In line with this finding, [30] reported that soaking beans in water results in a lighter color due to greater pigment leaching. Furthermore, the addition of pandan leaves to the natural flavor product did not significantly impact L^* and b^* values ($p > 0.05$), when comparing between ST and ST+PL treatment or NA+ST and NA+ST+PL treatments. In contrast, the addition of pandan leaves to the oat milk flavor product caused a loss of L^* and slightly increased b^* values ($p \leq 0.05$), when comparing between ST and ST+PL treatment or NA+ST and NA+ST+PL treatments. This could be due to the presence of yellow-green pheophytin.

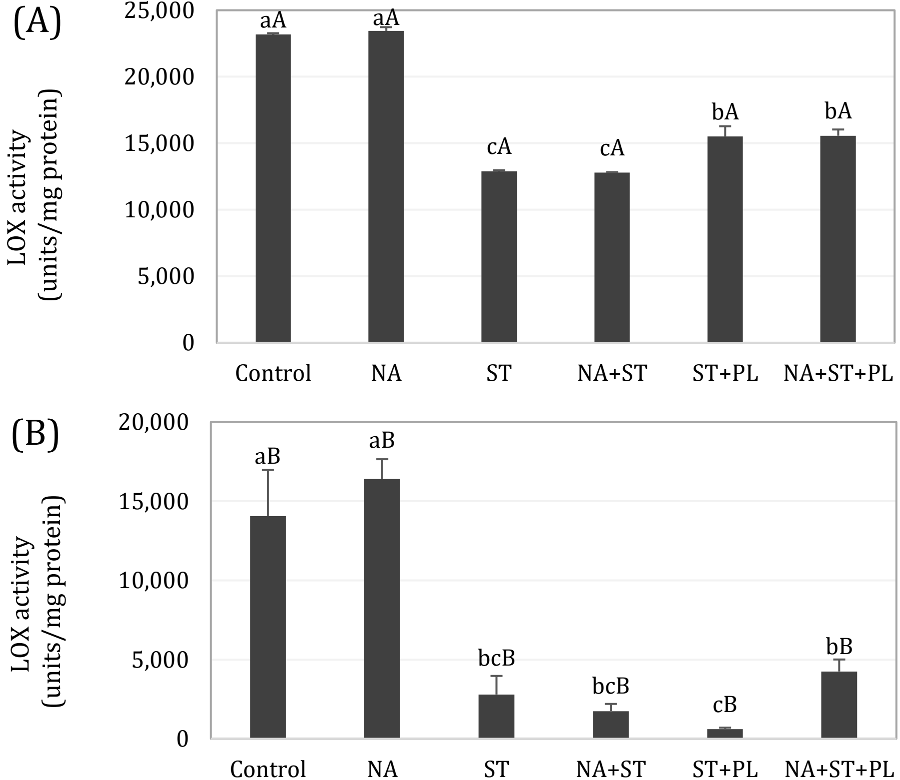

Figure 1. Lipoxygenase activity of the mung bean beverage. Natural flavor (A) and oat milk flavor (B). The data are presented as the means ± standard deviations (n = 3). The different small letters indicate significant differences in the means among the treatments in the same flavor, whereas capital letters indicate significant differences in the means between the product flavors in the same treatment ($p \le 0.05$).

Table 2. Color parameters of the mung bean beverage

Treatment	Lightness (L*)	Redness (a*)	Yellowness (b*)	Chroma (C*)	Hue angle (h°)
Natural flavor					
Control	45.03 ± 0.25aB	-4.93 ± 0.15dB	9.83 ± 0.88bB	11.00 ± 0.82bB	-1.10 ± 0.03aA
NA	42.71 ± 0.06bB	-5.36 ± 0.03eB	11.08 ± 0.09aB	12.31 ± 0.09aB	-1.12 ± 0.00aA
ST	27.13 ± 0.09cB	-3.29 ± 0.18bcA	10.76 ± 0.27aB	11.25 ± 0.31bB	-1.27 ± 0.01bcA
NA+ST	25.05 ± 0.38dB	-3.09 ± 0.09abA	10.67 ± 0.26aB	11.11 ± 0.24bB	-1.29 ± 0.01cdA
ST+PL	26.80 ± 0.34cB	-3.51 ± 0.18cA	10.89 ± 0.29aB	11.44 ± 0.33bB	-1.26 ± 0.01bA
NA+ST+PL	24.65 ± 0.18dB	-2.89 ± 0.13aA	10.96 ± 0.12aB	11.33 ± 0.11bB	-1.31 ± 0.01dA
Oat milk flavor					
Control	62.45 ± 0.04aA	-3.51 ± 0.09bcA	17.37 ± 0.04deA	17.72 ± 0.05cA	-1.37 ± 0.00bB
NA	62.24 ± 0.05abA	-3.76 ± 0.03dA	17.21 ± 0.06eA	17.62 ± 0.06cA	-1.36 ± 0.00aB
ST	61.00 ± 0.26cA	-3.57 ± 0.05cA	17.67 ± 0.13bcA	18.02 ± 0.13bA	-1.37 ± 0.00bB
NA+ST	62.00 ± 0.08bA	-3.22 ± 0.01aA	17.51 ± 0.17cdA	17.80 ± 0.16cA	-1.39 ± 0.00dB
ST+PL	60.11 ± 0.15dA	-3.45 ± 0.06bA	17.87 ± 0.11abA	18.20 ± 0.11abA	-1.38 ± 0.00cB
NA+ST+PL	60.11 ± 0.11dA	-3.84 ± 0.06dB	17.91 ± 0.16aA	18.32 ± 0.16aA	-1.36 ± 0.00aB

* The data are presented as the means ± standard deviations (n = 3). The different small letters indicate significant differences in the means among the treatments in the same flavor, whereas capital letters indicate significant differences in the means between the product flavors in the same treatment ($p≤0.05$).

Figure 2. The mung bean beverage: natural flavor (A) and oat milk flavor (B)

3.4 Total phenolic content and antioxidant activity

Table 3 presents the content of total phenolics and antioxidant activity measured by DPPH and ABTS assays. Data were expressed as the result of the control and two favorable treatments in the sensory tests (data not shown) in each of the product flavors, which were both steam-treated. As shown in Table 3, higher levels of TPC and antioxidant activity in oat milk flavor were observed. This was probably due to oat bioactive phytochemicals (β-glucans, phenolic compounds, and phytic acid) [31]. Loss of total phenolic content and antioxidant activity for both flavors was found. According to the natural flavor, the NA+ST+PL treatment contained higher TPC and antioxidant activity measured by DPPH and ABTS assays than the ST treatment ($p≤0.05$). These could be due to the release of phytochemicals from the pandan leaves added to the process. There was a 27.27, 4.55, and 22.05% loss of TPC, DPPH, and ABTS activity in NA+ST+PL treatment, respectively. Meanwhile, the NA+ST+PL treatment of the oat milk flavor also contained higher TPC and antioxidant activity measured by DPPH and ABTS assays than the ST+PL treatment. It was found that the loss of TPC, DPPH, and ABTS activities of the oat milk flavor in NA+ST+PL treatment accounted for 20.88, 4.44, and 16.40%, respectively. Loss of phenolics and antioxidant activity in plant-based beverages could be induced by thermal degradation of polyphenols [32].

Table 3. Phenolic and antioxidant activity of the mung bean beverage

Treatment	TPC (mg GAE/mL)	DPPH (µmol TE/mL)	ABTS (µmol TE/mL)
Natural flavor			
Control	0.55 ± 0.02aB	0.44 ± 0.00aA	11.88 ± 0.43aB
ST	0.32 ± 0.02cB	0.39 ± 0.01cB	7.63 ± 0.55cB
NA+ST+PL	0.40 ± 0.02bB	0.42 ± 0.01bA	9.26 ± 0.39bB
Oat milk flavor			
Control	0.91 ± 0.12aA	0.45 ± 0.00aA	14.21 ± 0.79aA
ST+PL	0.63 ± 0.06bA	0.42 ± 0.00bA	10.28 ± 0.32cA
NA+ST+PL	0.72 ± 0.06bA	0.43 ± 0.01bA	11.88 ± 0.28bA

*TPC, total phenolic content; DPPH, 1,1-Diphenyl-2-picrylhydrazyl free-radical scavenging assay; ABTS, 2,2'-azino-bis(3-ethylbenzthiaz-oline-6-sulfonic acid) free-radical scavenging assay. The data are presented as the means ± standard deviations (n = 3). The different small letters indicate significant differences in the means among the treatments in the same flavor, whereas capital letters indicate significant differences in the means between the product flavors in the same treatment (treatment of ST was compared with ST+PL) ($p \leq 0.05$).

4 CONCLUSIONS

Processing treatment and formulation of mung bean beverages (natural and oat milk flavors) affected LOX activity, color, TPC, and antioxidant activity. Results revealed that mung bean LOX was inactivated effectively by steaming (ST, NA+ST, ST+PL, and NA+ST+PL), while soaking in 0.5% $NaHCO_3$ could not significantly affect enzyme inhibition. The results suggested that ST treatment is practical and effective to inactivate LOX activity in mung bean beverages. In contrast, a thermal process caused color changes and loss of TPC and antioxidant activity. However, adding pandanus leaves (NA+ST+PL treatment) could enhance the level of phenolics and antioxidant properties of the natural flavor product ($p \leq 0.05$) when compared to ST treatment. This study shows the applicability of conventional processing for removing beany flavor and improving bioactive compounds in the development of mung bean beverages. Nonetheless, consumer acceptability studies and improvements in product color and stability during storage are worth further study.

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EFFECT OF MICROWAVE AND XANTHAN GUM ON FUNCTIONAL PROPERTIES OF DUCK EGG WHITE POWDER

AP-P157

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Keywords

Microwave, Xanthan gum, Egg white powder, Foaming properties, Protein

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ABSTRACT

The application of industrial egg white powder has been limited due to its relatively low functional properties. However, microwave treatment is a novel and efficient way to improve the functionality of egg white powder. The primary emphasis of this study was the functional properties of duck egg white powder (DEWP) treated with microwaves in the presence of xanthan gum. DEWP treated with different microwave energy levels (0 watts (control), 8 minutes at 300 watts and 4 minutes at 600 watts) and xanthan gum at three different levels (0%, 0.5%, 1%) were combined in an experiment to study the functional properties of egg white powder. The solubility, viscosity, foaming capacity, foaming stability and the optic image of DEWP were investigated. The microwave-treated DEWP in the presence of xanthan gum showed higher solubility, viscosity, foaming capacity and foaming stability than untreated DEWP. The results of the optic imaging showed smaller bubbles. The foaming capacity of DEWP after microwave treatment was increased from 3.12 L/mg to 4.82 L/mg in the presence of xanthan gum. This study highlighted the benefits of the application of microwave treatment plus the incorporation of xanthan gum in the functional modification of proteins.

1. INTRODUCTION

Poultry eggs are high in nutrients and proteins that are essential for human health, in addition to their great functional features such as water holding capacity, gelation, foaming and emulsion [1]. Duck eggs are the second most popular food after chicken eggs. However, egg white has limitations in terms of taste, smell, color and texture, meaning that many people would prefer not to consume it. Duck eggs are identical to chicken eggs in term of their structure and chemical makeup and duck egg whites are also highly nutritious and contain the same essential amino acids found in chicken egg whites. Raw materials used in agriculture may gain value and this may also encourage consumers to eat more duck eggs [2].

Egg whites are a very nourishing food and are frequently utilied in culinary products that need to be fluffy, including bakery goods, because they also foam well [3]. However, the shelf life of liquid egg whites is limited. Long-distance transportation is rendered impossible as a result, which poses a challenge for the food business [4]. Egg whites that are liquid have therefore been converted into a dry powder in an effort to decrease these restrictions and increase the likelihood of

them being used more often. This leads to more profitable egg white production. Spray-drying is a common technique used in the production of egg white powder due to the brief duration and low power usage [5]. The functional characteristics of egg white protein powder that cause foaming are reduced
by this spray-drying process due to the usage of high temperatures, which partially destroys the natural conditions [6]. Freeze-drying technology has also been used in the production of egg white powder. The food's quality can be preserved, but it takes a long time, costs a lot and uses a lot of energy [7]. However, compared to spray-drying techniques, it has a better influence on foam production and foam stability [8].

Microwave cooking is a simple and safe physical approach as there is an electromagnetic heating mechanism in microwave technology [9]. Wang et al. [10] reported that using microwaves can enhance foaming and adsorption at the interface of the liquid and gaseous phases. It was discovered that egg white powder, when microwaved for 6 minutes at 440 watts, develops a structure similar to that of natural proteins, providing flexibility and aiding in foam formation. It promotes rapid diffusion and adsorption between the gas and liquid phases, which is advantageous for foam formation. The ability of egg white protein to foam can be increased by changing the structure of the egg white powder in a moderate microwave.

Polysaccharides are a simple way to increase the viscosity of liquids and change the properties of proteins. Anionic polysaccharides are produced by the bacteria *Xanthomonas campestris*. Studies have investigated the usage of xanthan gum, which has a high molecular weight of 1–50 x 10^6 g per mol and is very viscous, to enhance the foaming qualities of egg white powder as this creates a strong wall of air bubbles that may contribute to the stability of the foam [8].

Thus, the goal of this study is to investigate how to employ xanthan gum hydrocolloid in conjunction with microwave technology and freeze-drying duck eggs to improve the foam formation of egg white protein powder for the purpose of assessing and determining the optimal conditions.

2. MATERIAL AND METHODS

2.1 Material

Duck egg was purchased from CP Freshmart.

2.2 Duck egg white powder preparation

This recipe, which was modified from the method used by Katekhong and Charoenrein [11] calls for separating the egg whites from the yolks and slurry stirring to guarantee that the egg white sample is consistent and to separate the egg white terminal (Chalazae). After that, the egg white protein samples were put into a freeze drier and the powdered egg white protein was kept at 25°C in a foil bag. Duck egg white power was called DEWP.

2.3 Microwave and xanthan gum of duck egg white powder

This recipe, which was modified from Wang et al. [10] and Li et al. [8] Put 20 g of egg white protein powder on a plate, heat it for 8 minutes at 0 watts, 8 minutes at 300 watts, and 4 minutes at 600 watts. Then, add 0.5, 1%, and 0% of the egg white protein powder's mass to the xanthan gum.

2.4 Solubility

The biurate technique Morr et al. [12] was used to evaluate all samples with varying microwave levels and xanthan gum amounts. Samples of egg white powder were taken and distilled water was added. The solution was then concentrateto 14% per weight by mass and the samples were stirred at 250 rpm for 30 minutes. Then, 30-minute-old pre-centrifugal solution samples were reacted with biurate at 540 nm. Furthermore, the solubility was calculated by contrasting it with the typical graph created using serum ovalbumin solution:

Solubility (%) = (Protein content in supernatant/The total protein content) *100

2.5 Foaming

The egg white powder's foaming was determined using the microwave technique in conjunction with xanthan gum, as outlined by the method of Wang et al. [13] After adding a sample of egg white powder to distilled water, the mixture was concentrated to 14% of the mass by weight. It was then stirred for 30 minutes at 250 rpm and beaten for 3 minutes at a speed of 5 with a mixer to create the foam. The measuring cylinder was filled with foam by the spoonful and the foam was weighed to calculate its volume. The stability of the foam was also assessed; after 30 minutes, the amount of foam was measured and the formulated foam stability and foam formation capacity was calculated.

$$FC = V_1/m$$

$$FS\ (\%) = V_2/V_1$$

2.5 Viscosity

Distilled water was added to a sample of powdered egg white, resulting in a solution with a 50% concentration per weight by mass. After being left for half an hour, the Brookfield 42-millimeter probe size was used to measure the volume of a 1 milliliter sample at room temperature, beginning with a shear rate of 38.4-107.5 1/s.

2.6 Optic image

After adding a sample of egg white powder to distilled water, the mixture is concentrated to 14% of the mass by weight. It is stirred for 30 minutes at 250 rpm and then beaten for 3 minutes at a speed of 5 with a mixer to create foam. The bubbles were spread on a slide, magnified 40 x under a microscope and their sizes were measured at random.

2.7 Statistical analysis

Statistical analysis was carried out using IBM SPSS statistic (version 25) and the results were finally expressed in the form of mean ± Standard deviation. The least significant difference (Duncan) test at a confidence interval of 95% was further used to compare the means.

3. RESULTS AND DISCUSSION

3.1 Solubility

The solubility characteristics of proteins serve as markers for the degradation of proteins. Figure 1 illustrates the egg white protein's solubility. The protein in egg whites became much more soluble after 8 minutes of a 300-watts microwave treatment. Wang et al. [10] claimed that the accelerated melting from microwaves is caused by the influence of heat-induced electromagnetic fields. The protein therefore becomes considerably longer than it was when it was spherical and exhibits more hydrophilic groups. 4 minutes at 600 watts of microwave radiation significantly reduces thawing. The protein therefore becomes extensively denatured, loosens linearly and generates a hydrophobic reaction. Aggregation and precipitation also occur. However, studies where xanthan gum was added at 0.5% and 1% with an increase in solubility and microwave use at levels of 300 watts for 8 minutes and 600 watts for 4 minutes might greatly enhance protein solubility, in accordance with the experiment of Chang et al. [14]. The functional qualities of proteins are enhanced when glycosylation processes are induced using microwaves. As xanthan gum is a negatively-charged polysaccharide, using microwaves can therefore make egg white proteins more soluble [15]. As a result, the protein becomes more widely distributed and more soluble.

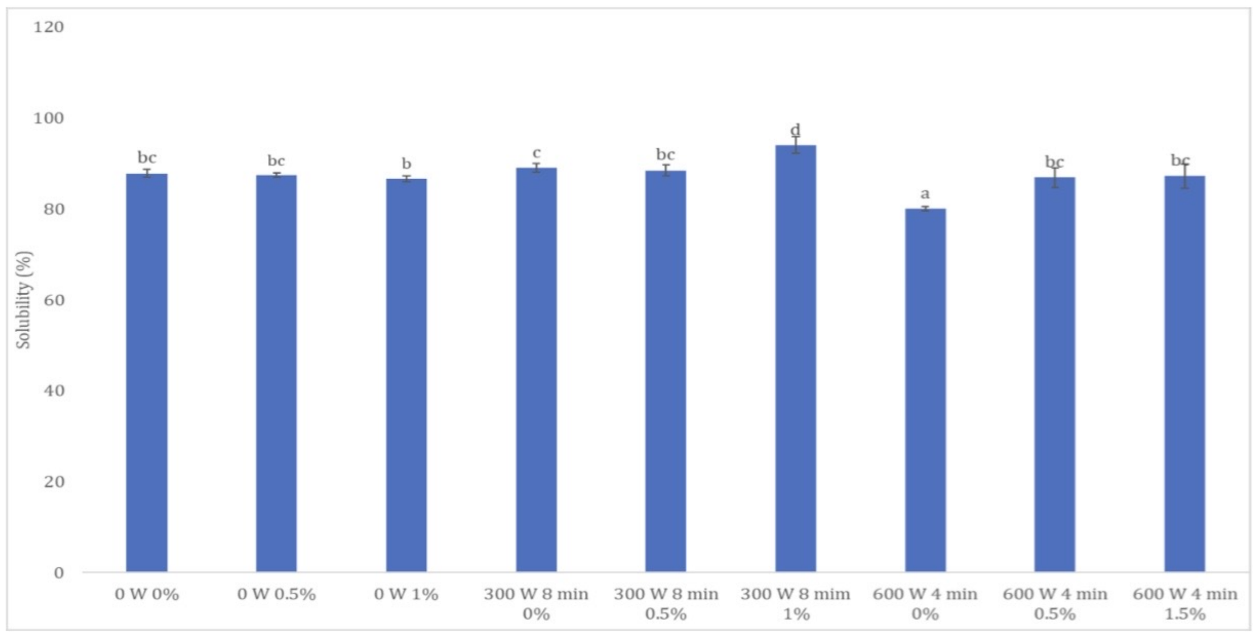

Figure 1. The experiment involved varying the quantities of microwaves and xanthan gum to test the solubility of egg white powder. The average result is shown ± standard deviations. A statistically significant difference of 0.05 between various samples is represented by different letters (a-d).

3.2. Foam capacity

Properties of viscosity are linked to the capacity to generate foams. The solubility is shown in Figure 2. Following tests at 0 watts, 300 watts for 8 minutes and 600 watts for 4 minutes in conjunction with 0.5% and 1% xanthan gum, the foaming capability of egg white powder rose dramatically. However, there was not enough viscosity to prevent proteins from moving to the continuous phase. The negative charge of xanthan gum may cause the surface charge of the protein molecule to increase and proteins do not combine as a result. This makes a positive difference in the shift to the continuous phase. Microwave treatment for 8 minutes at 300 watts and 4 minutes at 600 watts has a statistically significant impact on the potential for foam capacity growth. Li et al. [16] state that studies have been conducted on the enhancement of egg white protein content with the use of microwaves in conjunction with the addition of a phosphate group. Microwave radiation causes spherical proteins to become less rigid; because of its increased elasticity, air bubbles may be swiftly encircled [17].

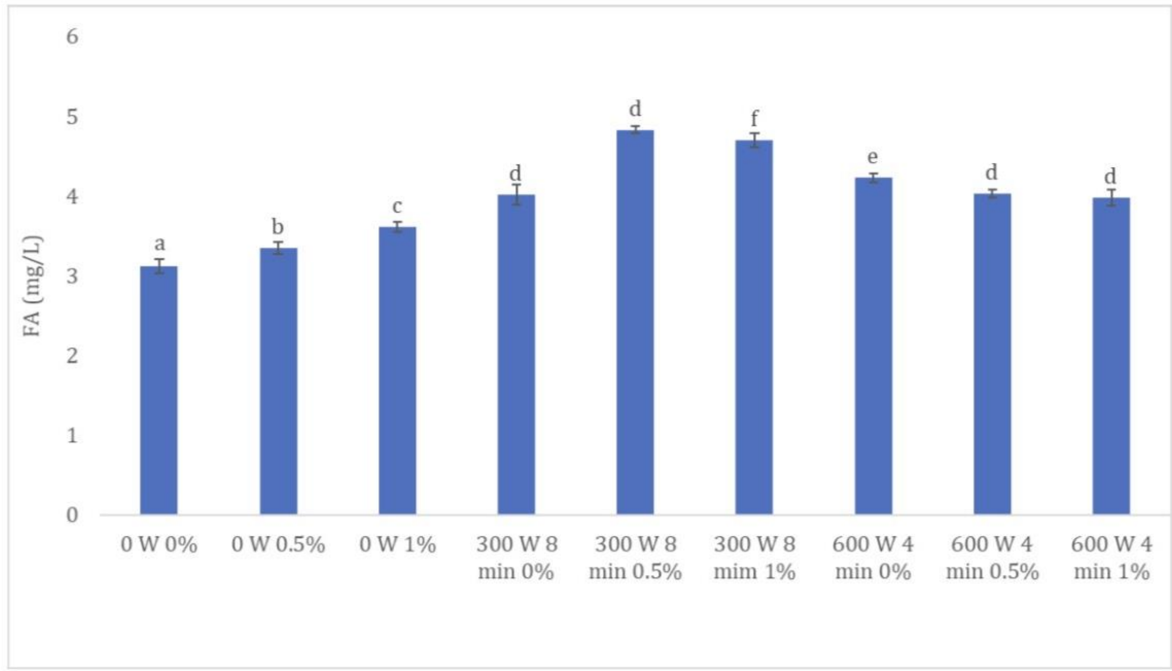

Figure 2. The experiment involved varying the quantities of microwaves and xanthan gum to test the foam capacity of egg white powder. The average result is shown ± standard deviations. A statistically significant difference of 0.05 between various samples is represented by different letters (a-f).

3.3 Foam Stability

As can be seen in Figure 3, viscosity in the system increases with the addition of xanthan gum and microwave radiation, hence influencing the stability of the foam. Consequently, compared to the egg whites that were not microwave-tested, the bubbles exhibited greater stability and the xanthan gum addition reached statistical significance. This translates to a reduced bubble structure, as shown in Figure 5, and the system's high viscosity shown in Figure 4 could be the result of heat, electromagnetic radiation from microwaves and xanthan gums. According to Li et al. [8] xanthan gum can be used to improve foam stability, connecting the continuous phase of water and air into a robust structure. The viscosity will prevent the air bubbles from collapsing, consequently increasing the foam's stability [18].

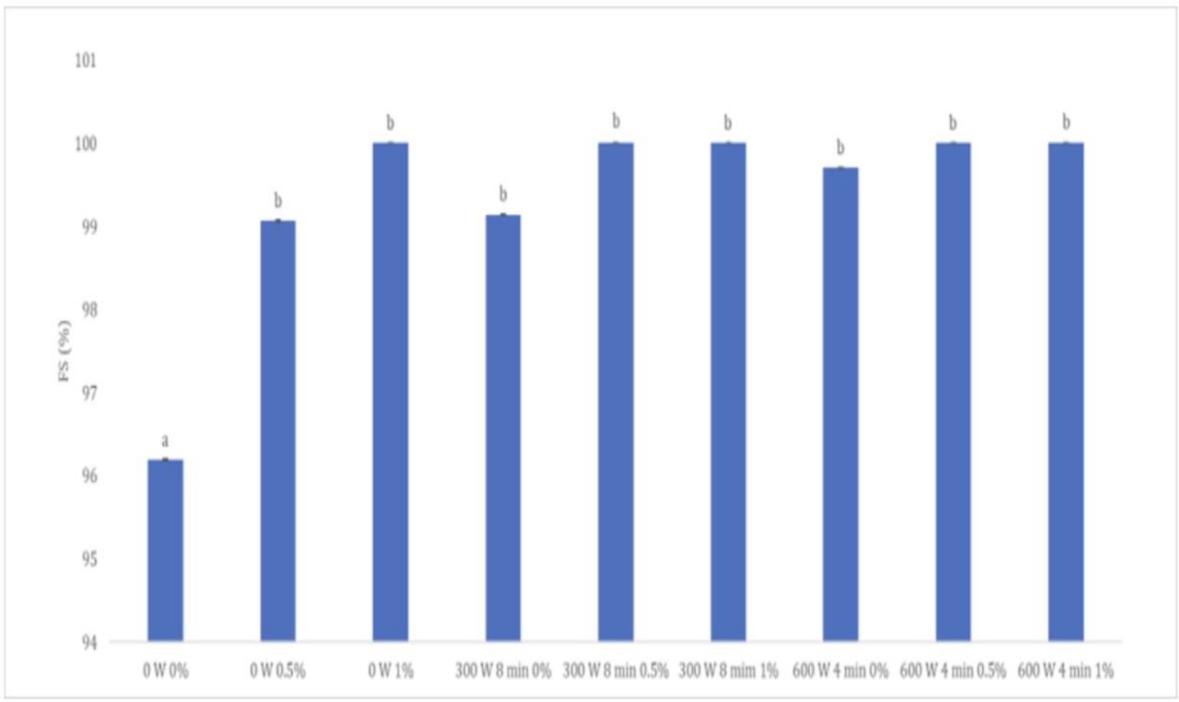

Figure 3. The experiment involved varying the quantities of microwaves and xanthan gum to test the foam stability of egg white powder. The average result is shown ± standard deviations. A statistically significant difference of 0.05 between various samples is represented by different letters (a-b).

3.4 Viscosity

Various microwave and xanthan gum studies have shown ways to make the egg white powder solution system more viscous, as seen in Figure 4, where a distinct shear rate is obtained from the egg white solution. Consequently, when the shear rate increases, the egg white solution's viscosity falls. Wang et al. [10] indicated that the increase in viscosity following microwave studies might be attributed to spherical protein molecules in their native condition, when proteins are denatured by low heat, such as that created by microwave radiation. Consequently, proteins with a spherical structure only slightly denature. As a result, the protein is distributed throughout different fluid layers and the molecules might be connected in different fluid layers. NsenGiyumva and Alexandridis [19] describe xanthan gum as a high molar mass polysaccharide. This is consistent with the findings of Martínez-Padilla's [18] investigation into

the stability of foams containing xanthan gum and whey protein. Conversely, viscosity rose after microwave treatment at 600 watts for 8 minutes as opposed to the same period when 0.5% and 1% xanthan gum was added. This might be because the more denatured form of the protein has less interaction with xanthan gum. Moreover, viscosity tends to decrease from its initial value as shear force increases. The shear force rupturing the protein chains holding the system together causes a reduction in viscosity.

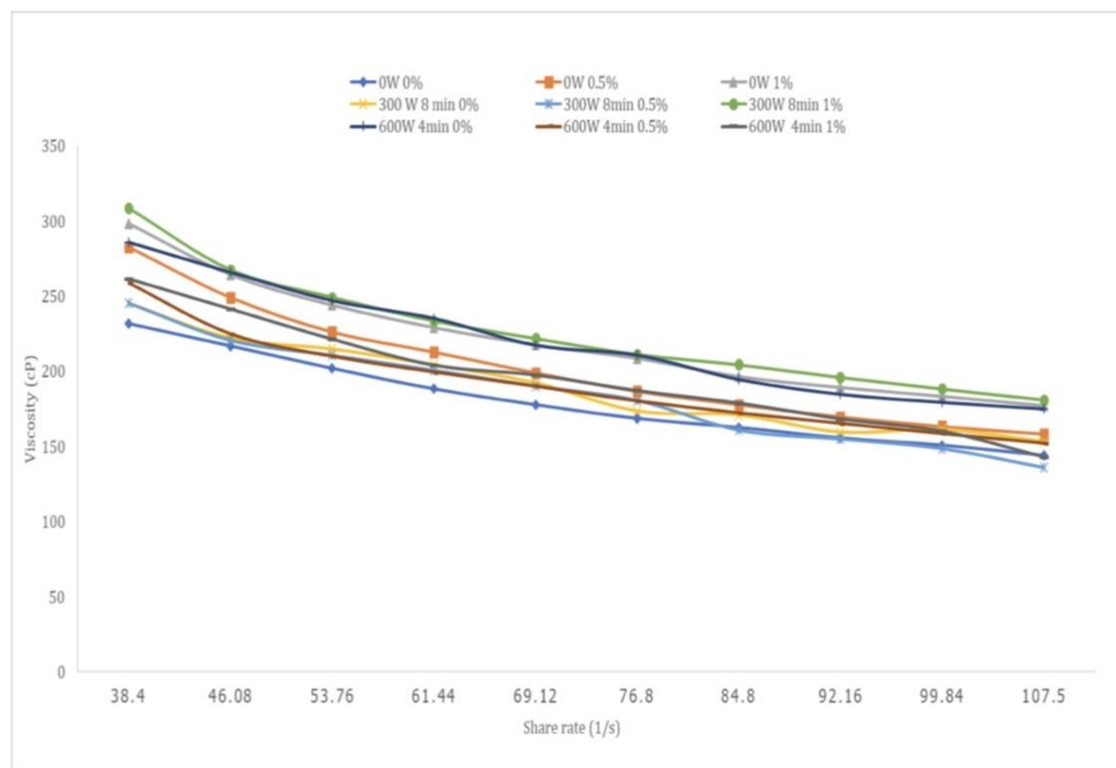

Figure 4. The experiment involved varying the quantities of microwaves and xanthan gum to test the viscosity of egg white powder; the findings revealed a mean (n=3).

3.5 Microstructure of foaming

Figure 5 shows that following the microwave and xanthan gum trials, the bubble size was lower than the sample at 0 watts of 0% xanthan gum. Smaller bubble sizes have a favourable effect on foam production and foam stability; the size of the bubbles is connected to these two concepts. The findings of research by Wang et al. [10] showed that the usage of microwaves led to reduced bubble sizes; similarly, trials utilising moderate microwave levels produced smaller bubble sizes than non-microwave experiments. In contrast to not adding xanthan gum, the experiment which involved adding xanthan gum resulted in an increase in viscosity, which decreased the size of the bubbles [8]. Zhang et al. [20] suggest that substantial air conditions may lead to aggregation as the modest size of the bubbles can slow down both the air bubble bursting and the foam collapse.

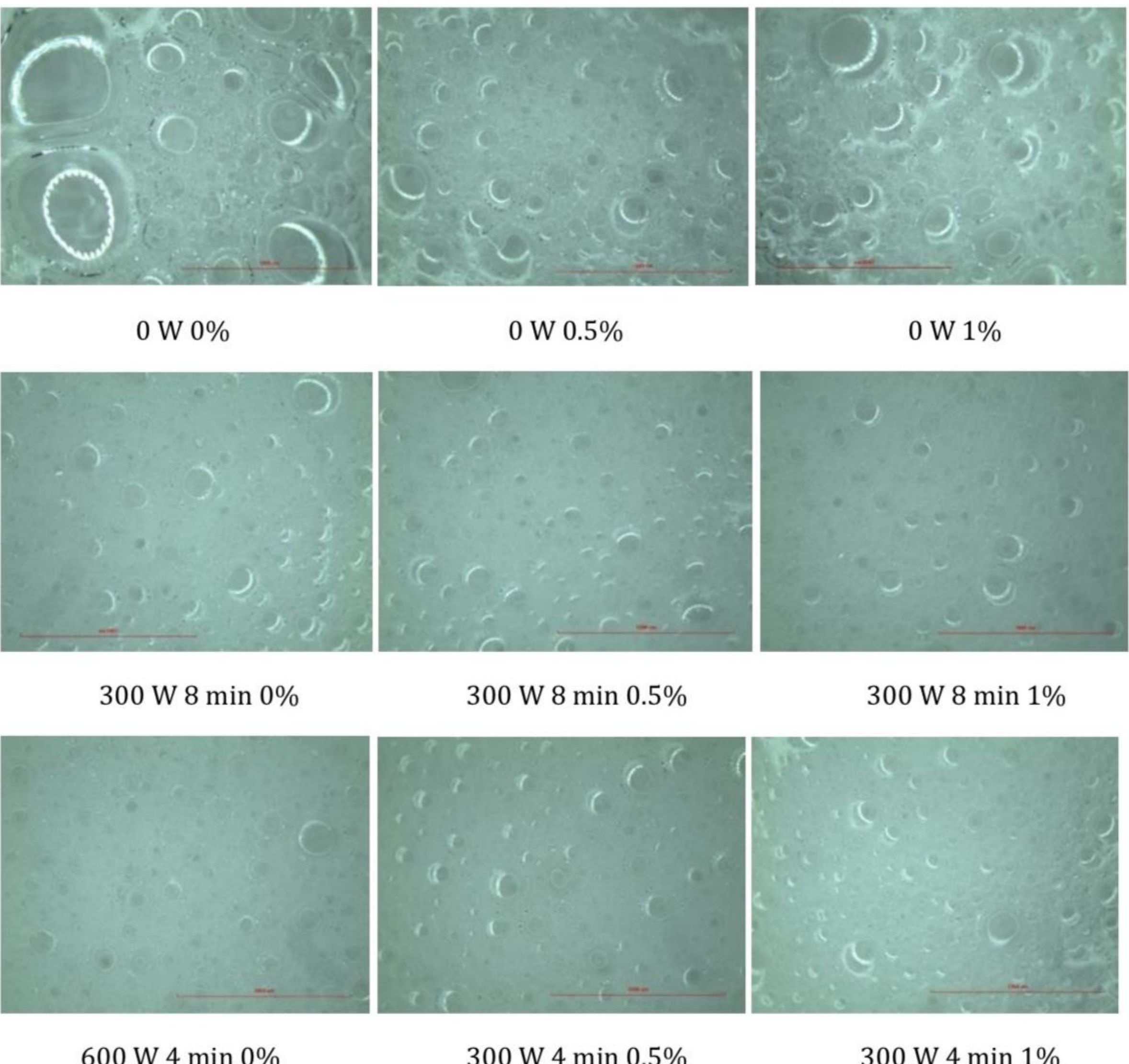

Figure 5. Egg white powder bubble microstructure (40x) experimented with at varying microwave and xanthan gum concentrations.

4. CONCLUSIONS

When using xanthan gum and microwave treatments, the native protein found in egg whites may be made more functional and flexible as the proteins will be partially denatured by microwave radiation, negatively charged xanthan gum and high molecular weight. In the continuous phase, this leads the gas bubbles to move and surround the protein, thus affecting the properties of the egg white, resulting in increased viscosity and foam production. This causes the outer wall of the bubble to thicken, which changes the stability of the foam compared to duck egg powder and xanthan gum samples that were tested in a microwave.

5. ACKNOWLEDGEMENTS

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Characteristics and nutrition values of cereal bar fortified with Asian sea bass bone bio-calcium powder.

AP-P171

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Keywords

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Asian sea bass bone
Cereal bar
Calcium fortification

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ABSTRACT

Bio-calcium powders were extracted from Asian sea bass bone by heat-treated alkaline with fat removal and bleaching supplementary method. Cereal bars (CBs) were fortified with produced bio-calcium at 3 levels: (1) increased calcium (IS-Ca; calcium ≥10% Thai RDI), (2) good source of calcium (GS-Ca; calcium ≥15% Thai RDI), and (3) high calcium (H-Ca; calcium ≥30% Thai RDI) which were consistent with the notification of the Ministry of Public Health, Thailand: No. 445; Nutrition claim issued in B.E. 2023. Moisture content, water activity, color, calcium content and FTIR analysis of bio-calcium powders were measured. Dimension, color, water activity, pH and texture of fortified CBs were determined. Produced bio-calcium could be classified as a dried food with light yellow-white color. Calcium contents in bio-calcium powder was 23.4% (w/w). Dimension, weight and color except b* and ΔE* values of fortified CBs were not different ($P > 0.05$) from those of the control. Fortifying of bio-calcium resulted in harder texture CBs. An increase of fortified bio-calcium amounts decreased carbohydrate and fat but increased of protein, ash and calcium in the fortified CBs. Shelf life of CBs was to be shorten by fortification of bio-calcium powder because of the increment of moisture, water activity and pH. Yield of bio-calcium production was 40.30%. Production cost of bio-calcium was approximately 7,416 Bth/kg while cost of fortified CBs increased almost 2-3 times compared to the control. Calcium contents in IS-Ca (921.12 mg/100g), GS-Ca (1,287.10 mg/100g) and H-Ca (2,639.70 mg/100g) cereal bars could be claimed as increased calcium, good source of calcium and high calcium, respectively. In conclusion, production of cereal bar fortified with Asian sea bass bone bio-calcium powder as a fortified food was possible. However, checking the remained hazardous reagents in bio-calcium powder must be carried out before using in food products and analysis of calcium bioavailability, sensory acceptance and shelf life of the developed products should be determined in further studies.

1. INTRODUCTION

Cereal bars (CBs) are considered convenient and popular foods and play a role in response to health and natural perception for consumers (1). The global CBs market has been becoming gradually increase and spreading from America and Europe to Asia-Pacific region including Thailand. [1–5]. Generally, CBs are bar-shaped food products, made by pressing mixture of cereals and dried fruits which are held together by binder ingredients, cut, and shaped in the form of a bar [1]. The preparation of CBs can be broadly divided into two methods: (i) Hot process and (ii) Cold process. In hot or oven process of CBs making all ingredient and baked whereas in cold process all the ingredients are made into bar shape after mixing then stored in cool temperature. As nutritional viewpoint, CBs may be classified into four types: fibrous, energy, diet (light), and protein bars [2]. Physicochemical and nutritional qualities of CBs are varied by a wide variety of ingredients that the bars made from. The benefit of CBs is proposed as a great source of fiber, sufficient protein, fat, and carbohydrate especially high sugars that the bars become a quality source of energy. Nevertheless, CBs are rarely considered as a good choice for a source of minerals such as calcium and phosphorus for health-conscious consumers suggesting by limitation of reports in market analysis and current trends of CBs [3-5]. In Thailand, CBs products are categorized in a group of desserts that defined 30 grams for their serving size [6] and recently accepted as alternative snack products by various age consumers especially officers in urban area and sport persons.

Asian sea bass (*Lates calcarifer*) is native species cultured in the coastal area of Thailand. The cultured Asian sea bass has been broadened mainly to the Central and Eastern areas of Thailand and is mostly marketed in form of frozen fish and fillets to consuming in the country and exporting abroad. According to data in 2021, the initial production of Asian sea bass from aquaculture is 49,060.2 tons and the expected production in 2022 is 53,725.5 tons, with an increase of 9.5 percent from the previous year [7]. Recently, fish bones plenty obtained from fish industry waste known as a by-product has gained interest for bio-calcium production which is used as an alternative raw material in the production of calcium supplements to create value-added food products because they contain high calcium and phosphorus [8-10]. Fish bones generally contain high calcium and phosphorus, depending on the species, and comprise several minerals e.g. iron, magnesium, copper, zinc, and others (8). The calcium in the fishbone is in the form of calcium phosphate similarly to the composition of human bones and teeth [10].

Fortification of minerals and vitamins in foods is commonly accepted for daily intake [11]. With an increasing of healthy product demands in markets, several fortified food products with health-promoting ingredients have been developed including egg-shell bio-calcium supplemented biscuit [12], bio-calcium Asian sea bass enriched bread [10] and tuna bone bio-calcium fortified cracker [13]. According to cereal bars are on-to-go and ready-to-eat products which is a good vehicle for healthy food products whereas utilization of fish bone calcium can reduce environmental problems and increase the value of these by-products [9]. Calcium fortification in cereal bars with bio-calcium Asian sea bass is not only improving CBs nutritional quality but also enhancing fishery waste value. Nevertheless, fish bone bio-calcium fortification might contribute to characteristics and quality of cereal bars. This study was aimed to develop cereal bar fortified with Asian sea bass bone bio-calcium powders in three levels of calcium contents (1) increased calcium (calcium ≥10% Thai RDI), (2) good source of calcium (calcium ≥15% Thai RDI), and (3) high calcium (calcium ≥30% Thai RDI) which were consistent with the notification of the Ministry of Public Health, Thailand: No. 445; Nutrition claim issued in B.E. 2023 [6]. Characteristics and chemical compositions of fortifying products were determined. Costs of fortified bio-calcium CBs production in a laboratory scale was calculated to evaluate possibility of commercial production.

2. MATERIAL AND METHODS

2.1 Material

Cereals including oats (McGerrett Brand, Australian Oats, corn flakes (Thanyathippattana Co., Ltd, Bangkok, Thailand.), almonds (Heritage Brand, Heritage Snacks & Food Co., Ltd.) were purchased from supermarkets in Bangkok, Thailand. Sweeteners (sorbitol syrup and maltitol syrup), maltodextrin and tricalcium phosphate ($Ca_3(PO_4)_2$) were ordered from Krungthepchemi, Bangkok, Thailand. Sunflower oil (Naturel Brand, Lam Soon (Thailand) Public Co., Ltd.) and sunflower lecithin (NNP Foods Supply Co., Ltd.) were purchased from bakery shops in Bangkok, Thailand. Sea bass bonds were provided by Khaopong Farm Co., LTD., Chachoengsao, Thailand.

2.2 Preparation of Asian sea bass bone bio-calcium powder

Asian sea bass bone bio-calcium powder was prepared by heat-treated alkaline with fat removal and bleaching supplementary method [14]. Firstly, Asian sea bass bones were boiled in boiling water and washed with clean water to remove flesh, blood and contaminants. Then, the fish bones were soaked in 2M sodium hydroxide (NaOH) with a bone to solvent ratio 1:10 (w/v) at 50°C for 30 min. To remove proteins the fish bones were washed with clean water again, then dried at 50°C for 12 hr in a hot air oven and crushed to reduce the sizes of fish bones using a food grinder. After that, crushed bones were soaked in 99% hexane with a bone to solvent ratio 1:10 (w/v) and stirred continuously for 60 min to remove fats. The hexane was drained out and placed the crush bones overnight at room temperature to evaporate hexane. Next, dried crush fish bones were soaked in 2.5% (v/v) sodium hypochlorite (NaClO) for 30 min for bleaching and then immersed in 2.5% (v/v) hydrogen peroxide (H_2O_2) for 60 min. After that the fish bones were cleaned with DI water for 10 min and dried again at 50°C for 12 hr in a hot air oven. Finally, the dried fish bones were ground with a pin mill to get Asian sea bass bone bio-calcium powder. Calcium contents in the bio-calcium powder was analysed by an In-house method TM-CH-105 based on AOAC (2019); 984.27 [15] (AMARC, Bangkok, Thailand) measuring calcium contents by an inductively coupled plasma-optical emission spectrometer. Moisture content and color of the bone powder were determined by a hot air oven method (AOAC, 2000) [16] and the CIE system (Konica Minolta CR-400), respectively. Bio-calcium powders were subjected to Fourier Transform Infrared Spectroscopy (FTIR) using the ATR-FTIR model in accordance with the protocol of co-adding 64 scans at 400–4000 cm^{-1} with step resolutions of 4 cm^{-1} to record the spectral value of the sample using OPUS 3.0 software (Bruker, Ettlingen, Germany).

2.3 Preparation of cereal bars fortified with Asian sea bass bone bio-calcium powder

A controlled formulation of cereal bars was modified from Souiy et al., 2022 [17] which were composed of 15% coarse baked oats, 15% finel ground baked oats, 10% cereal corn flakes, 5% raw and natural medium diced almonds, 11% sorbitol syrup, 40% maltitol syrup, 1% sunflower oil, 0.5% sunflower lecithin power and 2.5% maltodextrin. Cereal bars were prepared by a hot process making by mixing dried ingredients (coarse baked oats, finely ground baked oats, cereal corn flakes, raw and natural medium diced almonds) together, holding the dried mixture with the mixture of binder ingredients (sorbitol, maltitol, maltodextrin, sunflower, lecithin, sunflower oil), moulding in a stainless tray (40 mm width x 50 mm length x 15 mm thickness) and baking in an oven at 150°C for 25 min. To fortified bio-calcium powder in cereal bars, regarding to the notification of the Ministry of Public Health, Thailand: No. 445 Nutrition claim issued in B.E. 2023 [6], Asian sea bass bone bio-calcium powder was added in three levels of calcium contents: 1) high calcium level contained calcium not less than 30% of the Thai RDIs (10.33 g bio-calcium powder/100 g material), 2) good source of calcium level contained calcium not less than 15% of the Thai RDIs (5.33 g bio-calcium powder /100 g material), 3) increased calcium contained calcium not less than 10% of the Thai RDIs

(3.67 g bio-calcium powder /100 g material) in comparison with reference foods. The Thai Reference Daily Intakes (Thai RDIs) of calcium is 1000 mg [6]. After baking and cooling at room temperature, CBs were packed and stored in an airtight container at room temperature for less than 1 day before their quality determinations.

2.4 Determination of characteristics and chemical compositions of cereal bars

2.4.1 Dimension and weight

Sizes of cereal bars (CBs) were measured in width (mm), length (mm) and thickness (mm) by a vernier caliper. Weights of CBs were determined by a digital balance of 4 digits.

2.4.2 Color

Colors of CBs were measured by using a Konica Minolta CR-400 colorimeter reported values in the CIE system showed in lightness (L*), redness/greenness (a*) and yellowness/blueness (b*) values. Total difference in color (ΔE*) was measured as per the following equation:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

where ΔL∗, Δa∗ and Δb∗ are the differences between the corresponding color parameters of the sample and that of controlled CBs.

2.4.3 Moisture content, Water activity and pH

Moisture content in CBs was determined by a hot air oven method (AOAC, 2000) [16]. Water activity and pH values of CBs were determined by a water activity meter (4TEV, Aqualab, Pullman, WA, USA) and a pH meter (SevenCompact S220, METTLER TOLEDO, Switzerland), respectively.

2.4.4 Carbohydrate, fat, protein, ash and calcium contents

CBs were determined in total carbohydrate, total fat, protein, ash, and calcium contents at AMARC, Bangkok, Thailand. Total carbohydrate included fiber was determined by the method of Darryl M. Sullivan & Donald E. Carpenter [18]. Total fat and ash were determined by AOAC (2019) method [15]. Protein and calcium were determined by and In-house method TM-CH-017 based on AOAC (2019) 992.23 and In-house method TM-CH-105 based on AOAC (2019), 984.27, respectively.

2.4.5 Texture

Texture qualities of CBs were determined by a texture analyzer (TA.XTPlus Texture Analyzer, Stable Micro Systems, UK) and carried out by A three Point Bend Rig (HDP/3PB) probe reporting in fractureability, hardness, crispness and stickiness. Testing speed was set at 10 mm/s.

2.5 Statistical analysis

Experiments were carried out by using completely randomized design (CRD). Data were presented as Mean ± Standard Deviation (SD) and statistically assessed by One-way analysis of variance (ANOVA). Differences among samples were examined by Tukey's test was used at a significance level of 0.05 using the Statistical Package for Social Science (IBM SPSS Statistics,IBM, New York, USA).

3. RESULTS AND DISCUSSION

3.1 Characteristics of Asian sea bass bio-calcium powder

Bio-calcium powders produced from Asian sea bass bones was presented in Fig. 1a. The powder contained very low moisture (7.93±1.01%) and low water activity (0.45±0.01) which could be classified in a dried food groups which was difficult in microorganism growth. It showed a light

yellow-white powder with 95.07±3.83, -0.26 ± 0.05, 12.13 ± 0.53 of L*, a* and b* values, respectively, similarly to bio-calcium produced from Asian sea bass scales [20] and bones fortified in bread [10]. The brightness was caused by bleaching in the production [8, 21] indicating that the pigments in fish bones might be oxidized or destroyed by NaOCl or H2O2. Decomposition of both agents results in the oxidizing agent formation, which is able to destroy chromatophore. OH• is a strong nucleophile with the potential in breaking bonds of chromatophores. Thus, chromatophores were destroyed or could not absorb the visible light (22). while the light yellow was probably resulted from non-enzymatic browning reaction occurred by remained protein in fish bones and heating in drying process [21]. Calcium content of Asian sea bass bio-calcium powders was 23.4% (w/w) of the bone powder which was in comp

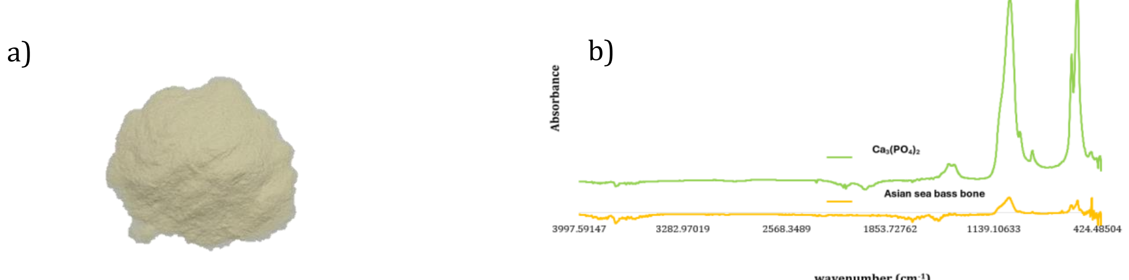

Figure 1. Asian sea bass bio-calcium powder (a) and FTIR spectra at wavenumbers of 400–4000 cm^{-1} of Asian sea bass bone powder (yellow line) and tricalcium phosphate ($Ca_3(PO_4)_2$) (green line)

FTIR spectra of bio-calcium from Asian sea bass bones and tricalcium phosphate ($Ca_3(PO_4)_2$) present in Fig. 1b. FTIR analysis of the bio-calcium powder showed two sharp peaks at 1026 cm^{-1} attributed to the P-O stretching range and 560 cm^{-1} attributed to the O-P-O bending range [20, 23] and shoulders nearly the peaks which were similar spectra to those of $Ca_3(PO_4)_2$ (Fig. 1b), but the analysis had the difference in amplitude. Cao et al., 2020 [24] reported that the ranges of 1122 cm^{-1} and 567 cm^{-1} represented PO_4^{3-} ions and Nilsuwan et al., 2023 [20] presented that the range of 1400 cm^{-1} exhibited to the C-O stretching vibrations. Those appeared FTIR spectra suggested that this bio-calcium definitely contained calcium and phosphate in comparable to previous studies [10, 19, 21].

3.2 Characteristics of cereal bars as affected by bio-calcium fortification

3.2.1 Dimension, Weight and Color

Dimension, weight and color of cereal bars (CBs) fortified with bio-calcium from Asian sea bass bones are shown in Table 1. Width and thickness of fortified CBs decreased when the level of bio-calcium powder increased but there was no difference among all samples tested and control ($P > 0.05$) indicated by appearances of fortified products shown in Fig. 2. This suggested that the fortified CBs were less expansion after baking. Decreasing of width and thickness was also observed in development of whole wheat crackers fortified with tuna bone bio-calcium powder [13]. Increasing of bio-calcium powder fortified in CBs increased particles which dispersed in the solid phase and density of the product system while the liquid phase was limited resulting in high compactness and difficult to spread of CBs during baking. Weights of fortified CBs was more than that of the control and increased when the level of fortified bio-calcium increased with no significant difference ($P > 0.05$) suggested that fortified CBs weights were less affected by bio-calcium adding in the level of increased calcium (IS-Ca), good source of calcium (GS-Ca), and high calcium (H-Ca). Surface color of fortified CBs was not different from control ($P > 0.05$) except b* values (Table 1). Fortified bio-calcium CBs showed more yellowness than the control because of non-enzymatic browning reaction of remained proteins in bio-calcium powder and heating occurred during baking. ΔE* values among fortified CBs were not significant difference ($P > 0.05$). Nevertheless the highest ΔE* value was observed in GS-Ca sample (7.92 ± 4.20) probably caused by the lowest value of L* and

b* of GS-Ca compared to IS-Ca and H-Ca samples. Although the dimension and surface color of cereal bars was not affected significantly by bio-calcium fortification in the level of IS-Ca, GS-Ca and H-Ca, the appearance of those samples was noticeably changed (Fig. 2). Cereal pieces in fortified CBs were clearly observed in IS-Ca and GS-Ca suggesting more brittle bars while gaps between cereal ingredient ware hardly noticeable in H-Ca suggesting more stiff and dense structure of the bars.

Table 1. Characteristics of cereal bars fortified with bio-calcium powder at different levels

Sample	Control	IS-Ca	GS-Ca	H-Ca
Width (mm)	37.67 ± 0.62^{a}	38.33 ± 0.85^{a}	38.25 ± 0.83^{a}	37.92 ± 0.86^{a}
Length (mm)	48.33 ± 0.85^{a}	48.83 ± 0.37^{a}	48.25 ± 0.72^{a}	48.42 ± 0.76^{a}
Thickness (mm)	14.17 ± 0.80^{a}	14.25 ± 0.43^{a}	14.17 ± 0.55^{a}	14.08 ± 0.49^{a}
Weight (g)	22.39 ± 1.32^{a}	24.37 ± 1.53^{a}	23.01 ± 0.83^{a}	23.44 ± 0.73^{a}
Color				
L*	55.17 ± 3.36^{a}	54.19 ± 3.14^{a}	51.95 ± 3.89^{a}	54.85 ± 3.55^{a}
a*	5.89 ± 0.75^{a}	6.00 ± 1.26^{a}	5.91 ± 1.16^{a}	5.42 ± 0.88^{a}
b*	24.21 ± 1.92^{b}	22.14 ± 3.43^{ab}	21.01 ± 3.31^{a}	21.83 ± 2.09^{ab}
ΔE*		5.54 ± 2.86^{a}	7.92 ± 4.20^{a}	4.83 ± 1.91^{a}
Texture				
Fractureability(g)	247.20 ± 26.35^{a}	376.98 ± 67.03^{a}	297.45 ± 46.95^{a}	340.63 ± 89.00^{a}
Hardness (g)	411.83 ± 68.55^{a}	775.66 ± 191.23^{b}	738.14 ± 128.40^{b}	823.02 ± 61.02^{b}
Crispness (g)	2.50 ± 0.50^{a}	2.75 ± 0.83^{a}	3.00 ± 0.71^{a}	3.50 ± 1.50^{a}
Stickiness (g)	-86.12 ± 10.73^{b}	-127.37 ± 5.76^{a}	-92.60 ± 11.33^{b}	-109.79 ± 11.94^{ab}

* Control: Cereal bars without Bio-Ca powder fortification; IS-Ca: Cereal bars fortified with bio-calcium powder in the level of increased calcium; GS-Ca: Cereal bars fortified with bio-calcium in the level of good source of calcium; H-Ca: Cereal bars fortified with bio-calcium in the level of high calcium. Different lowercase letters in the same column indicate significant differences (P > 0.05).

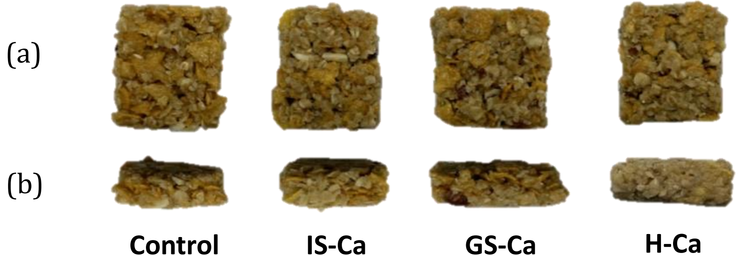

Figure 2. Appearances of cereal bar products fortified with bio-calcium powder at different levels; (a) Top view of cereal bars, (b) Side view of cereal bars. Control: cereal bars without Bio-Ca powder fortification; IS-Ca: cereal bars fortified with bio-calcium powder in the level of increased calcium; GS-Ca: cereal bars fortified with bio-calcium in the level of good source of calcium; H-Ca: cereal bars fortified with bio-calcium in the level of high calcium.

3.2.2 Textural properties

Textural properties of fortified CBs compared to the control is showed in Table 1. Fractureability and crispness of fortified samples were more than those of the control with no difference for all tested. Fractureability and crispness of samples increased when amount of bio-calcium increased. Hardness of fortified CBs was increased significantly compared to the control corresponding to the decreasing of width and thickness (Table 1) and appearance of fortified CBs (Fig. 2). This suggested that an increase of bio-calcium powder which was low solubility solid particles strongly incorporated with other ingredients such as lecithin, maltodextrin and cereals in

the fortified CBS. This incorporation might obstruct the formation of gel network and interfere the stabilization of emulsion system in the products. Consequently, the harder fortified CBs leading to more difficult breaking (Table 1). These textural properties were also observed in whole wheat crackers added with tuna bone bio-calcium [13]. Additionally, harder breads texture when adding Asian sea bass bio-calcium increased was reported by Siangchin et. al., 2023 [10].

3.3 Chemical compositions and nutrition values of cereal bars as affected by bio-calcium fortification.

In this study, CBs were fortified with Asian sea bass bio-calcium in three calcium levels that could be claimed as increased calcium, good source of calcium and high calcium. The amount of bio-calcium powder for those calcium levels was calculated by following the notification of the Ministry of Public Health, Thailand [6]. Total carbohydrate (include fiber) of control, IS-Ca, GS-Ca and H-Ca were 78.07, 73.77, 72.97 and 71.01 g/100g, respectively. Total fat of control, IS-Ca, GS-Ca and H-Ca were 7.31, 7.83, 7.21 and 6.33 g/100g, respectively. Protein of control, IS-Ca, GS-Ca and H-Ca were 5.62, 6.47, 6.65 and 7.07 g/100g, respectively. Ash of control, IS-Ca, GS-Ca and H-Ca were 0.87, 3.37, 4.5 and 6.56 g/100g, respectively. Calcium of control, IS-Ca, GS-Ca and H-Ca were 36.7, 921.12, 1,287.10 and 2,639.70 mg/100g, respectively. It was found that carbohydrate and fat of fortified CBs was lower than those of the control while protein, ash and calcium were higher. The fortification of Asian sea bass bio-calcium reduced the ratio of oat, corn flake, almond and sweetener mostly provide carbohydrate and fat in CBs. Subsequently, an increase of protein, ash and calcium contents was clearly observed. It was notably observed that an increase of calcium contents in IS-Ca, GS-Ca and H-Ca samples was approximately 25, 35 and 72 folds, respectively, calculating based on calcium content in the control. Considering of serving size of CBs is 30 g [6], all fortified CBs developed in this study could be claimed as increased calcium, good source of calcium and high calcium cereal bars for IS-Ca, GS-Ca and H-Ca samples, respectively. Therefore, cereal bars fortified with bio-calcium powder from Asian sea bass in this study could be characterised as a fortified food. Additionally, Codex Nutrient Reference Values (Codex NRVs) [25] and Thai Reference Daily Intakes (Thai RDIs) [6] for calcium are 1000 mg, therefore, the daily consumption of 5, 3-4 and 2 pieces of IS-Ca, GS-Ca and H-Ca cereal bars, respectively, as approximately 23-24 g/piece of those fortified CBs (Table 1) could be achieved calcium requirement of the body.

Table 2. Chemical compositions of cereal bar fortified with bio-calcium powder at different levels

Sample	Control	IS-Ca	GS-Ca	H-Ca
Moisture (%)	6.65 ± 0.65^{a}	7.24 ± 0.98^{a}	7.47 ± 0.97^{a}	7.80 ± 1.87^{a}
Water activity (aw)	0.63 ± 0.04^{b}	0.57 ± 0.01^{a}	0.61 ± 0.02^{ab}	0.60 ± 0.02^{ab}
pH	5.96 ± 0.00^{a}	7.10 ± 0.00^{b}	7.33 ± 0.02^{b}	7.50 ± 0.14^{b}

*Control: Cereal bars without Bio-Ca powder fortification; IS-Ca: Cereal bars fortified with bio-calcium powder in the level of increased calcium; GS-Ca: Cereal bars fortified with bio-calcium in the level of good source of calcium; H-Ca: Cereal bars fortified with bio-calcium in the level of high calcium. Different lowercase letters in the same column indicate significant differences ($P > 0.05$).

In Table 2, moisture contents of all tested samples were not significant different (P > 0.05) in comparable with that of bio-calcium powder produced from Asian sea bass in this study (7.93±1.01% moisture). Lower water activity values were observed when adding bio-calcium compared to the control attributed to lower water activity (0.45±0.01) of bio-calcium. On the other hand, pH values were increased significantly (P > 0.05) with adding bio-calcium. It was probably caused by the remaining of proteins in the produced bio-calcium. However, in overall, IS-Ca, GS-Ca and H-Ca cereal bars could be classified in a dried food group attributed to difficult food spoilage [19]. To provide more information for utilization of Asian sea bass bone, cost of CBs production fortified with Asian sea bass bio-calcium in a laboratory scale was calculated. The cost calculation was concerned mainly raw materials and reagents without other expenses such as utility, machine, and wage. It was found that production of this fortified products was possible. Cost of the bio-calcium production was approximately 7,416 Bth/kg bio-calcium. Yield of bio-calcium production was 40.30%. Cost of IS-Ca, GS-Ca and H-Ca products and control was 11.07, 12.55, 18.48 and 6.62 Bth/pieces (23 g), respectively. However, the remaining of hazardous reagents such as NaOH, hexane, H_2O_2 and NaClO in bio-calcium must be seriously concerned. To be safe for consumer health, only bio-calcium without those hazardous reagents can be used for food fortification. In addition, analysis of calcium bioavailability, sensory acceptance and shelf life of the developed products should be determined in further study.

4. CONCLUSIONS

Produced bio-calcium powders could be classified in a dried food group with light yellow-white color and contained calcium and phosphate in their structure. Dimension, weight and color of fortified CBs were comparable the control. Harder texture, higher pH values and higher contents of protein, ash and calcium were presented in fortified CBs. Calcium contents of cereal bars fortified with bio-calcium powders at the levels of increased calcium (IS-Ca; calcium ≥10% Thai RDI), good source of calcium (GS-Ca; calcium ≥15% Thai RDI), and high calcium (H-Ca; calcium ≥30% Thai RDI) increased approximately 25 (921.12 mg/100g), 35 (1,287.10 mg/100g) and 72 (2,639.70 mg/100g) folds, respectively, compared to the control (36.7 mg/100g). As serving size of CBs is 30 g [6], all fortified CBs could be categorized as a fortified food and claimed as increased calcium, good source of calcium and high calcium cereal bars for IS-Ca, GS-Ca and H-Ca cereal bars, respectively. Regarding to Codex Nutrient Reference Values (Codex NRVs) [25] and Thai Reference Daily Intakes (Thai RDIs) [6] for calcium are 1000 mg, consumption of 5, 3-4 and 2 pieces (23-24 g)/day of IS-Ca, GS-Ca and H-Ca cereal bars respectively, could be accomplished calcium requirement of the human body.

5. ACKNOWLEDGEMENTS

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Total phenolic and flavonoid contents and free radical scavenging potential of honeys from different regions in Thailand

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ABSTRACT

This research studied the antioxidant effects of various types of honey samples from bee species of different areas in Thailand, namely longan honey from the northern region, Siam weed honey from the northeast, longan honey from the central region rambutan honey from the south, *Apis cerana* honey from the south and stingless bee honey from the eastern region. They were examined the reducing sugar content, total phenolic, and total flavonoid contents. The free radical scavenging activities were evaluated using DPPH and hydroxyl radical scavenging assays. The study found that longan honey from the central part showed the highest amount of reducing sugars around 9.612±0.001 mM. Stingless bee honey from the eastern region contained the highest level of total phenolic compounds by 2.699±0.011 mg gallic acid equivalent/ml. Additionally, stingless bee honey from the eastern region maintained the highest content of total flavonoids by 2.208±0.008 mg quercetin equivalent/ml. For DPPH scavenging activity, stingless bees honey from the eastern part strongly exhibited radical scavenging efficacy with an IC_{50} = 0.07% (v/v), when compared to the other types of honey. Furthermore, all types of honey were investigated for the hydroxyl radical scavenging capacity, stingless bee honey from the eastern region also demonstrated the maximal scavenging efficacy with an IC_{50}= 1.80%(v/v), closely comparable to that of vitamin C, used as a positive control. It was observed that stingless bee honey possessed higher levels of substances and antioxidant properties than honey from other regions, making it a potential candidate for developing functional food or cosmeceutical products.

1. INTRODUCTION

Honey is a natural product formed from the nectar of flowers by honeybees (*Apis mellifera*; Family: Apidae) [1]. Honey is the only insect-derived natural product with nutritional, cosmetic, therapeutic, and industrial values [2]. Honey was utilized as a natural sweetener from the ancient period since it has a high level of fructose (honey is 25% sweeter than table sugar)[3]. Moreover, the use of honey in beverages is also increasingly popular.[3] Nowadays, information on using honey to cure many human diseases can be found in general magazines, journals, and leaflets about natural products, suggesting various unknown activities [4]. Evidence indicates that honey can exert several health-beneficial effects including antioxidant [5], anti-inflammatory [5], antibacterial [6], antidiabetic [7], respiratory, gastrointestinal [8], cardiovascular, and nervous system [9] protective effects. Although honey is widely consumed in Thailand, there is still a lack of information on the antioxidant properties of Thai honeys. To date, this study has focused on determining the reducing sugar content, total phenolic and total flavonoid contents, and antioxidant properties (DPPH, FRAP, and hydroxyl radical scavenging activity) for the development of functional food products and enhancing the value of agricultural products in the future.

2. MATERIAL AND METHODS

2.1 Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) were purchased from Sigma-Aldrich, Germany. Ascorbic acid was obtained from Ajax Finechem, Australia. Folin-Ciocalteu's reagent was purchased from Carlo Erba, Germany. Gallic acid was provided from Fluka, Switzerland.

2.2 Honey samples

This research studied the antioxidant effects of various types of honey samples from bee species of different areas in Thailand, namely longan honey from Chiangmai, Siam weed honey from Ladda Farm, Chaiyaphum, longan honey from Charoen Honey Farm, Lopburi, rambutan honey and *Apis cerana* honey from Purinthorn Farm, Surat Thani, and the stingless bee (*Tetragonula pagdeni* Schwarz.) honey from Chantaburi, the eastern region of Thailand between October 2023 to January 2024 that kindly provided by the Stingless Bee Upbringing Community Enterprise Group at Chantaburi province. The samples were provided from Chiangmai, Chaiyaphum, Lopburi, Surat Thani, and Chantaburi provinces, all regions of Thailand. All honey samples were collected in sterile containers and stored in darkness at 5°C until use.

2.3 Determination of reducing sugar by DNS method

The total reducing sugar (RS) content was determined by applying the 3,5-dinitrosalicylic acid (DNS) method according to Cavaco et al. [10]. The method is based on the reduction of DNS by the reducing sugars present in the sample to generate 3-amino-5-nitrosalicylic acid, which results in the formation of a reddish-orange coloration which is measured spectrophotometrically at 540 nm. To this end, 0.2 mL of honey dissolved in water (50% v/v) was mixed with 2 mL of DNS reagent and 1 mL of distilled water and then incubated for 10 min in a boiling water bath. The mixture was allowed to cool to room temperature. Finally, the absorbance was measured at 540 nm using a UV/Vis spectrophotometer (Hitachi U-2000, Tokyo, Japan). Standard glucose solutions within the 0–1 mg/mL concentration range were used to obtain a calibration curve. The results were expressed as mM of glucose.

2.4 Determination of total phenolic content (TPC)

Folin–Ciocalteu method is the investigation for total phenolic content that was slightly modified testing from Shaghaghi, Manzoori, & Jouyban [11]. Briefly, an aliquot of 0.0125 to 0.2 mg/mL of gallic acid or 10%(v/v) of honey samples 125 µL were mixed with deionized water 0.5 mL, 7% sodium carbonate (Na_2CO_3) 1.25 mL and Folin–Ciocalteu reagent 125 µL. After that, the mixture was incubated at room temperature for 30 minutes in darkness before pipetting into the microplates of 200 µL per well. The absorbance was then measured with a microplate reader (Versamax, USA) at 750 nm. The absorbance value was continually compared with the standard curve of gallic acid and expressed as milligrams of gallic acid equivalent per mL of honey sample (mg GAE/ml sample).

2.5 Determination of total flavonoid content (TFC)

Total flavonoid content was examined by the aluminium chloride colorimetric method, which was slightly modified from Fattahi et al. [12]. An aliquot of 500 µL of quercetin at concentrations 0.03125 to 0.5 mg/mL or 50%(v/v) of honey samples mixed with 30 µL of 5% sodium nitrite ($NaNO_2$) as well as incubated for 6 min. Then, 30 µL of 10% aluminium chloride ($AlCl_3$) was added into the mixture and incubated for 5 min before the 200 µL of 1 M sodium hydroxide (NaOH) admixture and kept in the darkness for 15 min. The final volume of them was 760 µL. The mixture complete reaction was pipetted 150 µL into the microplate and measured absorbance at 510 nm using a microplate reader. The results of total flavonoid content were calculated from the standard curve and expressed as milligrams of quercetin equivalent per millilitre of honey sample (mg QE/mL of sample).

2.6 DPPH scavenging capacity

DPPH radical scavenging activity of the samples was carried out according to the method of Błaszczyk, Skolimowski, & Materac [13] with a slight modification. Honey samples were prepared at different concentrations ranging from 0.3125 to 5 %v/v, and ascorbic acid was used as a standard. Briefly, 50 µL of each sample or standard was mixed with 100 µL of 0.2 mM DPPH solution and allowed to react in the dark for 30 minutes. After that, the mixture was measured absorbance at 517 nm using a microplate reader before calculating the percent scavenging on DPPH radical as an equation 1. The results were expressed as an IC_{50} value (inhibitory concentration of antioxidant to scavenge 50% of DPPH radicals) computed from the linear equation of the DPPH radical arrest graph.

$$\text{DPPH scavenging activity (\%)} = (A0 - A1) / A0 \times 100 \quad (1)$$

Where A0 is the absorbance of the DPPH only
A1 is the absorbance of the DPPH + sample

2.7 Hydroxyl (OH) radical scavenging activity

The OH radical scavenging capacity in the honey samples was determined by the method of Bibi et al. [14], which was based on the Fenton reaction. Briefly, 2 mL of samples were transferred to the labeled test tubes. Then in each sample, 0.04 mL ferrous sulfate (0.02 M), 2.0 mL of phosphate buffer (0.2 M/pH 7.2), and 1 mL (0.04 M) 1, 10-phenanthroline were added. Afterward, the Fenton reaction was started by mixing 0.1 mL H_2O_2 (7 mM). The reaction mixture was incubated at room temperature for 5 min before taking absorbance at 560 nm. The percentage of OH radical scavenging effect was calculated by Equation (2).

$$\text{OH radical scavenging activity (\%)} = (A0 - A1) / A0 \times 100 \quad (2)$$

Where A0 is the absorbance of the OH radical
A1 is the absorbance of the OH radical + sample

2.8 Statistical analysis

The analysis was carried out in triplicate ($n = 3$), and all results were expressed as mean ± standard deviation. Honey varieties were compared using one-way ANOVA and the Turkey pairwise comparison test. All the statistical analysis was carried out using the Minitab version 17, embracing the significance level ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1 The reducing sugar content

The reducing sugar content of the honey samples by the DNS method ranged from 5.712±0.001 to 9.612±0.004 mM (Table 1). The results showed that the longan honey collected from Lopburi province contained the highest amount of sugar, whereas Siam weed honey from Chaiyaphum province found the lowest amount of sugar. Zawawi et al. [15] revealed that trehalulose was the dominant disaccharide in all 36 honey samples. Monosaccharides detected included glucose and fructose. The trisaccharide erlose was detected in small amounts, and sucrose was not detected in any of the stingless bee honey samples.

Table 1. The reducing sugar content, total phenolic, and total flavonoid contents of honey samples from different areas of of Thailand

Honey samples	Reducing sugar content (mM)	Total phenolic content (mg GAE/mL sample)	Total flavonoid content (mg QE/mL sample)
Longan honey from Chiangmai	6.831±0.020[b]	1.367±0.024[d]	0.639±0.025[d]
Siam weed honey from Chaiyaphum	5.712±0.001[c]	0.736±0.005[e]	0.575±0.015[d]
Longan honey from Lopburi	9.612±0.004[a]	1.862±0.002[c]	0.766±0.008[d]
Rambutan honey from Surat Thani	5.826±0.001[c]	2.124±0.006[b]	1.163±0.010[b]
Apis cerana honey from Surat Thani	6.081±0.001[b]	1.554±0.007[d]	0.905±0.019[c]
Stingless bee honey from Chantaburi	5.931±0.001[c]	2.699±0.011[a]	2.208±0.008[a]

*Results were stated as mean ± standard deviation; Means in the same column with different superscripts are significantly different at $P < 0.05$.

3.2 Total phenolic and total flavonoid contents

As a result of the phenolic compounds of six samples of honey collected from different regions of Thailand at 10%(v/v), the stingless bee honey from Chantaburi contained the highest TPC (2.699±0.011 mg GAE/mL sample) followed by the rambutan honey from Surat Thani, longan honey from Lopburi, *A. cerana* honey from Surat Thani, longan honey from Chiangmai and Siam weed honey from Chaiyaphum (2.124±0.006, 1.862±0.002, 1.554±0.007, 1.367±0.024, and 0.736±0.005 mg GAE/mL sample, respectively) (Table 1). The TPC of all samples was determined from a standard curve of gallic acid ($y = 2.744x-0.04551$, $r^2 = 0.97$).

For the total flavonoid content of all honey samples at 50%(v/v), the stingless bee honey from Chantaburi also showed the highest TFC (2.208±0.008 mg QE/mL sample) followed by the rambutan honey from Surat Thani, *A. cerana* honey from Surat Thani, longan honey from Lopburi, longan honey from Chiangmai and Siam weed honey from Chaiyaphum (1.163±0.010, 0.905±0.019, 0.766±0.008, 0.639±0.025, and 0.575±0.015 mg QE/mL sample, respectively) (Table 1). The TFC of all samples was determined from a standard curve of gallic acid ($y = 1.7757x-0.1.864$, $r^2 = 0.96$). We observed that the stingless bee honey had more TFC about 3 times than Siam weed honey.

Interestingly, high TPC and TFC were observed in stingless bee honey sample, suggesting the authenticity of all honey samples. The phenolic content in honey mainly depends on its floral origin; in fact, it can be used as a tool for classification and authentication, especially in the case of unifloral varieties [16]. Braghini et al. [17], for example, have previously reported the presence of phenolic compounds *p*-coumaric acid (1.00–64.3 μg/100 g), salicylic acid (8.02–98.0 μg/100 g), aromadendrin (8.00–303.0 μg/100 g) and taxifolin (3.80–282.0 μg/100 g) as the most common in honey tested. Other significant compounds often reported in stingless bee honey are gallic acid, kaempferol, naringin, luteolin, catechin, and apigenin [18]. The location of the beehives and or the geographical origin where the bees collect the nectar can influence the composition of the phenolic compounds of the honey since these compounds act in the plants as protectors against environmental stress, moderating the effects of the change of temperature, light level, water content, UV exposure, and mineral nutrient deficiency [19].

3.3 Antioxidant activities

Honey has long been utilized for medical and domestic purposes, but its antioxidant properties have come to light. Honey is increasingly popular as a source of antioxidants due to its high concentration of phenolic acids and flavonoids, which has been in high demand in the food industry. The antioxidant activity of the honey samples for their scavenging activities is presented in Table 2. Honey samples in this study were used at different concentrations ranging from 0.3125 to 5%(v/v). The results for DPPH scavenging activity, the stingless bee honey from Chantaburi (IC_{50} = 0.07%(v/v)) exhibited the strongest DPPH scavenging capacity when compared with other honey samples from other regions of Thailand (IC_{50} = 2.53, 3.98, 4.16, 4.56, and 5.30%(v/v) for rambuta

honey from Surat Thani, longan honey from Chiangmai, *A. cerana* honey from Surat Thani, Longan honey from Lopburi, and Siam weed honey from Chaiyaphum, respectively). The finding indicated significant ($p < 0.05$) strong scavenging activity of the stingless bee honey from Chantaburi in contrast to ascorbic acid (IC_{50} = 0.31%(v/v)). However, the IC_{50} values of the honey samples analyzed in the current analysis were higher than those of the other studies. The antioxidant activity in honey originates from nectar, pollen, or propolis, and substances that contain organic acids, vitamins, and enzymes are known to occur in honey [20].

For the results of OH radical scavenging capacity, the scavenging effect of all samples was found to be a concentration-dependent manner. The stingless bee honey from Chantaburi (IC_{50} = 1.80%(v/v)) possessed the strongest OH scavenging capacity when compared to other honey samples from other regions of Thailand (IC_{50} = 2.40, 2.90, 3.20, 6.40, and 9.60%(v/v) for *A. cerana* honey from Surat Thani, longan honey from Chiangmai, longan honey from Lopburi, Siam weed honey from Chaiyaphum, and rambutan honey from Surat Thani, respectively). Even the stingless bee honey from Chantaburi showed a strong OH radical scavenging capacity; nevertheless, the ascorbic acid exhibited stronger significant activity than the honey (Table 2). Beretta, Orioli, and Facino [21] indicated that phenolic acids and flavonoids were the main causes of the protective effect. These results provide unequivocal evidence that, through the synergistic action of its antioxidants, honey, by reducing and removing the reactive oxygen species (ROS), may lower the risks and effects of acute and chronic free radical-induced pathologies in vivo.

Table 2. Antioxidant activities of honey samples from different regions of Thailand

Honey samples	IC_{50} on DPPH radicals (%, v/v)	IC_{50} on OH radicals (%,v/v)
Longan honey from Chiangmai	$3.98±0.015^{b}$	$2.90±0.003^{c}$
Siam weed honey from Chaiyaphum	$5.30±0.012^{a}$	$6.40±0.001^{b}$
Longan honey from Lopburi	$4.56±0.015^{a}$	$3.20±0.002^{c}$
Rambutan honey from Surat Thani	$2.53±0.006^{b}$	$9.60±0.003^{a}$
A. cerana honey from Surat Thani	$4.16±0.030^{a}$	$2.40±0.002^{c}$
Stingless bee honey from Chantaburi	$0.07±0.005^{d}$	$1.80±0.008^{d}$
Ascorbic acid	$0.31±0.001^{c}$	$1.00±0.003^{e}$

*Results were stated as mean ± standard deviation; Means in the same column with different superscripts are significantly different at $P < 0.05$.

4. CONCLUSIONS

In conclusion, our current findings showed that the stingless bee honey from Chantaburi was rich in phenolic and flavonoid contents and possessed potent scavenging effects against DPPH and OH radicals. Our current findings suggest that stingless bee honey has high medicinal value and might be useful in treating and managing many diseases. Future in vivo intervention studies are warranted to explore further the bioavailability and bioefficacy of stingless bee honey bioactive compounds and the beneficial health effects of stingless bee honey in humans when applied topically or consumed orally. With higher levels of active substances and antioxidant properties of stingless bee honey from Chantaburi than kinds of honey from other regions of Thailand, it is a potential candidate for developing functional food or cosmeceutical products in the future.

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Enhancing Okara Protein Concentrate Production using Ultrasonic Pretreatments

AP-0175

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ABSTRACT

Okara, rich in protein but limited in food applications due to extractionchallenges, was investigated for enhanced okara protein concentrate (OP) production using ultrasonic pretreatment under alkaline conditions (pH 11.5). Ultrasonic pretreatment significantly enhanced OP production in alkaline extraction (pH 11.5). Compared to controls, ultrasonic pretreatment increased protein content (up to 76.65%), protein recovery (up to 45.12%), and extraction yield (up to 19.93%). This suggests effective disruption of okara cell walls for improved protein release. Pretreatment duration optimization is crucial to avoid protein degradation. The pretreatment also improved OP functionality. Particle size decreased with pretreatment time, indicating better dispersion. Zeta potential increased, suggesting enhanced protein stability. These changes have the potential to improve subsequent extraction efficiency and functionality. Additionally, protein solubility, water holding capacity (WHC), and oil holding capacity (OHC) all increased with pretreatment time, suggesting improved functionalities for food applications. Ultrasonic pretreatment appears to be a valuable method for improving both production and the functional properties of OP. Further research is needed to optimize pretreatment parameters and explore the impact on foaming properties for broader food applications. This technology can potentially unlock OP as a valuable ingredient in the food industry.

1. INTRODUCTION

As the global population surges, plant-based protein sources are gaining significant attention due to their sustainability advantages compared to animal protein production [1]. Among these, soybeans stand out for their exceptional qualities, particularly their high-quality protein content. They boast a complete essential amino acid profile, meaning they contain all eight essential amino acids that the human body cannot synthesize on its own [2]. Additionally, soybeans exhibit digestibility comparable to animal protein sources like cow's milk, meat, and eggs. The processing of soybean seeds into staple products like soymilk and tofu has witnessed a surge in consumption, particularly among lactose-intolerant individuals and those mindful of cholesterol levels, reflecting shifting dietary preferences [3].

Okara, a byproduct of soymilk and tofu production, presents a challenge: vast underutilization despite its abundance. Every kilogram of soybeans yields roughly 1 kg of wet okara, translating to a staggering 1.4 billion tons globally [4]. Primarily discarded or used for animal feed in Asian countries like China, Japan, Korea, and Singapore [5], this underutilization signifies a missed opportunity for waste management and sustainable protein sources. However, okara holds immense

potential as a sustainable and cost-effective source of plant protein. It boasts a protein content ranging from 27-38% (dry weight basis), showcasing its nutritional value [6]. Okara, a byproduct of soymilk and tofu production, boasts a complete essential amino acid profile and okara exhibits promising in vitro digestibility, reaching up to 80% [7]. making it a promising plant-based protein source. Soymilk processing removes water-soluble proteins, leaving okara with less-extractable ones [8]. Okara's protein (OP) is tightly bound to cell walls such as cellulose, hemicellulose, and lignin, hindering separation [9]. Additionally, harsh heat (>80 °C) during soymilk production can significantly reduce OP functionality compared to milder temperatures (25 °C) [10]. While Ma et al. (1997) observed a yield increase of up to 53.4% at 80 °C. However, the latter extraction conditions may cause protein denaturation and aggregation as the denaturation temperatures of the two major soybean proteins, (glycinin and β-conglycinin) are approximately 82 °C and 68 °C, respectively [10]. Conventional alkaline extraction (pH 9.0) struggles with defatted okara due to fiber entanglement. Extreme alkaline treatment offers a solution. Under high pH, proteins partially unfold while retaining structure (molten globule state), potentially improving functionality [4]. However, balancing yield and protein integrity is crucial. Pretreatments like homogenization or ultrasound might be explored to optimize protein accessibility while minimizing denaturation This two-step approach could unlock okara's potential [12,13]

Ultrasonication technology is gaining attention for improving protein extraction efficiency while reducing solvent and energy use. The cavitation mechanism in ultrasonication aids in cell disintegration, enhancing extraction yields but potentially altering protein structure [14]. Despite benefits in lab-scale studies, industrial applications require optimization in reactor design and energy consumption. Ultrasonication not only aids extraction but also enhances protein properties like solubility and emulsification, expanding their industrial utility. Advancements in ultrasonic technology are enhancing extraction systems and promoting wider adoption in industrial protein extraction processes [15,16]

This research aims to optimize the use of ultrasonic pretreatment to enhance protein concentrate production from okara in alkaline conditions. The study hypothesizes that ultrasonication can effectively disrupt okara cell walls, leading to enhanced protein release, increased protein content and recovery rates, and the preservation of functional properties. The investigation seeks to provide insights into the potential of ultrasonic pretreatments in improving the efficiency of okara protein concentrate production.

2. MATERIAL AND METHODS

2.1 Raw material

Wet okara, a byproduct of soymilk production from Dairy Plus Company Limited, was used in this study. The manufacturer provided information on the soymilk extraction process: soybeans were cooked in hot water at 80 - 90 °C, ground into a slurry, and okara was separated using a decanter centrifuge. Upon arrival at the laboratory, the okara was dehydrated in a forced-air oven (UMAC, UM-Oven 120 L) at 60 °C for 10 h to achieve a final moisture content of approximately 5% (wet weight basis). The dried okara was then ground using a medium-sized fine grinder (DXFILL MACHINE, DXM-2000) and sieved through a 50 mesh sieve. Finally, the okara powder was stored in aluminum foil bags at 4 °C. All chemicals used in this research were of analytical grade and were purchased from Sigma-Aldrich (UK) and Merck (USA).

2.2 Alkaline extraction of okara proteins

Dried okara was mixed with distilled water at a 1:10 (w/v) ratio. Protein extraction was performed under alkaline conditions by adjusting the pH to 11.5 with 2 M NaOH at 60 °C with continuous stirring for 90 min. The mixture was then centrifuged at 10,000 rpm for 30 min (CRYSTE, VARISPIN 15, Korea). The supernatant, containing the extracted protein, was acidified with 2 M HCl to reach the isoelectric point (pI) for protein precipitation. The precipitate was washed twice with distilled water at a 1:5 (w/v) ratio by centrifugation at the same conditions used previously. The washed pellet was then re-suspended in distilled water, and its pH was adjusted to 7.0 with 2 M NaOH. The neutralized protein was frozen, followed by freeze-drying. The resulting powder was

ground and stored at 4 °C until further analysis.

2.3 Ultrasonic pretreatments assisted alkaline extraction of okara proteins

The dried okara was reconstituted with distilled water at a ratio of 1:10 (w/v). The resulting mixture was subjected to ultrasonication using an ultrasonic bath (Elma E30H) operating at 37 kHz and maintained at 29 °C. Ultrasonication was performed for durations of 15, 30, and 45 min. The mixture was performed under alkaline conditions by adjusting the pH to 11.5 with 2 M NaOH at 60 °C with continuous stirring for 90 min. Protein extraction was carried out under the same conditions as the previous method.

2.4 Determination of protein content in okara extracts

The protein content was determined using the Kjeldahl method AOAC 2010. Protein content, protein recovery and extraction yield were calculated using the following equations :

$$\text{Protein content (\%)} = \frac{Nitrogen\ Content\ (g) \times Conversion\ Factor (5.71) \times 100}{Sample\ Weight\ (g)} \quad (1)$$

$$\text{Protein recovery (\%)} = \frac{mass\ of\ protein\ in\ protein\ extract\ (g)}{mass\ of\ protein\ in\ OP\ (g)} \times 100\% \quad (2)$$

$$\text{Extraction yield (\%)} = \frac{mass\ of\ protein\ extract\ (g)}{mass\ of\ OP\ (g)} \times 100\% \quad (3)$$

2.5 Zeta potential and particle size

The zeta potential of protein isolates was determined using a laser Doppler velocimetry and phase analysis light scattering technique (Malvern Zetasizer Nano-ZS, model ZEN3600, Malvern Instruments Ltd., UK). Samples (0.5% w/v) in distilled water were equilibrated for 5 minutes. One milliliter aliquots were then transferred, without agitation, to an electrophoresis cell (model DTS 1060C, Malvern Instruments Ltd., UK). Measurements were performed in triplicate at 25 °C and the average value reported. The particle size of OP solutions with a concentration of 1% (w/v) were prepared in deionized water, followed by 2 hours of stirring before being diluted tenfold. The particle size of OP was meticulously determined using Dynamic Light Scattering (DLS) on a Malvern Zetasizer Nano ZS instrument (Malvern) under controlled conditions at 25 °C. The measurements were conducted at a scattering angle of 173° and a wavelength of 633 nm to ensure accurate assessment of particle dimensions and characteristics.

2.6 Functional properties

The functional properties of the OP were evaluated, including protein solubility at pH 7, water holding capacity (WHC), oil holding capacity (OHC), flow behavior, and foaming properties (foaming activity index, FAI; foaming stability index, FSI). Methods from Tao et al. (2019)[17] were combined for these analyses. Emulsifying properties (emulsifying ability index, EAI; emulsifying stability index, ESI) were determined following the method of Zhu et al. (2017) with slight modifications. Briefly, a 10 mg/mL OP solution (45 mL) and soybean oil (15 mL) were mixed and homogenized at 10,000 rpm for 2 minutes using an Ultra-Turrax T25 (IKA, Staufen, Germany). A 500 μL aliquot of the freshly prepared emulsion was diluted with 50 mL of 0.1% SDS solution, and its absorbance (A_0) at 500 nm was measured (control: 0.1% SDS solution). The diluted emulsion's absorbance (A_{30}) was measured again after 30 minutes to determine EAI and ESI using the following equations :

$$EAI\ (m^2/g) = \frac{2 \times 2.303 \times A_0 \times N}{C \times \varphi \times 1000} \quad (4)$$

$$ESI\ (min) = \frac{A_0}{A_0 - A_{30}} \times 30 \quad (5)$$

where N is the dilution factor (100), C is the protein concentration in the solution (0.01 g/mL), and φ is the oil volume fraction (0.25).

2.7 Statistical analysis

All experiments were performed in triplicate, and data are presented as mean ± standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA) with IBM SPSS Statistics version 25 (SPSS Inc., Chicago, USA). Significant differences between means ($p < 0.05$) were Duncan's new multiple range test.

3. RESULTS AND DISCUSSION

3.1 Protein content, protein recovery and extraction yield of OP extract.

This study examined the potential of ultrasonic pretreatment (Control, 15, 30, and 45 min) to enhance protein concentrate production from okara under alkaline conditions at pH 11.5 as shown in Table 1. Ultrasonication time significantly influenced protein content (%), recovery (%), and extraction yield (%) ($p < 0.05$). Compared to the control (0 min), protein content increased from 73.14% to 76.65%, and protein recovery increased from 38.06% to 45.12%, including extraction yield increased from 16.73% to 19.93% with extended sonication (45 min). These observations suggest that ultrasonication disrupts the okara cell wall matrix, facilitating protein release and potentially enhancing extractability. This aligns with existing research demonstrating the effectiveness of ultrasound in augmenting protein extraction from diverse sources, such as legumes and oilseeds [18]. The mechanism of action likely involves cavitation phenomena generated by ultrasound waves, which create microscopic bubbles that collapse rapidly, disrupting cell walls and releasing intracellular components [19].

However, optimizing the process is crucial. While extended sonication generally improved protein content and recovery in our study, a potential drawback exists. Extended treatment times might lead to protein degradation due to excessive heat generation during the cavitation process [20]. Therefore, further research is necessary to determine the optimal treatment duration that balances maximizing protein yield with mitigating potential drawbacks. This optimization could involve investigating the impact of additional parameters like sonication amplitude and temperature control during the process. Future studies exploring the impact of ultrasonication on the functional properties of the extracted protein concentrate would provide valuable insights into its overall quality and potential applications. Evaluating factors like solubility, emulsifying capacity, and foaming properties would be crucial for understanding the suitability of the protein concentrate in various food or industrial applications.

Table 1. Protein content, protein recovery and extraction yield of OP extract

Ultrasonic time (min)	Protein content (% dry basis)	Protein recovery (%)	Extraction yield (%)
Control	73.14 ± 0.84^{b}	38.06 ± 0.32^{d}	16.73 ± 0.53^{c}
15	71.04 ± 0.02^{c}	44.03 ± 0.11^{b}	19.93 ± 0.55^{a}
30	73.86 ± 0.53^{b}	41.96 ± 0.31^{c}	17.99 ± 0.67^{b}
45	76.65 ± 1.32^{a}	45.12 ± 0.13^{a}	18.24 ± 0.43^{b}

* The results are presented as the mean ± standard deviation (SD) of three independent measurements. Significant differences ($p < 0.05$) between means within the same column are indicated by different superscript letters following Duncan's new multiple range test.

3.2 Zeta potential and particle size

The results of particle size and zeta potential measurements provide crucial insights into the impact of ultrasonic pretreatment on the characteristics of OP. Zeta potential reflects the electrostatic repulsion between particles in a liquid. High values (positive or negative) indicate greater stability in emulsions, while low values promote aggregation. The pH of the medium is the most important factor that affects the zeta potential. The zeta-potential was carried out at pH 7, away from the isoelectric pH (pH 4) of soy proteins and the pH for most food applications [21]. The values of the particle size of the OP obtained by alkaline extraction and ultrasonication are presented in Figure 1a. The particle size analysis reveals a consistent trend of reduction with increasing durations of ultrasonic pretreatment. Specifically, compared to the control (alkaline extraction), the particle size decreased from 368 nm to 256 nm, 267 nm, and 220 nm after 15, 30, and 45 minutes of ultrasonic pretreatment, respectively. This reduction in particle size indicates effective disruption of the okara cell walls by ultrasonic waves, potentially leading to improved dispersion and enhanced accessibility of proteins for extraction [10]. Moreover, the zeta potential of the samples (Figure 1b.) Similarly, the zeta potential measurements demonstrate noteworthy changes associated with ultrasonic pretreatment. While the control exhibited a zeta potential of -30 mV, the pretreated samples displayed values of -36 mV, -45 mV, and -38 mV after 15, 30, and 45 minutes of ultrasonic treatment, respectively. The observed increase in negative zeta potential values suggests enhanced stability of the protein dispersions, likely attributed to improved protein-protein repulsion and reduced aggregation. These alterations in particle size and zeta potential could be indicative of conformational and structural changes in proteins induced by ultrasonic pretreatment, which could significantly influence subsequent protein extraction efficiency.

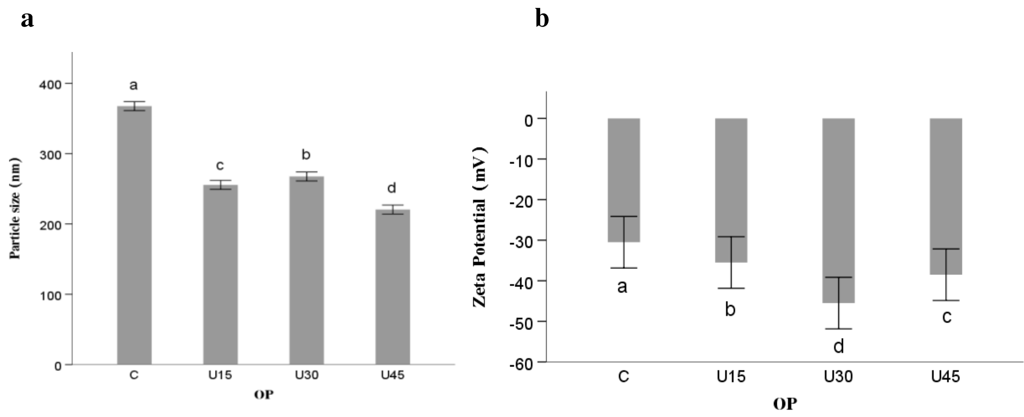

Figure 3. (a) Particle size and Zeta potential (b) of OP and OP extracted at different ultrasonic time (15, 30 and 45 min). Means and standard deviation are from triplicate determinations. Different letters denote significant difference ($p < 0.05$) as determined by Duncan's new multiple range test.

3.3 Protein solubility and water/oil holding capacity (WHC/OHC)

Protein solubility is a critical functional property profoundly influencing other functionalities like emulsification, foaming, and rheological behavior. To ensure reliable measurements and avoid interference from the protein's isoelectric point (pI), where the net charge is zero and solubility is minimal [22], the solubility in this study was determined at pH 7.0. The results of solubility, water holding capacity (WHC), and oil holding capacity (OHC) shed light on the effect of ultrasonic pretreatment on the functional properties of OP. The solubility of OP(C) was around 40.06%, while that of all ultrasonic pretreatment were higher ($p<0.05$). The difference might be due to the fact that OP(C) rise in solubility indicates enhanced protein dispersion and dissolution, likely facilitated by the disruption of protein aggregates and improved hydration due to ultrasonic treatment [15]. In comparison with U30, the solubility of U15 decreased by 7.41%. In contrast, the solubility of U45 increased by 7.53% compared with U30, which could be attributed to changes in zeta potential and particle size. An increase in negative zeta potential would enhance electrostatic repulsion between protein molecules, leading to improved hydration and dispersion stability [23]. Furthermore, the decrease in particle size likely leads to an increase in the total surface area available for interaction with water molecules. This enhanced protein-water interaction could promote improved solubility and dispersion stability [24].

Similarly, WHC and OHC also demonstrated a consistent upward trend with increasing ultrasonic pretreatment time. WHC increased from 9.23 g/g in the control to 13.56 g/g, 14.75 g/g, and 15.62 g/g after 15, 30, and 45 minutes of pretreatment, respectively, while OHC rose from 6.89 g/g in the control to 7.23 g/g, 10.15 g/g, and 8.19 g/g, respectively. The increase in WHC and OHC suggests improved water and oil binding capacities of the proteins, likely due to enhanced protein solubility and altered protein structure induced by ultrasonic pretreatment [25]. These findings underscore the potential of ultrasonic pretreatment in enhancing the functional properties of okara protein, making it more suitable for various food applications where water and oil retention are crucial.

3.4 Foaming properties

The impact of ultrasonic pretreatment duration on the foaming properties of okara protein varies. While there's a slight increase in foam activity index (FAI) at 45 minutes (24%) compared to the control (18%), no consistent trend is observed across all durations. Conversely, foam stability index (FSI) shows a decreasing trend with shorter pretreatment times (15 and 30 minutes), potentially due to factors like increased particle size or altered protein interactions at the air-water interface [26]. However, FSI somewhat recovers at 45 minutes (52%). Although longer pretreatments have been linked to increased protein solubility, their influence on FAI appears less pronounced compared to FSI, suggesting other factors like protein unfolding or aggregation due to ultrasonic treatment may play a more significant role [15].

3.5 Emulsifying properties

The emulsifying activity index (EAI) and emulsifying stability index (ESI) are commonly employed metrics to assess protein emulsifying properties, and the results for OP are provided in Table 2. Significant variations were detected in the emulsifying properties of OP ($p < 0.05$). A 15-minute pretreatment significantly increased EAI compared to the control, likely due to reduced particle size and potential surface hydrophobicity changes [27]. However, EAI at longer durations (30 and 45 min) exhibited a diminished or negative effect, suggesting increased protein aggregation at higher ultrasonic exposure times, which might hinder interfacial activity (Nor Afizah and Rizvi, 2014). Conversely, ESI showed a slight decrease across all pretreatments, potentially due to altered protein interactions or exposed hydrophobic groups upon unfolding [15].The impact of ultrasonic pretreatment duration on the foaming properties of okara protein varies.

Table 2. The solubility, water holding capacity (WHC), oil holding capacity (OHC), foam activity index (FAI), foam stability index (FSI), emulsion activity index (EAI) and emulsion stability index (ESI) of OP extract

	C	U15	U30	U45
Solubility (%)	40.06 ± 0.23^{d}	51.36 ± 0.86^{c}	58.77 ± 0.65^{b}	66.30 ± 1.06^{a}
WHC (g/g)	9.23 ± 0.85^{d}	13.56 ± 0.39^{c}	14.75 ± 0.46^{b}	15.62 ± 0.77^{a}
OHC (g/g)	6.89 ± 1.32^{c}	7.23 ± 0.66^{c}	10.15 ± 0.52^{a}	8.19 ± 0.94^{b}
FAI (%)	18 ± 1.96^{c}	19 ± 0.55^{b}	17 ± 0.21^{d}	24 ± 0.82^{a}
FSI (%)	55 ± 0.85^{a}	42 ± 0.74^{d}	44 ± 0.81^{c}	52 ± 1.23^{b}
EAI (%)	50 ± 0.23^{d}	69 ± 0.52^{a}	52 ± 1.23^{c}	65 ± 0.87^{b}
ESI (min)	43 ± 0.21^{a}	40 ± 0.30^{b}	38 ± 0.74^{d}	39 ± 0.23^{c}

*The results are presented as the mean ± standard deviation (SD) of three independent measurements. Significant differences ($p < 0.05$) between means within the same row are indicated by different superscript letters following Duncan's new multiple range test.

4. CONCLUSIONS

The study demonstrates that ultrasonic pretreatment effectively enhances okara protein concentrate production under alkaline conditions, leading to increased protein content, recovery rates, and extraction yield by disrupting okara cell walls. Optimization of the ultrasonic pretreatment process is essential to balance maximizing protein yield with mitigating potential drawbacks like protein degradation, while smaller particle size and higher zeta potential indicate improved dispersion and stability of the extracted proteins. Ultrasonic pretreatment also positively affects okara protein's functional properties, including solubility and water and oil holding capacity, making it suitable for various food applications, although its impact on foaming and emulsifying properties requires further investigation. Overall, these findings highlight the potential of ultrasonic pretreatment to enhance okara protein concentrate production and functionality, offering opportunities for its utilization in food and industrial applications.

5. ACKNOWLEDGEMENTS

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Valorization of fish head by-product through enzymatic hydrolysis

AP-O180

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ABSTRACT

Fish heads, often discarded as waste in the fish processing industry, can be transformed into valuable products such as fish protein hydrolysates containing bioactive peptides. This study aims to explore the impact of varying enzymatic hydrolysis times and enzyme concentrations using Alcalase on the extraction yield, protein content, and antioxidant properties of hydrolysates derived from the heads of Salid fish, a species commonly consumed in Thailand. Additionally, we investigated the color properties and conducted FTIR spectroscopy analysis to further understand the characteristics of the hydrolysates. The fish head protein hydrolysates exhibited a yellow-brownish color. The results demonstrated that increasing both the enzyme concentration and hydrolysis time enhanced the protein extraction yield and antioxidant activity. The hydrolysates exhibited significant nutritional value with high protein content and demonstrated antioxidant properties across all samples. Given their biological activities, these hydrolysates have the potential to be used as natural additives in functional foods.

1. INTRODUCTION

In the processing industry, fish heads are often discarded despite their high protein and essential amino acid content. This is notably evident in the consumption patterns surrounding gourami fish(*Trichogaster Pectoralis*) or as known as "Salid Fish" in Thailand, where the head is usually not used [1]. This project aims to investigate the nutritional profile of fish [2], particularly focusing on the head of "Salid Fish", to assess their potential as natural food additives. The goal is to challenge current consumption habits and improve the economic value of what is often considered waste. The project will employ enzymatic digestion, specifically using the proteolytic enzyme alcalase to extract proteins [3] from fish heads. This method is chosen for its efficiency in breaking down proteins into smaller peptides or as you might know as “Protein Hydrolysate” , which are more easily absorbed by the body and have potential health benefits[4], such as antioxidant properties. These characteristics make these peptides suitable for use in natural preservatives or functional food ingredients.The main aim is to optimize the conditions for protein extraction from Salid fish heads through enzymatic hydrolysis, transforming an underutilized byproduct into a valuable food ingredient. This approach not only seeks to uncover the hidden

nutritional value of fish heads but also aims to improve their economic status. By collaborating with local fisheries, the project supports. sustainable food practices and contributes to more efficient food production and consumption systems.Overall, this research focuses on the sustainable valorization of fish waste, particularly fish heads, by leveraging enzymatic hydrolysis to produce bioactive peptides. This endeavor aligns with effortsenhance the sustainability and economy of the food industry, promoting the use of all parts of the fish and reducing waste.

2. MATERIAL AND METHODS

Part 1: The Extraction Phase

1.1. Preparation of Fish Heads:

Given that the fish heads, primarily composed of skull and bones, present challenges for conventional extraction methods so they were ground to a creamy texture. This process facilitates more efficient protein extraction.

1.2. Enzymatic Hydrolysis:

The prepared fish heads underwent enzymatic hydrolysis with 1:4 fish to water ratio , following a plan that varied the enzyme concentration across three levels (2%, 4%, and 6%) and hydrolysis time across three durations (1, 1.5, and 2 hr.), resulting in nine treatment combinations. These samples were then placed in a water bath maintained at 65°C. Throughout hydrolysis, the pH of the mixture was stabilized at 8 by the use of hydrochloric acid and sodium hydroxide, optimizing conditions for the alcalase enzyme. To halt enzyme activity, the mixture was subsequently heated to 90°C for 2 minutes and then cool down to prevent denaturing of the protein from extensive heat exposure .

1.3. Separation Process:

A centrifuge was utilized to separate the supernatant from the mixture, effectively removing oil, bone, and scales. To further purify the supernatant, it was filtered through a fine cloth.

Part 2: Determination of Physicochemical Properties and Antioxidant Activity

2.1. Protein Content Analysis:

The combustion method was employed to determine the protein content of the extracted protein. This technique involves burning the sample with oxygen and gasses containing nitrogen oxide and then collected in a ballast tank until a specified pressure is reached then Helium is used as a carrier and an aliquot of combustion gas containing nitrogen oxides is reduced to nitrogen which indicates protein composition.

2.2. Antioxidant Capacity Assessment:

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method, known for its efficiency and minimal sample requirement, was used to evaluate antioxidant properties. This involves mixing the DPPH compound, a free radical agent, with the samples. A spectrophotometer measured the inhibition rate, where a higher percentage indicates better antioxidant properties. The results were compared against Trolox, a well-regarded form of vitamin E, for its antioxidant capabilities. Starting with mixing the supernatant with ethanol and centrifuge the mixture to avoid the sediment of the protein when mix with ethanol, left with clear yellow solution, then this protein solution is mix with DPPH and then tested inside the spectrophotometer machine.

2.3 The FTIR Spectroscopy Analysis involves directing infrared radiation into the compound and examining the peaks corresponding to specific vibrations, which signify the presence of the compound of interest which in this study we're looking for the peak at 1630 cm^{-1} and at 1570 cm^{-1} which belongs to amide I and amide II region representing the presence of protein.

3.RESULTS AND DISCUSSION

The results of the experiment study found that Salid fish heads extracted with 4% enzyme concentration gave the highest antioxidant property at 77.7%. When compared to Trolox at 95.6%(Table 1). Meanwhile, the protein content of water diluted hydrolysate protein contains around 3000 mg of protein per 100 g of diluted supernatant(Table 2), which will be discussed below.

Table 2. Antioxidant property hydrolysate of fish head hydrolysate

Enzyme concentration	Time(hour)	DPPH (IHB%)	significant
	1	68.50±0.21	**e**
2%	1.5	60.03±0.30	g
	2	50.53±0.18	i
	1	76.26±0.34	c
4%	1.5	77.72±0.10	b
	2	63.95±0.21	a
	1	59.30±0.37	f
6%	1.5	70.74±0.45	h
	2	67.13±0.37	d
Trolox(control)		95.96	

*Where **IHB%** is the percentage of the free radical agent that is neutralized

The study shows that the antioxidant property peaks around those treatments treated with 4% enzyme concentration, neutralizing up to 77.72 percent of free radical agent, which is also made from the latest badge of ingredient so the freshness of the ingredient might affect the performance of the antioxidant agent.

Table 2. Protein content of fish head

Enzyme concentration	Time(hour)	Protein (%)	significant
	1	2.6870±0.04	a
2%	1.5	2.7167±0.03	a
	2	2.5433±0.07	b
	1	2.3300±0.08	d
4%	1.5	2.4767±0.04	bc
	2	2.4833±0.06	bc
	1	2.4467±0.05	bc
6%	1.5	2.3933±0.09	cd
	2	2.2367±0.03	e

The fish head protein hydrolysate contains approximately 2.48±0.15%. This concentration remains relatively stable and does not vary significantly with increased hydrolyzing time. Although 2.48% might seem low, it is understandable considering that the head of Salid fish mainly consists of skull and bones with very little meat.

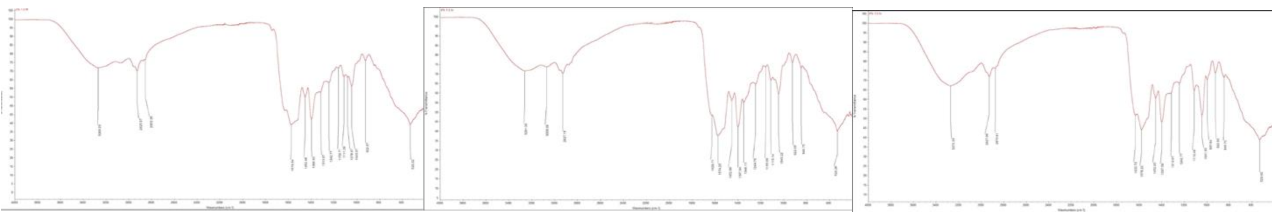

Figure1. FTIR spectra of a sample of 2%, 4%, and 6% enzyme concentration

From FTIR experiment, it is displayed the amino function groups within the protein (Fig.1). The peak at 1570 is also linked to the protein fraction, involving N–H deformation and C–N stretching

vibrations from amide II and the samples shows a broad band at 1630, which belongs to C–O stretching vibrations from the amide I region. All these results show the presence of protein which confirms the result from the protein content in table 2.

4. CONCLUSIONS

Fish head protein hydrolysate has shown good nutritional value with high protein content, and all samples exhibited antioxidant activity. Considering the biological activities, these fish head protein hydrolysates are potential natural additives for functional foods.

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ULTRASOUND-ASSISTED EXTRACTION OF BIOACTIVE COMPOUNDS FROM TAMARIND SEED

AP-P201

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Ultrasound Assisted Extraction, Tamarind Seed, Bioactive compounds.

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ABSTRACT

Ultrasound-assisted extraction (UAE) has been identified as an effective method for extracting natural bioactive chemicals from a variety of plant sections. This study evaluated the antioxidant activity, phenolic content, and flavonoid content of *Tamarindus indica L.* seeds by varying the temperature (30°C, 40°C, and 50°C) and the ethanol concentration (30%, 40%, and 50%). Additionally, the proximate analysis was established. It was shown that the most prevalent compositions were protein (14.66±0.06) and carbohydrates (73.61±0.10). Furthermore, the optimal extraction of phenolic compounds required 30% ethanol and a temperature of 40°C (6.18 mg GAE/g). An association between the sample's free radical scavenging and reducing power processes was suggested by the investigation's notable positive correlation between DPPH radical scavenging activity and FRAP reduction potential. Variations in the study's solvent content and temperature, however, had no discernible impact on the antioxidant activities. This could be the result of additional factors that have not been thoroughly examined or restrictions in the spectrum of these criteria that were selected. Further investigations examining a wider range of solvents, temperatures, or other pertinent parameters may provide additional insight on the variables affecting these antioxidant activities. On the other hand, 50°C and 40% ethanol showed to be the best conditions for antioxidant activity. It was determined that tamarind seeds have antioxidant activity and a meaningful bioactive content.

1. INTRODUCTION

In the current market, there is a significant increase in demand for healthy foods, especially those made from plant material. Many bioactive molecules that are well-known for their antioxidant qualities, like flavonoids and phenolic compounds, are being thought to be the main forces behind the market shift. The tamarind (*Tamarindus indica L.*), a dicotyledonous fruit belonging to the monotypic Fabaceae (Leguminosae) family, is notable among these foods.

The exotic fruit tamarind is often used to make jelly, fruit pulp, dehydrated fruit, and other sweet treats. Fruit processing generates wastes such seeds, which make up 25–40% of the entire fruit and must be disposed of, which causes issues for the environment and financial losses (Martins et al., 2022). However, the by-products are rich in bioactive materials including fatty acids, phenolics, and polysaccharides as well as important nutrients like proteins, carbs, vitamins, and minerals. For example, the linoleic, oleic, palmitic, and stearic acids found in tamarind seeds add to the fruit's well-known health advantages. These advantages include reducing diarrhea and constipation and avoiding heart disease, cancer, and other cardiovascular diseases. (Martins et al., 2022)

Food items are usually extracted using conventional methods like maceration and extraction with a Soxhlet device. These methods involve the use of several organic solvents in conjunction with different sample preparation procedures, extraction times, and temperatures. Nevertheless, they frequently call for lengthy extraction times, which may cause the bioactive substances included in the extracts to degrade or become inactive (Dorta et al., 2013). As a result, to guarantee the production of safer products and lessen the influence of the extraction process on the environment, there is an increasing focus on developing environmentally friendly extraction techniques. There are many extraction methods that use ultrasonic waves with frequencies between 20 kHz and 100 MHz, such as ultrasound-assisted extraction (UAE). The cavitation bubbles produced by ultrasound-assisted extraction collapse, producing a strong shear force. Ultrasound-assisted extraction often operates in the frequency range of 20 to 1,000 kHz. The release of bioactive molecules is facilitated by the breakdown of the cell wall or membrane within the matrix caused by cavitation, which is aided by this shear stress (Kumar et al., 2020). These techniques need little heat or energy input, which allows for quick extraction, reduced solvent usage, and effective low-temperature extraction of thermally labile substances. Therefore, UAE presents a chance to minimize any health concerns and lower energy consumption while increasing the output of required phytochemicals from plant materials (Shen et al., 2023). UAE thus positions itself as a strong substitute for conventional extraction techniques, providing an economical and ecologically friendly extraction process.

UAE has demonstrated effectiveness in removing phytochemicals from a variety of food sources, such as longan seeds (Chindaluang & Sriwattana, 2014; Wen et al., 2020) and mango kernels (Hayat et al., 2023). Carrera et al. (2012) have utilized it for the purpose of extracting phenolic components from grapes.

Previous investigation investigated on the extraction of bioactive substances from tamarind seeds using organic solvents and ultrasound-assisted extraction (UAE). (Ha et al., 2022; Martins et al., 2022)

To the finest of the authors' knowledge, though, relatively few research have explicitly examined the ideal UAE conditions with reference to the chemical makeup and phytochemical characteristics of tamarind seed extract. Therefore, the purpose of this study was to investigate how the extraction temperature and solvent concentration affected the

amount of total phenolic content, amount of total flavonoid content, and amount of antioxidant activity throughout the UAE extraction process. The study also sought to determine the ideal UAE tamarind seed extraction parameters.

2. MATERIAL AND METHODS

2.1 Materials

Tamarind served as the raw material in this research. It was procured from a local market in Bangkok, Thailand. This study utilized mature tamarinds characterized by their uniform size and brown to reddish-brown meat colour.

2.2 Methods

Preparation of Tamarind Seed Powder

The seeds upon being manually separated and cleaned with distilled water, the tamarind fruit seeds were blanched to eliminate any toxins and anti-nutrients. They were then dried in an air-circulating oven at 50°C for 24 hours, or until the moisture content was less than 5%. A grinder was used to grind the dry seeds until the average particle diameter was 0.40 mm. In order to stop the ground samples from absorbing moisture and being exposed to sunlight, they were finally vacuum packed in aluminum bags.

UAE Extraction Process

Finely ground tamarind seeds (10 g) were mixed with ethanol (powder: ethanol = 1:10 w/v) in different proportions (30%, 40%, and 50%). For the extraction procedure, the mixture was sonicated at temperatures of 30°C, 40°C, and 50°C for a predetermined period of 10min in an ultrasonic bath. The final residues were obtained by concentrating the TSE samples until they were completely dry using a rotary evaporator at a regulated temperature of 50°C and lower pressure. After that, 100 mL of distilled water was added to the dried samples. Until further usage, all sample extracts were kept in glass vials at 8°C.

Determination of proximate composition

The seeds' moisture, lipid, and ash contents were analytically determined using the standard procedures provided by AOCS (AOCS, 1993). The Kjeldahl method, as reported by AOAC (AOAC, 1995), was utilized to ascertain the protein content, and the difference between the value obtained from the sum of moisture, lipids, proteins, and ash was used to quantify the total carbohydrates.

Determination of Total Phenolic Contents

The Folin-Ciocalteu test was used to measure the Total Phenolic Contents (TPC) of tamarind seed extracts (TSE), with some modifications made to the methodology developed by Kushwaha & Verma (2017). In brief, a 0.3 mL extract sample was transferred and diluted with distilled water to make 1.5 mL. The Folin-Ciocalteu reagent (2 mL) was then added, well mixed, and incubated for 10 minutes. After which, 1.5 mL of 10% Na_2CO_3 solution was added, and the combination was incubated at room temperature in the dark for a further 60 minutes. At 765 nm, the absorbance was measured as a light blue tint emerged. A calibration curve was developed using gallic acid as the standard in order to determine the total phenol

content in milligrams gallic acid equivalents per grams (mg GAE)/g.

Determination of Flavonoid Content

The total flavonoid content (TFC) was assessed using the aluminium chloride colorimetric method, as described by Munhoz et al., (2014), with slight adjustments. In summary, 1 mL of the sample and 1 mL of a 10% (w/v) aluminium chloride solution were mixed. Using a spectrometer set to detect absorbance at 430 nm, the solution was incubated for 30 minutes at room temperature. The calibration curve was constructed using quercetin as the standard. The findings are given in milligrams of quercetin equivalents per grams (mg QE/g).

Evaluation of antioxidant activity by DPPH Scavenging Assay

A 0.1 mM DPPH solution was made in order to assess the tamarind seed extract's (TSE) capacity to scavenge free radicals. 2 mL of DPPH solution and 0.2 mL of TSE samples made up the reaction mixture. It was then allowed to sit at room temperature for thirty minutes. The absorbance was then measured at 515 nm. To calculate the free radical scavenging activity, the following formula was used:

$$\text{Percentage Inhibition} = \{(Abs_{control} - Abs_{sample}) / Abs_{control}\} \times 100$$

Where $Abs_{control}$ = absorbance of DPPH solution and $Abs_{control}$ = absorbance of sample.

Evaluation of antioxidant activity by Ferric Reducing Antioxidant Power Assay

By measuring the amount of Fe^{3+} that was converted to Fe^{2+}, the reducing power of the tamarind seed extract was established. 2.85 mL of FRAP solution was mixed with 0.15 mL of sample volume. Acetate buffer (300 mM, pH=3.6), 20 mM $FeCl_3$, and TPTZ solution (20 mM TPTZ in 0.04 mM HCl) were combined in a ratio of 10:1:1 to create the FRAP solution. The FRAP solution was warmed to 37.5°C before use. After completely mixing the sample and FRAP solution, the combination was incubated for 30 minutes. The absorbance was then measured at 595 nm. The following formula was used to determine the reduction potential:

$$\text{Percentage Inhibition} = \{(Abs_{control} - Abs_{sample}) / Abs_{control}\} \times 100$$

Where $Abs_{control}$ = absorbance of FRAP solution and $Abs_{control}$ = absorbance of sample.

Statistical analysis

The experiments were structured using a comprehensive three-level, 2-factor (3^2) full factorial design. The independent factors examined included three varied temperatures (x_1: 30, 40 and 50°C), and three different solvent concentrations (x_2: 30, 40 and 50% ethanol concentration). The dependent responses encompassed TPC, TFC and antioxidant activity by FRAP and DPPH assay of the TSE. Analysis of variance, followed by post-hoc test "Tukey", were employed to assess the effects of the independent variables on the dependent variables. Differences between means were considered significant at $p \leq 0.05$. Minitab v18 software was used for performing data analysis.

3. RESULTS AND DISCUSSION

3.1 Proximate Analysis of fresh seeds

The proximate composition of tamarind seed is shown in Table 1. According to the findings, fresh *Tamarindus indica L.* seeds had a moisture content of 44.60%. The moisture content of the seeds dropped to 6.81% after they were dehydrated for 24 hours at 50°C in an oven dryer to obtain the extract. According to proximate composition investigation, the tamarind seeds had a higher carbohydrate content (73.61%) compared to the findings of Khairunnuur et al. (2009), who concluded it to be 61.15%. The contents of fat (2.91%) and protein (14.66%) were similar in the two investigations. Notably, compared to Khairunnuur et al. (2009) finding on the moisture content of the seed (20.45%), the moisture content in the present study (6.81%) was substantially lower. The difference most likely results from different drying conditions used during sample preparation. Although there was a significant amount of protein (14.66%) and a high level of total carbohydrates (73.61%), the lipid and ash contents were low at 2.91% and 2.01%, respectively. Protein content of dried tamarind seed powder was 14.66±0.06%. The values can be employed as a component in baking flours because they are higher than the protein level of wheat flour (13.4%), as reported by Nwosu (2013). Additionally, Shilini and Murthy (2015) found that raw tamarind seed has an 18.8% protein content. The nutritional relevance of the tamarind seed lies in its protein content. As a result, consuming it will surely raise the amount of protein consumed overall. It was discovered that grounded tamarind seeds may be a possible dietary fibre source. This characteristic helps in the management of cardiovascular disease and diabetes. According to MAHAJANI's (2020) analysis, 8.6% of crude fibre is found in tamarind seeds.

Table 1. Proximate composition of tamarind seed

Composition	**Mean±SD (%)**
Moisture content	6.81±0.05
Lipids	2.91±0.01
Proteins	14.66±0.06
Ashes	2.01±0.02
Total Carbohydrates	73.61±0.10

3.2 *Optimization of the experimental conditions*

3.2.1 Effect of Ethanol Concentration and Temperature on Bioactive Properties of the Extract from Tamarind Seeds

3.2.1.1 Effect of Temperature and Ethanol Concentration on Total Phenolic Content

It is evident that both temperature and ethanol concentration significantly influenced the total phenolic content. The average and standard deviation, which indicate the extent of value dispersion, were calculated and are presented in Table 2.

Table 2. Effect of ethanol concentration and temperature on bioactive properties of the extract from tamarind seed.

Properties	Ethanol Concentration (%)	Temperature (°C)		
		30	40	50
Total Phenolic Content	30	2.21^{Cc}±0.05	6.18^{Aa}±0.07	3.41^{Cb}±0.04
	40	6.04^{Aa}±0.05	4.51^{Bc}±0.03	5.38^{Ab}±0.07
	50	3.91^{Ba}±0.03	3.26^{Cc}±0.04	3.75^{Bb}±0.03
Total Flavonoid Content	30	1.13^{Ba}±0.09	1.73^{Cb}±0.07	1.03^{Cb}±0.04
	40	1.50^{Bb}±0.11	1.92^{Aa}±0.09	1.87^{Ba}±0.06
	50	2.08^{Aa}±0.11	1.39^{Bb}±0.08	2.11^{Aa}±0.11
Radical Scavenging Activity (%)	30	60.17^{Cc}±0.34	89.96^{Aa}±0.44	85.35^{Bb}±0.38
	40	89.27^{Aa}±0.34	87.50^{Bb}±0.43	88.53^{Aa}±0.24
	50	88.12^{Bb}±0.30	80.35^{Cc}±0.27	89.37^{Aa}±0.43
Reducing Potential (%)	30	47.60^{Cc}±0.70	132.54^{Aa}±0.49	72.52^{Cb}±0.70
	40	118.85^{Ab}±1.60	75.67^{Bc}±1.45	142.70^{Aa}±0.51
	50	91.53^{Bb}±0.32	63.31^{Cc}±0.46	96.01^{Ba}±0.58

*The values in the table represent the mean ± standard deviation. Values sharing different uppercase and lowercase letters within the same column and row, respectively, indicate significant differences ($p \leq 0.05$).

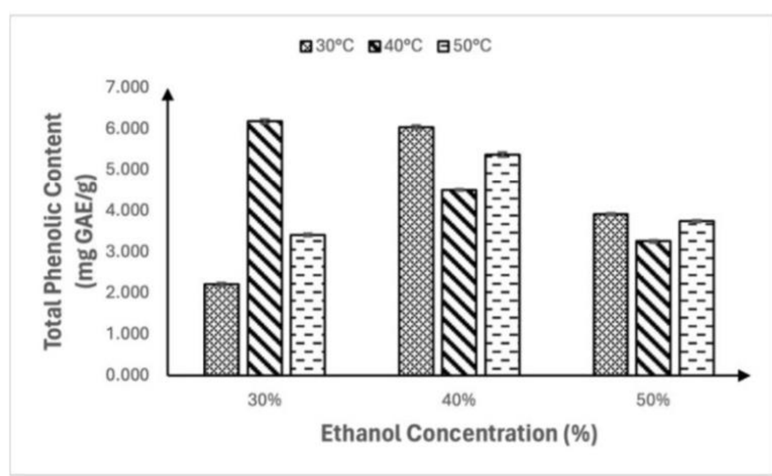

Figure 1. Total phenolic content of tamarind seed extract.

In the present study, extraction solvents were 30%, 40%, and 50% ethanol-water combinations. While ethanol is known to break the bond between solutes and plant matrix, water helps to increase the swelling of plant material. The improved mass transfer by diffusion that results from the greater permeability of plant tissues is responsible for the higher extractability of phenolic chemicals when water is added to ethanol. It was discovered that ethanol-water mixes were more successful in extracting polyphenols. The ethanol content had the biggest impact, as Table 2 illustrates. When the ethanol content rose from 30% to 40%, the yield of total polyphenols increased from 2.21 to 6.04 mg GAE/g at a temperature of 30°C (Figure 1). The solubility of polar polyphenolic compounds was enhanced and mass transfer between the liquid and solid phases was increased by increasing the concentration of ethanol. These results are

consistent with earlier research that showed that adding water to alcohol had a synergistic impact that improved the effectiveness of phenolic component extraction from plant samples. Yang & Zhang (2008) saw a comparable outcome when rutin and quercetin were extracted from *E. Alatus (Thunb.).* They looked at ethanol concentrations of 40%, 50%, 60%, 70%, 80%, 90%, and 100%. Their findings showed that aqueous ethanol at 70% had a greater recovery rate.

Temperature affects many characteristics, including viscosity, diffusivity, solubility, and surface tension, which can affect the extraction yield of the total phenolic component. It appears that the extraction temperature had an impact on the overall phenolic content. The maximum total phenolic content was 2.21 mg GAE/g after 10 minutes at 30°C. Next, the yield rose to 6.18 mg GAE/g at 40°C, and then fell to 3.41 mg GAE/g at 50°C as the temperature rose. Because temperature has an impact on both solute and solvent, it increases UAE yield. A temperature increase increases the solute's solubility and desorption capacity on the one hand, but also lowers the solvent's viscosity, increasing the solvent's diffusivity in the tissue matrix on the other. However, a weaker cavitation effect could result in a yield reduction from additional temperature increases. (Kumar et al., 2020)

3.2.1.2 Effect of Temperature and Ethanol Concentration on Total Flavonoid Content

It was found that solvents had a similar effect on flavonoid compounds as they did on phenolic compounds. At 30°C, the production of flavonoid components rose as ethanol concentration increased. In particular, the yield increased to 2.08 mg QE/g from 1.73 mg QE/g. At a temperature of 50°C and a concentration of 50%, the maximum yield of 2.11 mg QE/g was obtained (Table 2).

Due to the influence of extraction temperature, a similar pattern was seen in flavonoid compounds as in phenolic compounds. The yield slightly increased from 30°C to 40°C at a 30% ethanol concentration, then slightly decreased from 40°C to 50°C.

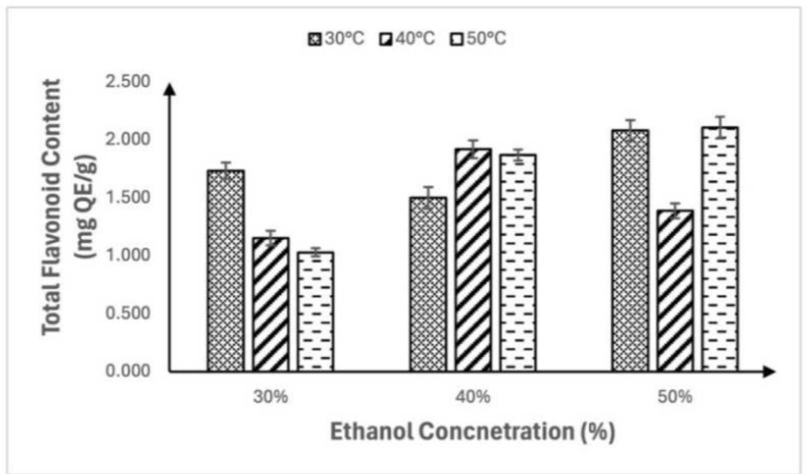

Figure 2. Total flavonoid content of tamarind seed extract

3.2.1.3 Effect on Antioxidant Activity

DPPH Radical Scavenging Activity

It has been observed that the antioxidant capacity assay is greatly impacted by the solvent selection, however not equally across all solvents. For example, solvents with high polarity have been reported to provide lower values for the extent of reduction in the DPPH assay, one of the most widely used assays for assessing the antioxidant activity of phenolic compounds. (Molyneux, 2004)

There was a significant effect of extraction temperature on antioxidant activity. As seen in Figure 3, activity increased significantly as the temperature rose from 30°C to 40°C and then somewhat decreased at 50°C. These changes may be explained by the equilibrium principle, which states that greater temperatures may accelerate the rate of extraction, achieving the maximum recovery of phenolic component content and, as a result, a higher concentration of antioxidant compounds. For antioxidant activity, a 30% ethanol concentration showed a similar pattern.

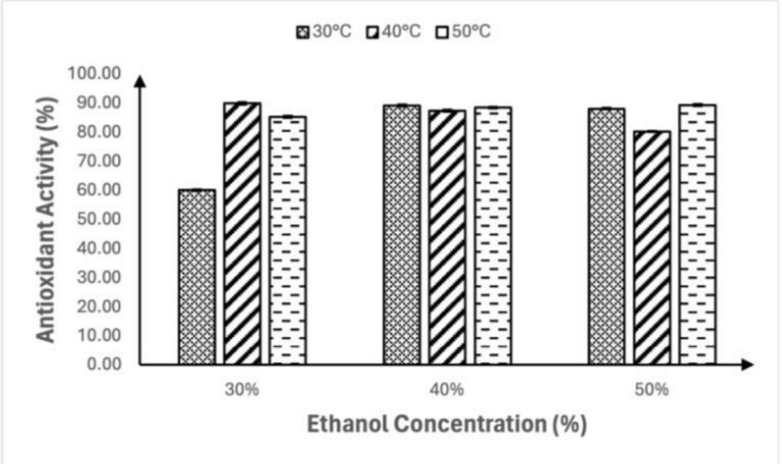

Figure 3. The antioxidant activity by DPPH (%Inhibition) of tamarind seed extract

Ferric Reducing Antioxidant Potential (FRAP)

A modified Fe^{3+} to Fe^{2+} reduction assay was used to measure the extract's reducing power, which can be viewed as evidence of its antioxidant activity. The Fe^{3+}/ferricyanide combination is reduced to the Fe^{2+} form by antioxidants in the sample. Remarkably, Table 2 shows that the ethanol concentration at 40% exhibited the maximum reduction potential of 142.70%. The reducing potential at 30°C and 40% concentration was 118.85%, which was shown to be substantially different ($p < 0.05$). At 30°C, Figure 4 shows that the reducing power increased significantly as the ethanol content increased, going from 30% to 40% before showing a little decline at 50%. The yield fluctuated between 47.60% and 118.85% before falling to 91.53%. The rising polarity of the solvent is the cause of this variance. (Turkmen et al., 2006)

Table 2 shows that the reducing potential at a 30% concentration first increased noticeably from 30°C to 40°C before declining at 50°C. This phenomenon's fundamental cause is similar to what was found in the DPPH Assay.

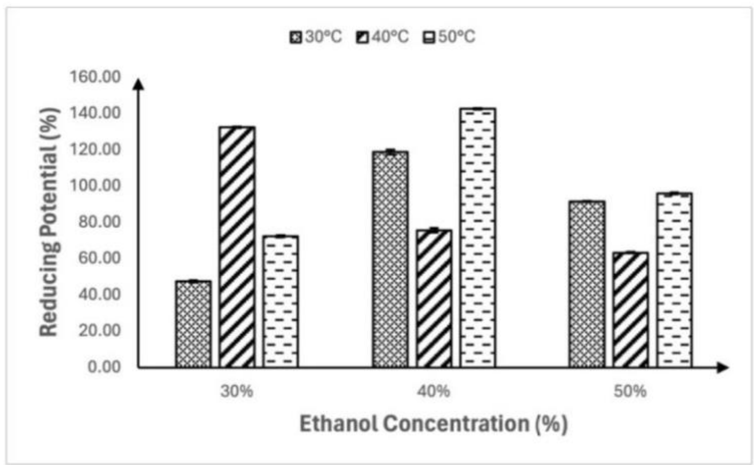

Figure 4. The reducing potential (%Inhibition) of tamarind seed.

The optimal conditions for ultrasound tamarind seed extraction at 50°C and 40% ethanol were found to be 88.53±0.24 and 142.70±0.51, respectively, based on the results of an antioxidant activity test employing DPPH and FRAP assays. Both the total phenolic and total flavonoid contents were found to be significantly enhanced under these conditions.

Relationship between DPPH and FRAP Assay

The correlation between the two antioxidant activity assays, ferric reducing antioxidant power (FRAP) and DPPH (2,2-diphenyl-1-picrylhydrazyl), was examined in this investigation. A moderately substantial positive connection (Pearson correlation coefficient = 0.6936) was found between the two approaches in the data. This implies that they capture some of the same antioxidant characteristics. This could be because antioxidants participate in ferric ion reduction (FRAP) and free radical scavenging (DPPH) by serving as electron donors. Antioxidant-rich samples that function through pathways relevant to both assays are probably going to exhibit a higher correlation. On the other hand, there may be less of a link between samples that have a greater diversity of antioxidant processes. Nevertheless, the composition of the sample may also affects how strong this correlation is. Notably, the association does not prove that the same antioxidants are involved in both activities, and both tests have their limits (Ahoua et al., 2012).

4. CONCLUSIONS

The results of extracting several bioactive elements from tamarind seed were outlined in this study. With the assistance of ultrasound, the ideal extraction parameters were identified. In particular, it was shown that 50°C and 40% ethanol were the ideal extraction conditions for bioactive compounds having antioxidant activity. These findings highlighted temperature and ethanol content as the critical factors. These results will be further developed by characterizing extracts from a

compositional perspective and concentrating on bioactive components. Furthermore, additional experiments will investigate the biological characteristics in other fields with the goal of expanding the usefulness of the recovered substances.

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Effects of Banana flour as wheat flour substitution on physicochemical properties in Financier

AP-O208

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ABSTRACT

Thai society has an increasing number of Non-Communicable Diseases (NCDs) patients, paralleling the rising demand for baked-goods. Banana flour, made from whole unripe green bananas, is a type of resistant starch with key properties such as a low glycemic index, high nutritional value, and many digestive system benefits. The aim of this study was to focus on the physicochemical properties of adding different amounts of banana flour on French almond-egg white cakes, known as Financier. The physiochemical measurements included color analysis, texture profile analysis, water activity, and internal structure imaging using a stereoscopic microscope camera. Results showed that when adding more banana flour in Financier recipes, the color values of L*, a*, and b* and water activity (a_w) decreased significantly ($p < 0.05$) which water activity (a_w) value decreased from 0.67 to 0.59. For texture profile analysis (TPA) indicated that chewiness value and gumminess value significantly increased from 2.16 to 2.96 and from 2.60 to 3.37, respectively ($p < 0.05$). Images from stereoscopic microscope showed that fewer and smaller air cell structure can be seen in a high ratio of banana flour adding in Financier recipe. Therefore, from these results can be concluded that as the proportion of banana flour increases, the Financiers become darker in color and firmer and denser in texture. Financiers with adding more banana flour can prolong shelf life due to the reduction of free water content. In future research, it might be possible to change some other ingredients such as sugar or butter to make the financiers more appealing to NCDs patients.

1. INTRODUCTION

In Bakery world, million tons of wheat flours are used; all-purpose, bread flour, and cake flour, each tailored for specific uses. Wheat flour is a staple ingredient in many baked goods, but its role in the diet of non-communicable disease (NCDs) patients is complex. Wheat flour has many beneficials, though refined wheat flour has a high glycemic index, causing spikes in blood sugar, which is a concern for diabetics and NCDs patients. Despite these drawbacks, wheat flour remains essential in baking due to its unique properties. Gluten provides elasticity and structure, contributing to the texture of bread, cakes, and pastries. In 2022, the market value of bakery using wheat flour is significantly high, approximately 44,000 million baht [1][18]. As the bakery market

evolves, there should be a growing demand for gluten-free and alternative flours as well, however wheat flour remains foundational due to its unique baking characteristics.

In Financier, the small-French almond cake, are normally made with all-purpose flour. It has a soft interior and a crispy exterior, made with ground almonds or hazelnuts, egg whites, brown butter, and icing sugar [2]. Financier has a long shelf life at room temperature, portable, and conveys luxury. It could be energy-boosting desserts, providing protein, fat, and carbohydrates without excessive calories. Their simple preparation, heat resistant and variety of flavours make them ideal as gifts. If developed to be low-calorie with added nutrients, they could gain popularity among health-conscious Thai people.

Resistant starch is a type of carbohydrate that cannot be digested in the stomach or small intestine. Instead, it passes into the large intestine and acts as dietary fibers which helps to improve overall digestive health, reduces cholesterol levels and stabilizes insulin [3]. These pros are beneficial for people with NCDs like diabetes and heart disease. Common types of resistant starch used in Thailand in these days included banana flour, potato starch, rice flour, starch from specific nuts etc. Banana flour, used in this experiment, is a gluten-free flour made from whole unripe green NAM-WA Thai bananas. It's rich in resistant starch and contains low glycemic index, making it a good choice for NCDs patients or those seeking to maintain steady energy levels. It's versatile in baking, offering a subtle banana flavor and a smooth texture. Often used in gluten-free and grain-free recipes [4]. Table 1. shows main nutritional contents in unripe banana flour compare with wheat flour. This information shows that banana flour contains more ashes, fibers and resistant starches contents [5].

Table 1. Chemical compositions of wheat flour and unripe banana flour

Materials/ Parameters	Moisture %	Crude protein%	Ash%	Crude fiber %	Resistant starch%
Wheat flour	11.25	11.2	0.66	0.42	11.72
Banana flour	12.05	4.2	2.5	6.3	53.12

*Reference: Mona M. K. et al. 2017. [5]

This study focus on physicochemical effects of using banana flour instead of wheat flour in variant amount on Financier. Banana flour were then incorporated into Financier batter, resulting in changing of free water contents, color, cellular structure and textural properties. The banana flour replaced wheat flour at the levels of 0%, 50%, and 100%, designated as Ba 0 (control recipe), Ba 0.50, and Ba 1.0 with three replicates for each experiment. The results of this study should guide essential findings for the development of Financier recipe using banana flour to improve its nutritional contents as well as optimal appearances and taste.

2. MATERIAL AND METHODS

2.1 Ingredients

The ingredients used in this study included; Banana flour (house-made in KMITL School of Food Industry, Bangkok, Thailand), All-purpose flour (T55, GRAND MOULINS DE PARIS made in France, protein content 10-11%), Brown butter (Unsalted butter, West gold, Home Fresh Gold, Thailand), Egg white (CP-Pasteurized Egg white bottle, Makro, Thailand), Icing-sugar (Lin, TRR group, Thailand), Baking powder (Imperial Bakers' choice Double Action Baking Powder, Thailand).

2.2 Financier preparation and baking procedure

In this experiment, whole unripe green NAM-WA banana flours were used and prepared by

following steps. Unripe bananas were washed, sliced into pieces, and dried at 60-65 degrees Celsius in tray dried until the moisture content was less than 10%. Then, the dried bananas were ground using blender and pin mill machine to achieved the particle size about 80 mesh. This banana flour was used to mix with other dry ingredients for the next step.

The recipe of this Financier was described by Ecole Tsuji, institute of Patisserie, Tokyo, 2003. Ingredient used in these recipes were summarized in Table 2. Financier was prepared by whisking method, mixing egg white with sugar following by mixing all dry ingredients and wet ingredient, the brown butter. To bake, added the batter into the mold, about 27 grams per each. Baked at 190-200 degrees Celsius for 10-12 minutes or until it became golden brown. Let the financiers cooled for about 1-2 hours at room temperature before measuring its qualities. [2]

Table 2. Formulas of Financier prepared by different amount of banana flour

Ingredients	**Ba 0**	**Ba 0.5**	**Ba 1.0**
Brown butter	26.50%	26.50%	26.50%
Egg white	26.50%	26.50%	26.50%
Almond powder	14.12%	14.12%	14.12%
Icing sugar	21.70%	21.71%	21.70%
Baking powder	0.59%	0.59%	0.59%
All-purpose flour	**10.59%**	**5.29%**	**0.00%**
Banana flour	**0.00%**	**5.29%**	**10.59%**
Total %	100%	100%	100%

*Ba 0 = Banana flour 0% (control recipe), Ba 0.5= Banana flour 50%, Ba 1.0 = Banana flour 100%

2.3 Physiochemical experiments

Financiers from step 2.2 were prepared into two different ways for physicochemical analysis:

- For water activity analysis: cut the financiers into approximately 0.5cm x 0.5cm x 0.5cm (W x L x H), weighting about 4-5 grams.

For texture, color, and internal structure analysis: Take the entire piece with size about 2 cm. x 7 cm. x 4 cm. (W x L x H). This size is used for measuring texture properties, color and cross sectioned for internal structure imaging and height measurement with a stereoscopic microscope.

2.3.1 Water activity (a_w) analysis

Measure the water activity by using a Water Activity Meter (Aqualab 4, USA). Read the a_w value and recorded. This method was adapted from Alexandra M.B. et al., 2021[6]. All samples were performed in triplicated.

2.3.2 Color analysis

For each sample replication, top side of Financiers were cut off to make its total height about 3.5 cm. and then measured in the position of top, bottom, left, and right-side by using a Konica Minolta Chroma Meter, Model CR-400. The color values are represented as followed: L* designated brightness, ranging from 0 (black) to 100 (white); a* indicates red and green tones; and b* signifies yellow and blue shades. This method was adapted from Jum-Soon Park et al., 2010 [8]. All samples were performed in triplicated.

2.3.3 Texture analysis

Sample was prepared similarly to the topic of 2.3.2. Evaluate the texture of Financier using a Texture Analyser (Stable Micro Systems TA-HD plus) with a cylinder probe (P/75). Compress each piece to 50% of its original height. Set the pre-test speed to 1.00 mm/s, the test speed to 2.00 mm/s, and the post-test speed to 5 mm/s. Measure hardness, springiness, chewiness, adhesiveness cohesiveness and gumminess. The distance between Financier and texture analyser should be 5 mm. This method was adapted from Alexandra M.B. et al., 2021 [6]. All samples were performed in triplicated.

2.3.4 Cellular structure analysis

Financiers were cut in cross section with perpendicular to their base (vertical axis), height was measured with stainless ruler and then captured and recorded a photo. For cellular structure analysis, cut Financier in half lengthwise (cross section). Images of the internal structure for analysis the internal cellular with a Stereoscopic microscope with a digital camera, Model E200, at magnifications of x0.67, x1, and x2 were then captured to use for observation the air cells dispersion and their size. This method was adapted from C. Segundo, 2016 [7].

2.4 Statistical & Data analysis

Statistical analysis of the results was completed using the SPSS program (IBM SPSS Statistics 23, International Business Machines Corporation, Armonk, NY, USA). Significant differences were assessed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test at a 95% significance level ($p<0.05$) [15].

3. RESULTS AND DISCUSSION

3.1 Water activity of Financiers

The water activity of Financier for all treatments are presented in Table 4. The result showed that an increasing of banana flour in the Financier recipe significantly reduced the free water content (a_w) ($p < 0.05$) which decreased from 0.67 in control recipe (Ba 0) to 0.61 in Ba 0.5, and 0.59 in Ba 1.0, respectively. As mentioned in Table 1, banana flour contains more dietary fibers and resistant starches than wheat flour, therefore water activity content of Financiers adding with banana flour can be lower value because it was reported that unripe banana flour as a source of resistant starch in bakery product have the greatest effect on reducing water activity [5].

Table 3. Color and water activity values of Financier at different amount of banana flour

Treatments	Parameters			
	L*	a*	b*	a_w
Ba 0	41.55 ± 0.61^a	14.51 ± 0.99^a	13.93 ± 0.37^a	0.67 ± 0.035^a
Ba 0.5	40.49 ± 0.46^b	11.76 ± 0.28^b	12.62 ± 0.15^b	0.61 ± 0.01^b
Ba 1.0	37.54 ± 0.36^c	10.26 ± 0.15^c	9.44 ± 0.53^c	0.59 ± 0.01^b

*Different superscripts in the same column indicate significant differences between values according to Duncan's multiple-range test ($p < 0.05$). Ba 0 = Financier with 0% Banana flour (control recipe); Ba 0.5 = Financier with 50% Banana flour; Ba 1.0 = Financier with 100% Banana flour. Values are the means of triplicate determinations ± SD, n=3.

3.2 Color analysis

While color parameter was studied, it was founded that adding more banana flour into Financier significantly related to its colors ($p < 0.05$). Banana flour quantities were expected to be a main factor of the browning levels in Financiers, considering from its originally dark color. As shown in Figure 1,2 and Table 3, L*, a* and b* value of sample adding more banana flour ranged from 0% to 50% and 100%, the average color values decreased significantly ($p < 0.05$), contrast to the work of

P. Ngamnikom et al. [15] mentioned that yellowness in brownie is a bit higher. It could be said that adding more banana flour in Financier led to darker in color and also lessen redness, yellowness and blue, respectively. Apart from the dark color of banana's peel, this incident might cause by the
nutritional contents in banana flour. The higher proportions of banana flour were added, the more contents of ashes, dietary fibers, resistant starch, and phenolic compound would be high, leading to darker in Financiers color [7][9]. Moreover, the greater amount of banana flour would explain the brown color of both crust and crumb due to Maillard and Caramelization reactions [10].

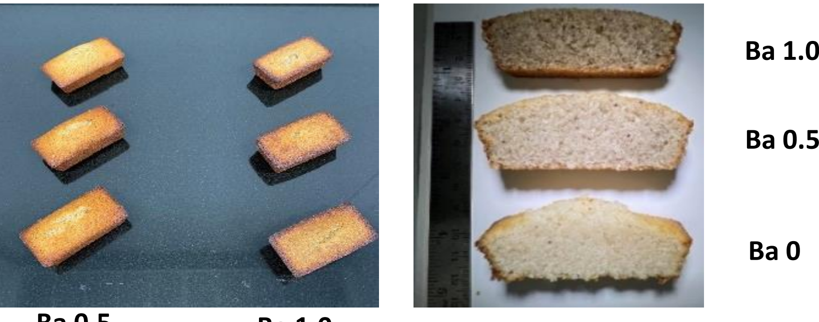

Figure 1. Physical characteristics of Financier in different banana flour contents.

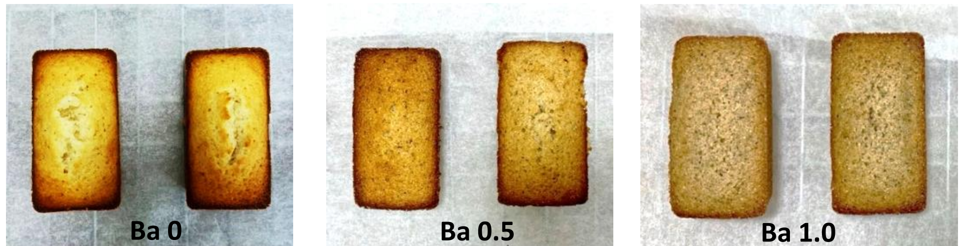

Figure 2. Color characteristics of Financier in different banana flour contents

3.3 Texture profile analysis

Stable Micro Systems TA-HD plus texture analyzer was used to determine the hardness, springiness, chewiness, adhesiveness, cohesiveness, gumminess of the Financier samples after baking. The results in Table 4 showed that the control recipe (Ba 0) has the lowest values of chewiness, adhesiveness, gumminess, as shown 2.16, -9.78(g/sec), 2.60, respectively. These values became higher when adding more banana flour ($p < 0.05$), similarly results from P. Ngamnikom et al., 2023 [15] who used the unripe banana flour instead of wheat flour in brownie products. In case of hardness parameter, Ba 0.5 has the lowest value at 4.23 g compared with other samples ($p < 0.05$). On the other hand, springiness parameter did not show significantly differences which was similar to the result of unripe banana flour in brownie product as well [15]. This can be concluded that adding more banana flour into Financier recipe, could lead to denser and firmer with a tighter crumb due to lower quantity of gluten contents [7] [11]. Lacking of gluten also reduces carbon dioxide expansion, which directly affects to the Financier's quality [12]. Baked goods with banana flour may require additional binders, such as xanthan gum and guar gum or mixed with other gluten-free flours, such as rice flour or leavening agents to improve texture properties as it does not provide the elasticity of gluten.

Table 4. Textural properties of Financier at different amount of banana flour

Properties	Treatments		
	Ba 0	**Ba 0.5**	**Ba 1.0**
Hardness (g)	6.57 ± 0.15^{a}	4.23 ± 0.36^{b}	6.40 ± 0.03^{a}
Springiness ^{ns}	0.86 ± 0.04	0.85 ± 0.03	0.80 ± 0.03
Chewiness	2.16 ± 0.11^{b}	2.61 ± 0.09^{ab}	2.96 ± 0.45^{a}
Adhesiveness (g/sec)	-9.78± 0.71^{b}	-12.52±0.68^{b}	-52.17±13.85^{a}
Cohesiveness	0.58 ± 0.01^{b}	0.64 ± 0.02^{a}	0.58 ± 0.03^{b}
Gumminess	2.60 ± 0.11^{b}	2.92 ± 0.22a^{b}	3.37 ± 0.52^{a}
Height (cm.)	3.32 ± 0.1^{a}	3.12 ± 0.1^{b}	2.5 ± 0.08^{c}

*Ba 0 = Financier with 0% Banana flour (control recipe); Ba 0.5 = Financier with 50% Banana flour; Ba 1.0 = Financier with 100% Banana flour. Results are presented as mean ± standard deviation. Within a row, means with different superscripts are significantly different ($p < 0.05$), n=3.

3.4 Cellular structure analysis

The cellular structures of Financier at different ratios of banana flour were studied by using stereoscopic at 3 different levels of magnification, x0.67, x1.0, x2.0, as shown in Figure 3. It was found that increasing amount of banana flour affected to decrease amount and size of air cell structure. In addition, color as seen in Figure 3 was also became more brownish. These can be implied that adding more banana flour into the Financier recipe can give higher consistency of the batter due to its increasing fiber contents. This incident can decrease air bubbles movements and coalescences, leading to a smaller air cell structure in the Financier [7]. Therefore, it might be indicated that banana flour does not promote good expansion and elasticity in bakery product [12]. However, using this unripe green banana flour still promotes higher height than that of replaced by other starches, such as corn starch [13] [14].

In addition, more added banana flour significantly related to the height of the Financier ($p < 0.05$) as shown the result in Table 4 and Figure 3. It was clearly seen that the wheat flour used as ingredient in Financiers recipe were significantly shown the higher height ($p < 0.05$) than that of the Financiers adding with banana flour of all ratios. The heights of Financier in Ba 0, Ba 0.5, and Ba 1.0 were about 3.3 cm., 3.0 cm., and 2.6 cm., respectively. In short, increasing the amount of banana flour led to shorter height in Financiers due to its lower gluten content, which affected the expansion of the Financier structure. Additionally, more fiber contents in unripe banana flour possess a higher water-binding capacity than wheat flour. This reduces the amount of water available for other ingredients like starch, thereby influencing the characteristics of the products, such as shorter height and firmer texture. [16][17]

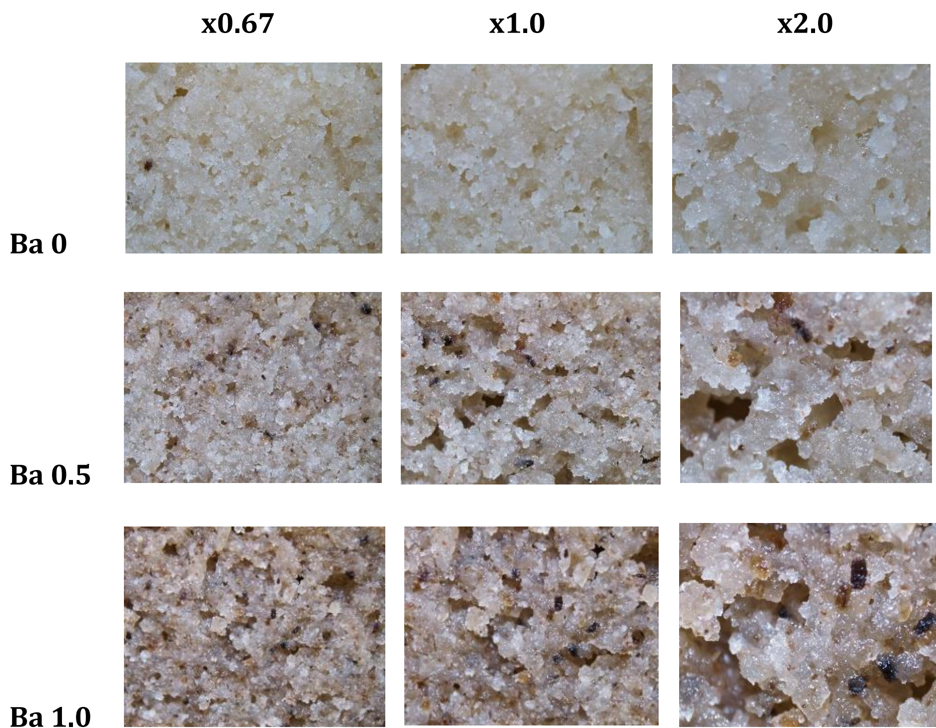

Figure 3. Internal cellular structure of Financier replacing by banana flour

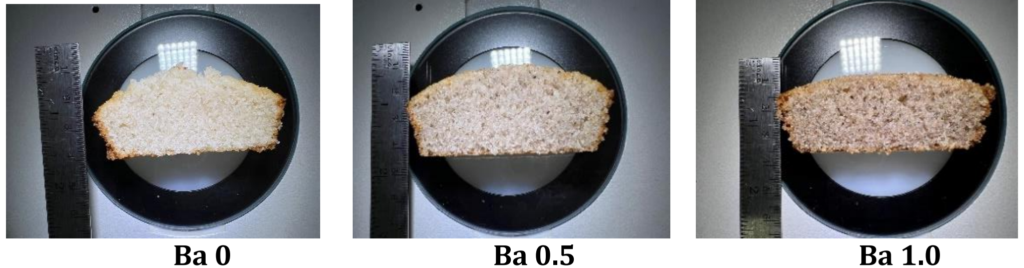

Figure 4. The height of Financiers in each formula

4. CONCLUSIONS

This exploratory work examined the effect of unripe banana flour as wheat flour substitution on physicochemical properties in Financier. The study indicated that unripe banana flour could be added to Financier up to the level of 50% (Ba 0.5) without significantly changed. However, it might not be preferrable to be added up to 100%, considered by the internal structure pictures, and texture properties. Even though, Financier with adding more banana flour (Ba 0.5 and Ba 1.0) can prolong the shelf life due to the reduction of free water contents, however, both recipes showed in less fluffiness, springiness, and higher firm in texture. Additionally, Financiers made with unripe banana flour might have slightly banana flavor, which could alter the intended taste profile. Despite its challenges, banana flour's health benefits and gluten-free properties make it an attractive option for healthier bakery products, especially for those managing blood sugar levels or following a gluten-free diet. In future research, it might be possible to change some other ingredients. For example, sugar and full-fat butter could be changed to other sweeteners, sugar alcohol and other fat replacers,

respectively. In order to make the financiers more appealing to NCDs patients by reducing the sugar amount and overall calories.

5. ACKNOWLEDGEMENTS

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Impact of Protein Concentration on the Stability of Emulsions and Characteristics of Plant-Based Fat Analogs

AP-O212

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ABSTRACT

This study explores the development of plant-based adipose tissue analogs in response to sustainability, health, and ethical concerns associated with meat consumption. The objectives are to investigate the impact of protein concentration on the stability of emulsions and characteristics of plant-based fat analogs. The study was conducted a 70 wt% palm oil-in-water emulsion stabilized by varying concentrations of soy protein isolate (SPI) (4, 6, and 8 wt%) at pH 7, using transglutaminase, and stored at room temperature over a period of 1-7 days. The evaluations included creaming stability, microstructure appearance, textural properties, and color analysis. The findings indicated that the emulsions were unstable across all protein concentrations. For the fat analogs, higher SPI concentrations were found to enhance hardness and cohesiveness but decrease springiness, resulting in a firmer and more cohesive texture. Further research is required to optimize the emulsion formulation and processing to achieve consistent, high-quality textures suitable for food products. This approach holds significant potential for advancing sustainable and ethical food production that meets modern consumer.

1. INTRODUCTION

As of November 2023, the global population stands at 8.1 billion, with projections indicating a rise to 9.7 billion by 2050 [1]. This growth poses a significant challenge to food production, necessitating increased efforts to meet the expanding demand. Concurrently, meat consumption has become a focal point of debate due to concerns about sustainability, health implications, and ethical considerations, including environmental impact, health risks, and animal welfare [2]. Additionally, studies have linked meat consumption to elevated risks of various conditions, including colorectal cancer and gastritis, as well as other diseases [3]. In response, plant-based animal fat analog have gained popularity due to their health benefits and contributions to environmental sustainability.

These fat analog, designed to mimic the properties and functions of animal adipose tissues, are created using synthetic plant-based materials [4]. Recent years have seen various methods employed for structuring these fats, including the Gel Emulsion technique (GE) [4], High internal phase emulsions (HIPEs) [5], and Transglutaminase (TG)-induced crosslinking (TGPC) [7]. GE, using oleogels, transforms liquid oils into semi-solid systems with viscoelastic and hydrophobic properties through organogelation, while emulsion gels form gel-like network structures with solid-like textural properties. Both have been used in meat product formulations as substitutes for saturated fats. However, these methods face challenges such as susceptibility to lipid oxidation during heating and regulatory limitations on certain structuring agents [9]. On the other hand, crosslinked fat crystal networks and transglutaminase (TG)-induced crosslinking involve the emulsification of plant-based lipids. TG are enzymes that catalyze the cross-linking reaction between the γ-carboxamide of a glutamine residue and the ε-amino group of a lysine residue. This process results in the formation of stable, insoluble macromolecular complexes through isopeptide bonds [8]. This process is followed by enzyme-driven covalent crosslinking, creating a bulk fat system that not only mimics the texture of animal fat but also aligns with sustainable food production methods by reducing animal-derived components. These plant-based lipids are better alternatives to animal fats, offering a lower level of saturated fat and cholesterol. From an ethical standpoint, this technology provides a responsible option for consumers who refrain from animal products, representing a significant advancement in food science for developing plant-based substitutes for conventional animal-based ingredients [9, 11, 12]. However, of the main ingredient was using fully hydrogenated canola oil, linked to adverse health effects due to its saturated and trans-fatty acid content [14].

Palm oil stands out as a favored choice in formulating plant-based fat analogs due to its unique properties. It contains a high proportion of saturated fats (44% palmitic acid), making it a suitable ingredient for creating solid or semi-solid fat analogs with a melting point around 35-45°C (95-113°F). Its exceptional oxidative stability, further enhanced by the presence of tocopherols and tocotrienols (forms of vitamin E) and carotenoids like alpha-carotene, beta-carotene, and lycopene, renders it less susceptible to rancidity and spoilage compared to unsaturated fats, which is paramount for ensuring shelf life and stability in food products. Notably, the specific composition of saturated fatty acids in palm oil, including palmitic acid, has been studied for its potential effects on cholesterol levels within a low-fat diet <30% energy intake [10]. Transglutaminase (TG) is a key enzyme that plays a crucial role in catalyzing the formation of covalent bonds between protein molecules, creating crosslinks. This enzymatic process involves the formation of cross-linking isopeptide bonds between the ε-amino group of lysine and the γ-carboxyamide group of glutamine residues in proteins. Consequently, a network of interconnected protein chains is formed, mimicking the structure of animal fat tissue. The application of TG in formulations brings about significant improvements in the hardness and elasticity of food products. It achieves this by inducing new cross-linking within the protein structure, enhancing substance cohesiveness, and catalyzing the formation of covalent bonds between protein molecules. The resulting crosslinks contribute to the cohesion and strength of the protein network, ultimately enhancing structural integrity and stability. This interconnected network effectively holds the protein matrix together, preventing it from breaking apart or losing its shape. The impact of the interconnected protein network extends to the texture of soy protein-based products, influencing attributes such as firmness, elasticity, and chewiness. Additionally, this network enhances the functional properties of soy protein, including emulsification, water-binding capacity, and gelation, thereby leading to improved product stability [13]. According to the previous study, structured lipids were prepared from protein suspensions with varying concentrations of soy protein isolate (SPI) and total fat, each having different solid fat contents derived from fully hydrogenated oil. Samples containing 6% and 8% SPI exhibited specific hardness values, and it was observed that higher concentrations of protein directly increased the hardness. Consequently, the size of the particles was found to increase with the firmness of the sample, which was in correlation with the amount of protein [5]

In this study introduced an innovative approach that involved structuring lipids from palm oil within a crosslinked protein matrix to more closely mimic animal fat tissue. The objectives included investigating the impact of protein concentration from 4% to 8% on the stability of emulsions and the characteristics of plant-based fat analogs, as well as evaluating the potential of these structured fats to replicate the elastic properties of animal fat.

2. MATERIAL AND METHODS

2.1 Materials

For this study, soy protein isolate was sourced from Yunan Road, Qingdao, China. The SPI sample contained 90.17% protein, 3.68% moisture, 0.08% fat, 4.84% ash and 1.23% carbohydrate. Palm oil, branded as YOK, was procured from a local wholesaler in Ubon Ratchathani, Thailand. Transglutaminase (TG), exhibiting an enzyme activity of 120 U/g as specified by the KFDA method, was obtained from YOTABIO, Kinry Food Ingredient Co., Ltd., in Shanghai, China. All chemicals used in the analysis of samples were of analytical grade.

2.2 Preparation of emulsion and plant-based fat analogs using transglutaminase

2.2.1 Emulsion preparation

A soy protein solution was prepared by dispersing soy protein isolate (SPI) at concentration of 4%, 6% and 8 % by weight into distilled water. The pH was adjusted to 7 using 1M HCl and 0.02 % sodium azide was added as an antimicrobial agent. The mixture was stirred overnight. Subsequently, oil-in-water (O/W) emulsions were created by blending 70% palm oil with these various concentrations of soy protein isolate. Additionally, a 30 wt% SPI solution was incorporated using high-shear homogenization at 15,000 rpm for 3 minutes. The emulsion was then sampled for analysis of creaming stability [6].

2.2.2 Preparation of plant-based fat analogs using transglutaminase

After preparing the emulsions, protein cross-linking was initiated by adding 10% transglutaminase of SPI used in the emulsions, followed by stirring. To further induce gelation through protein cross-linking, more SPI was added and stirred into the emulsions. The samples were incubated at 37°C, where transglutaminase (TG) remains highly active, allowing for effective protein cross-linking without denaturation. After incubation, the samples were refrigerated at 5°C overnight for subsequent analysis [6, 14].

2.3 Emulsions and plant-based fat analogs characteristics

2.3.1 Creaming stability measurement

Ten milliliters of emulsion samples were placed in glass test tubes and stored at room temperature for 24 hours prior to analysis. The susceptibility of the emulsion to creaming was determined by measuring the height of the boundary layer between the opaque droplet-rich layer at the top and the transparent or turbid, droplet-depleted layer at the bottom of the test tubes, following the method outlined by [14]. The results of the creaming were quantified using the following equation.

$$Creaming\ index = \left(\frac{Height\ of\ interface}{Height\ of\ total\ emuslsion}\right) \times 100 \qquad (1)$$

2.3.2 Microstructure

The microstructure of the emulsions was observed at room temperature using a ZEISS Primo Star microscope (Carl Zeiss Microscopy GmbH, 07745 Jena, Germany). Photomicrographs were taken at 40x and 100x magnifications with an Axiocam 105 color digital camera, and the images were analyzed using ZEN lite software. To preserve the integrity of the emulsion structures, samples were mounted on microscope slides and covered with a cover slip before observation under an oil immersion objective at 100x magnification.

2.3.3 Texture analysis

Texture analysis of samples was conducted using an Instron testing texture machine (Model TA.XT plusC, UK). Samples were placed in cylindrical glass containers, each weighing 50 grams (diameter: 33 mm; height: 5 cm). After overnight storage at 5°C, the samples were transferred to an ice box and then allowed to equilibrate to room temperature for 15 minutes. The analysis was performed under the following conditions: a pre-test speed of 2.0 mm/s, test speed of 2.0 mm/s, post-test speed of 2.0 mm/s, target mode set to strain, strain of 25%, and duration of 5 seconds. A trigger force of 5 g was applied, with tear mode set to auto and advanced options enabled. A cylindrical probe (diameter: 36 mm) was used. The force as a function of time was recorded after the probe deformed the samples twice by uniaxial compression at 2 mm/s to 25% of their original height. Hardness is defined as the maximum force exerted during the first compression cycle, springiness is the difference in recoverable specimen height between the first and second compression cycles, and cohesiveness is the extent to which the sample can deform before rupturing.

2.3.4 Color

The color of all of the samples was measured by Color Colorimeter (A60-1017-658, Herndon, VA 20190, USA). This instrument was employed to assess the CIE L*a*b color values of each sample.

2.4 *Experimental Design and Statistical Analysis*

The experimental design was implemented using a randomized complete block design (RCBD) with three replicates. For the statistical analysis, data were analyzed using ANOVA to determine the significant differences between the treatments at a 95% confidence interval ($p < 0.05$). Where significant differences were observed, a Duncan's multiple range test was conducted to compare the means of each treatment group utilizing SPSS software version 15.0.

3. RESUTS AND DISCUSSION

3.1 Influence of protein concentration on emulsion formation and stability

The purpose of this section was to determine the required amount of SPI to achieve stable emulsion formation. Figure 1 displays the appearance of samples in glass test tubes, stored at room temperature for 1, 2, 3, and 7 days for subsequent analysis. The result indicated that the emulsions at all tested protein concentrations were unstable, with no distinct creaming layer observed in the test tubes after 1 and 2 3, and 7 days at room temperature. These findings suggest that the protein present were insufficient to adequately cover the surfaces of the oil droplets formed in the hand homogenizer. The high shear rate of 15,000 rpm for 3 minutes may not have been sufficient to achieve the optimal particle size and emulsion stability. Furthermore, the critical ratio of protein to oil plays a significant role in forming stable emulsions. The SPI-to-oil ratio (30:70) used did not provide enough protein to adequately cover the oil droplets, leading to instability.

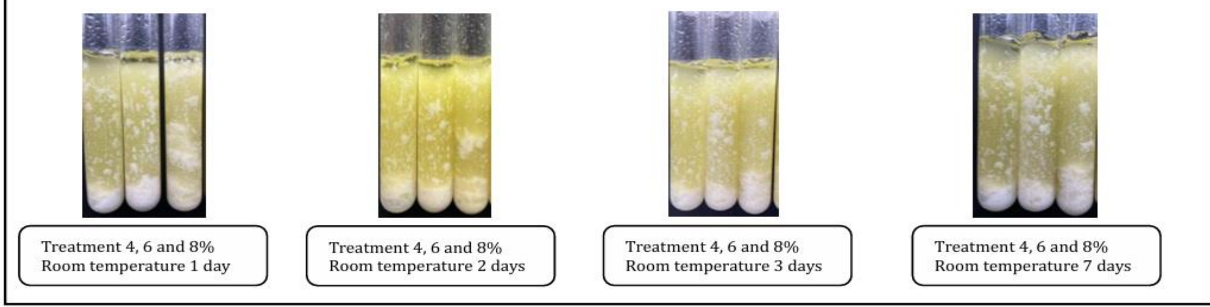

Figure 1. Visual observation of 70 wt % palm oil-in-water emulsion stabilized by different SPI concentration (4, 6, and 8 wt %) at pH 7 after storage at room temperature for 1, 2, 3, and 7 days

The lack of stability in the emulsions suggests that the electrostatic repulsion might not have been strong enough to prevent droplet coalescence [15]. However, the instability of the emulsions could be due to suboptimal particle size and viscosity, an inadequate protein-to-oil ratio, non-ideal pH conditions, insufficient electrostatic repulsion, or a combination of these factors. For future experiments, it is recommended to adjust these parameters and possibly incorporate other proteins or emulsifiers to enhance stability.

Even though the soy protein isolated at different concentrations ranging from 4-8% did not provide sufficient stability but showcases photomicrographs of a 70 wt% palm oil-in-water emulsion stabilized by various soy protein isolate (SPI) concentrations (4%, 6%, and 8%) at pH 7, was observed after storage at room temperature for 24 hours. As the concentration of SPI was increased, the size of the oil droplets in the emulsion decreased, leading to a more stable structure since smaller droplets are less prone to merging into larger ones. This is particularly important as the size of the droplets is a critical factor in the stability of emulsions [5]. Stability is also paramount when it comes to fat analogs like emulsions, which are designed to replicate the sensory qualities of fats in food products. A stable emulsion is essential for the right texture and mouthfeel, ensuring that the product resembles traditional foods containing fat. The study also noted that the reduction in droplet size, achieved by increasing SPI concentration, lessens the chance of coalescence, where droplets combine and cause phase separation, which enhances the emulsion's stability. For future research, should be recommended to closely monitor the stability of the emulsion under specific conditions, such as maintaining a neutral pH of 7 and storing the emulsion at room temperature for 24 hours. These factors play a significant role in the stability of the emulsion.

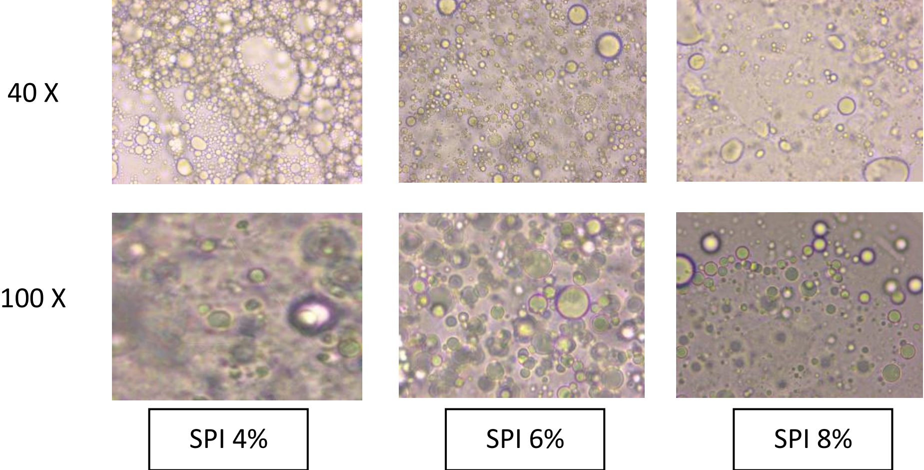

Figure 2. Photomicrographs of 70 wt % palm oil-in-water emulsion stabilized by different SPI concentration (4, 6, and 8 wt %) at pH 7 after storage at room temperature for 24 hours

3.2 Influence of protein concentration on plant-based fat analogs characteristics

Figure 3 illustrates that all plant-based fat analogs can form a similar soft structure, however, all samples exhibited oil release at room temperature (30-35 °C) after 2 days. The prolonged oil release may be attributed to the gel structure low capacity to retain both oil and water within the system, which likely destabilized the firm matrix observed in product. The destabilization resulted in evident phase separation after sample homogenization. The oil release of these samples can be scientifically explained by considering factors such as the protein-to-oil ratio and the activity of transglutaminase. The chosen 30:70 protein-to-oil ratio have been too low to form a stable emulsion capable of withstanding higher temperatures. Additionally, transglutaminase was incorporated to promote cross-linking within the protein structure, but the ambient temperature and the proportion used have been ideal for forming a stable network. The samples' inability to retain their structure at

room temperature, which is significantly higher than the refrigeration temperature of 5°C where they were initially stable, further indicates temperature sensitivity.

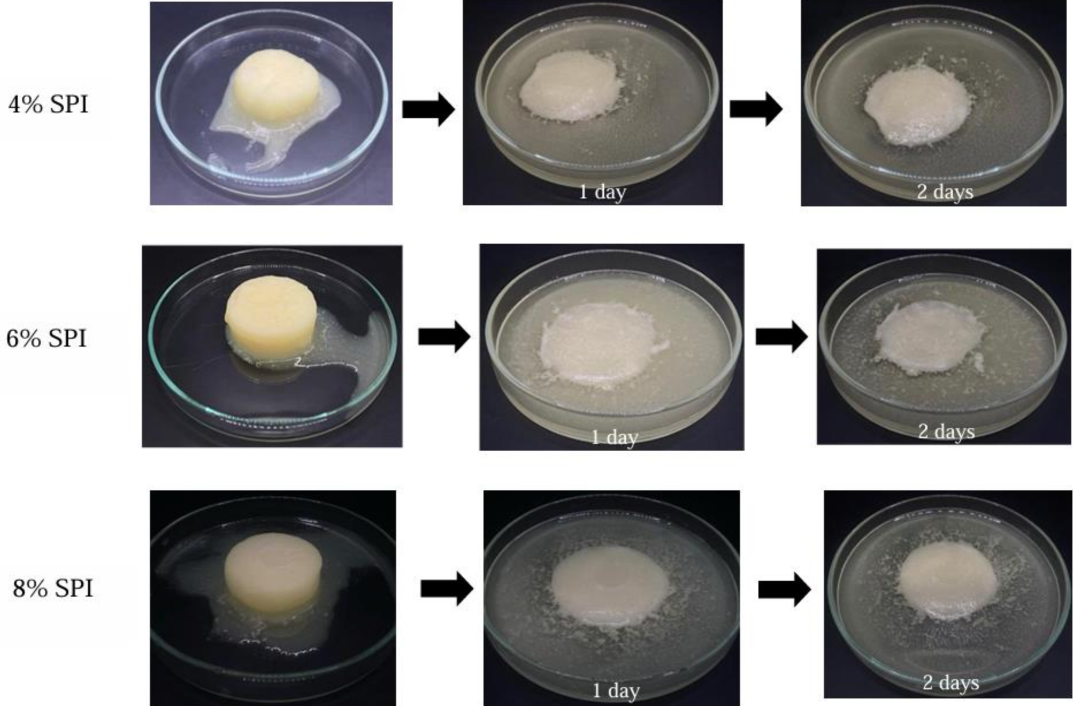

Figure 3. Plant-based fat analogs with 70% palm oil and varied SPI concentrations (4%, 6% and 8%) stabilized by transglutaminase crosslinking

Color characteristics were presented in table 1, specifically the lightness (L*), red/green value (a*), and yellow/blue value (b*), of plant-based fat analogs composed of 70% palm oil with varying concentrations of soy protein isolate (SPI) at 4%, 6%, and 8%. These analogs were solidified using transglutaminase crosslinking. The data indicates that as the SPI concentration increases from 4% to 8%, the lightness of the fat analogs also rises, moving from an L* value of 74.98 to 88.22. This trend suggests that higher SPI levels lead to a lighter shade of the analogs. Regarding the a* values, which denote the color's position between red and green, all samples exhibited negative values, signifying a greenish cast. However, this greenish cast diminishes as the SPI concentration grows, with the a* values becoming less negative, shifting from -5.33 at 4% SPI to -3.58 at 8% SPI. For the b* values, which reflect the color's position between yellow and blue, there was a minor increase from 13.65 at 4% SPI to 14.34 at 6% SPI, followed by a slight decrease to 14.27 at 8% SPI. These variations are likely due to the interaction emulsion soy protein and palm oil, as well as the stabilizing influence of transglutaminase on the emulsion. The texture profile study for soy protein isolate (SPI) emulsions, revealing that increasing the SPI content improves the textural properties in (table 2). Notably, the hardness of the emulsions, which indicates the force required for deformation, increases significantly in higher SPI levels. The emulsion with 4% SPI has a moderate hardness of 2603.37, indicating a consistent texture. The 6% SPI emulsion is firmer with a hardness of 3818.00, and the 8% SPI emulsion is the firmest, registering a hardness of 4542.67, albeit with some variation. The springiness, or the emulsion's ability to return to its original shape after compression, exhibits greater variability in the 4% SPI emulsion.

Table 1. Color (L*, a* and b*) of plant-based fat analogs with 70% palm oil and varied SPI concentrations (4%, 6% and 8%) stabilized by transglutaminase crosslinking

Sample	L*	a*	b*
4% SPI	88.21 ± 1.25^{a}	-2.76 ± 0.20^{a}	14.27 ± 0.59^{a}
6% SPI	85.93 ± 3.97^{a}	-3.58 ± 1.27^{ab}	14.58 ± 0.38^{a}
8% SPI	74.97 ± 4.76^{b}	-5.33 ± 1.03^{b}	13.65 ± 1.35^{a}

*The results are presented as the mean (n = 9) ± SD, and those with different letters are significantly different ($p < 0.05$) within the same columns.

In contrast, the 6% and 8% SPI emulsions show a significant reduction in springiness, with the latter suggesting a shift towards a more solid texture. Cohesiveness, the measure of resistance to subsequent deformations, also varies, with the 6% SPI emulsion displaying a notable increase, which may point to measurement inconsistencies or sample preparation variations. Gumminess and chewiness, which are derived from hardness, cohesiveness, and springiness, increase with SPI concentration. The 4% SPI emulsion shows moderate variability in these properties, while the 6% and 8% SPI emulsions have higher values, indicating a gummier and chewier texture. The 8% SPI emulsion is characterized by its firmness and cohesiveness. These results suggest that higher concentrations of SPI result in firmer and more cohesive emulsions, which could influence the sensory qualities of the final product. The use of transglutaminase in the process seems to have effectively produced stable emulsions with distinct textures. However, the variability observed, especially in springiness and cohesiveness, may need to be addressed to ensure uniformity in the product. Optimizing the emulsion formulation and processing conditions may help achieve the desired textural properties for specific culinary applications.

Table 2. The textural properties of plant-based fat analogs with 70% palm oil and varied SPI concentrations (4%, 6% and 8%) stabilized by transglutaminase crosslinking

Sample	Hardness	Springiness	Cohesiveness	Gumminess	Chewiness
4% SPI	2603.37 ± 69.45^{c}	0.50 ± 0.06^{a}	0.24 ± 0.04^{b}	667.41 ± 18.20^{c}	310.49 ± 9.42^{c}
6% SPI	3818.00 ± 25.48^{b}	0.54 ± 0.01^{a}	0.51 ± 0.7^{a}	814.60 ± 2.37^{b}	355.26 ± 4.69^{b}
8% SPI	4542.67 ± 21.40^{a}	0.11 ± 0.06^{b}	0.11 ± 0.22^{c}	956.44 ± 3.16^{a}	445.09 ± 7.69^{a}

*The results are presented as the mean (n = 9) ± SD, and those with different letters are significantly different ($p < 0.05$) within the same columns.

4. CONCLUSIONS

This study investigated the impact of protein concentration on the stability of emulsions and the characteristics of plant-based fat analogs. Our findings demonstrate that the concentration of Soy Protein Isolate (SPI) significantly influences the microstructure, texture, and color of the emulsions, which are critical factors in the development of plant-based fat analogs for food applications. The study introduces an innovative approach involving lipid structuring within a crosslinked protein matrix to closely mimic animal fat tissue. The objectives were successfully met by evaluating the potential of structured fats in replicating the elastic properties of animal fat. The process parameters, including the use of transglutaminase, were effective in producing stable emulsions with distinct textural characteristics.

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Effects of fish cultivation systems and cutting methods on quality of farmed hybrid catfish (*Clarias macrocephalus x Clarias gariepinus*) during frozen storage

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Protein properties

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ABSTRACT

The quality changes of fish during frozen storage, particularly concerning protein alterations, are influenced by fish cultivation systems and cutting methods. This study investigated these effects on farmed hybrid catfish (*Clarias macrocephalus x Clarias gariepinus*) over a three-month frozen storage period. Hybrid catfish, cultivated through conventional and biofloc systems, were processed using three cutting methods: whole fish without organs, steak, and fillet with skin. All samples were frozen at -35 ˚C and subsequently stored at -18 ˚C for three months. Physical and chemical properties, including drip loss, color (L*, a*, and b*), whiteness, water holding capacity (WHC), pH value, salt-soluble protein content (SSP), surface hydrophobicity (S_0), total sulfhydryl (TSH) content, and texture profile analysis (TPA), were analyzed. The results demonstrated that both cultivation systems and cutting methods significantly influenced the quality of the frozen fish. Whole fish samples exhibited lower drip loss compared to steak and fillet samples ($P<0.05$). WHC decreased as the storage period progressed, while pH values decreased, ranging from 6.79 to 7.16 after three months. L* and b* values decreased, whereas a* values increased during storage ($P<0.05$). Whiteness declined throughout the storage period. The study further revealed that cultivation systems and cutting methods affected SSP contents. A significant increase in S_0 and a decrease in TSH contents were observed. TPA decreased throughout storage, with biofloc-cultivated fish showing higher cohesiveness and springiness. Whole fish cuts retained superior texture over time. These findings suggest that biofloc technology enhances the quality of frozen fish, particularly in relation to protein changes.

1. INTRODUCTION

Hybrid catfish (*Clarias macrocephalus x Clarias gariepinus*) is a valuable freshwater fish in Thailand. In 2022, the production of catfish, including hybrids, reached approximately 107,584 tons, valued at 4,989 million baht (Fisheries Development Policy and Planning Division, 2023). Recently, modern farming technologies have been employed in hybrid catfish cultivation. One such innovation is biofloc technology, which presents several advantages over traditional extensive and semi-extensive aquaculture systems. Biofloc technology requires minimal or no water exchange, reduces environmental impact, recycles nitrogen compounds, synthesizes bacterial biomass, and provides a highly nutritious supplementary food source (Nugroho, Khakim, & Dewi, 2020).

The methods of fish cultivation, including species, feed, and environmental conditions, significantly influence the physicochemical properties and overall quality of the fish. Additionally, cutting methods prior to freezing are crucial for maintaining the quality of frozen fish products. Studies have shown that different cutting techniques influence the physicochemical, microbiological, and sensory characteristics of frozen fish, affecting overall quality and shelf life (Tabla, 2013; Liu et al., 2022). Moreover, storage temperatures, such as sub-cryoscopic conditions, can slow spoilage processes and extend the storage duration of frozen fish products, with cutting methods influencing the storage time before spoilage (Arkhipov, Kharenko, Bindyukova, Lavrukhina, & Syomushkina, 2022).

Freezing is a highly effective method for preserving fish, significantly extending shelf life. However, frozen storage can lead to chemical and physical changes, such as protein denaturation, enzymatic activity, fat oxidation, ice crystal formation, and drying out of the flesh (Shi et al., 2020). Prolonged storage weakens the water-binding capacity of fish, reducing its water-holding ability (Chan, Roth, Jessen, Jakobsen, & Lerfall, 2021). These changes can limit storage time and affect product quality. Quality deterioration in frozen fish is influenced by extrinsic factors, such as freezing rate, storage temperature, and temperature fluctuations, and intrinsic factors, like biochemical properties of the fish. Protein, the most abundant component in fish, plays a significant role in quality during frozen storage. Protein changes, such as myofibrillar protein denaturation, lead to textural changes and decreased protein solubility (Supawong et al., 2018). Protein oxidation reduces total sulfhydryl content (Zhou et al., 2022), and ice crystal formation can destroy the hydrophobic structure of salt-soluble proteins, exposing more hydrophilic groups and altering solubility (Yan et al., 2023).

The quality changes of hybrid catfish under different cultivation systems and cutting methods have not been studied extensively. Therefore, this research aims to examine the effects of fish cultivation systems and cutting methods on protein-related quality properties of farmed hybrid catfish during three months of frozen storage.

2. MATERIAL AND METHODS

2.1 Hybrid catfish

Conventional and biofloc-cultivated hybrid catfish (*Clarias macrocephalus x Clarias gariepinus*) with an average weight of 200±50 g were obtained from the aquaculture farm of Omega and Organic Farm Co., Ltd., located in Ubon Ratchathani, Thailand. Dead fish were transported by immersion in ice-cold water set at -10 ˚C and delivered to the laboratory within 2 hours. Upon arrival, the fish were chilled with flake ice (ice/fish ratio 1:1) in polystyrene boxes. Three types of fish cuts (Figure 1), including whole fish (without organs), steak (cut from across the fish's body), and fillet (with skin), were prepared. Six samples, comprising whole conventional-cultivated hybrid catfish (CW), conventional-cultivated hybrid catfish steak (CS), conventional-cultivated hybrid catfish fillet (CF), whole biofloc-cultivated hybrid catfish (BW), biofloc-cultivated hybrid catfish steak (BS), and biofloc-cultivated hybrid catfish fillet (BF), were individually quick-frozen at -35 ˚C using an Air Blast

Freezer (W10U, Coldine, Italy) and then vacuum-packed with polyamide and polyethylene (PA/PE) bags. The frozen fish samples were stored at -18±2 ˚C for 3 months. Samples were thawed at 10±2 ˚C for 14 hours for further analysis.

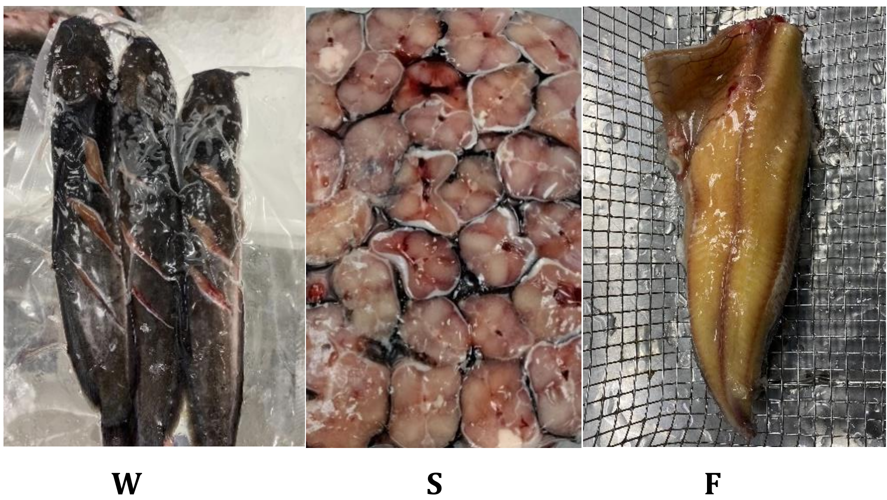

Figure 4. Three types of fish cuts. W=whole fish (without the organ), S= steak that cut from across the fish's body, F= fillet with skin.

2.2 Drip loss

Drip loss was determined according to the method of Sathivel et al. (2007) with slight modification. Samples were thawed at 10±2 ˚C for 14 h for further analyses. Each sample was weighted before freezing and after thawing process. The difference between the two-measured weights was recorded and calculated according to the following equation (1).

$$\text{Drip loss (\%)} = \frac{\text{weight before freezing - weight after thawing}}{\text{weight before freezing}} \times 100 \quad (1)$$

The measurements were taken 5 times.

2.3 Water holding capacity (WHC)

WHC was determined according to the method of Yu et al., (2022) with minor modification. Fish sample (2 g) was weighted accurately (W1) and wraped by two-layer filter papers (Whitman No.1), and then immediately centrifuge at 2,000 rpm (Hermle, Z36HK, Germany) at 4 ˚C for 10 min. After centrifugation, the sample was weighted again (W2). The weight loss after centrifugation was calculated as the equation (2).

$$\text{Water Holding Capacity (\%)} = \frac{W_2}{W_1} \times 100 \quad (2)$$

2.4 pH value

The pH value of frozen fish after storage was evaluated according to the method of Linn et al., (2022) with slight modification. Five gram of minced fish was homogenized with 50 mL of distilled water in 1 min using Ultra T25 homogenizer (Labortechnik homogenizer, IKA, Malaysia). The pH of

the homogenized sample was measured using pH meter (BP-10, Satorious, Germany). The measurements were taken 5 times.

2.5 Color measurement

Color parameters were measured using a colorimeter (A60-1017-658, Herndon,VA 20190, USA). and recorded as L^* (lightness), a^* (redness) and b^* (yellowness). The measurements were taken 5 times. Whiteness was calculated according to the following equation (3).

$$Whiteness = 100\sqrt{(100-L^*)^2 + a^{*2} + b^{*2}} \qquad (3)$$

2.6 Salt soluble protein content

The content of salt soluble protein was determined following the method of Balange and Benjakul (2010) and Endoo and Yongsawatdigul (2014). Fish sample was homogenized with 0.6 M NaCl, 20 mM sodium phosphate buffer (pH 7.0) at a ratio of 1:9 (w/v). The mixture was homogenized at 10000x g for 60s and then was centrifuged at 8,000 x g at 4 °c for 20 min. The supernatant was collected and then the salt soluble protein was determined by Bradford method using bovine serum albumin (BSA) as a standard (Bradford,1979). The measurements were taken 5 times.

2.7 Surface hydrophobicity

Surface hydrophobicity was determined by the method of Yongsawatdigul and Park (2002). Protein solution diluted to 0.1 to 1 mg/mL in an aqueous solution of 0.6 M NaCl, 20mM sodium phosphate buffer (pH7). Ten µL of 1-Anilinonaphthalene-8-sulfonic acid (ANS) 8 mM in 0.1 M sodium phosphate buffer, pH 7) was added to 2 mL of the diluted protein. The relative fluorescent intensity of ANS-protein conjugates was measured with spectrofluorometer (LS-55, PerkinElmer, UK) at 374 nm (excitation) and 485 nm (emission) wavelengths. Protein hydrophobicity was calculated from the initial slopes of plots of relative fluorescence intensity and protein concentration (w/v) using linear regression analysis.

2.8 Total sulfhydryl content

Total sulfhydryl content measured by Ellman's reagent according to the method of Monahan et al. (1995) with slight modification. Sample protein (4 mg/mL) was homogenized in solubilizing buffer (0.2M Tris-HCl, 2%SDS, 10mM EDTA, 8M Urea, pH 7). The homogenates were heated at 100 °C and centrifuged at 10,000 x g for 15 min. To 1 mL aliquot of supernatant was collected and added 0.01 mL Ellman's reagent (10mM 5,5-dinitrobis [2 nitrobenzoic acid] in 0.1M sodium phosphate buffer pH 7.2). The mixtures were incubated at 40 °C for 25 min. The absorbance of the mixtures was measured at 412 nm using a UV-visible spectrophotometer (PG instrument Limited, T60uv/visible, USA) and was used to calculate the total SH content using the extinction coefficient of 13600 M-1 cm.

2.9 Texture Profile Analysis (TPA)

Texture profile analysis was conducted using Texture analyzer (TA.XT plusC, UK) according to Xie et al. (2023) with minor modification. Samples were cut into 2x2x1 cm. Flat faced cylindrical probe of 36 mm diameter equipped with a load cell of 1 kg and a test speed of 1 mm/sec with a trigger force of 5 g. Samples were subjected to a double compression of 50%. The texture profile was

evaluated from the force-time plot hardness (g), adhesiveness(g/sec), springiness (mm), chewiness (g mm), gumminess, and cohesiveness The measurements were taken at least 10 times.

2.10 Statistical analysis

A completely randomized design was adopted to analyze the effects of various fish treatments. The data were analyzed for the degree of variation and significance of differences using an analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was applied to estimate the significance of differences among treatment mean values. The statistical significance was assessed at the 5% level ($P < 0.05$)

3. RESULTS AND DISCUSSION

3.1 Drip loss

Drip loss of conventional and biofloc cultivated hybrid catfish with various cuttings method during frozen storage for 3-months (Table 1), indicating that frozen storage affected weight changes and ability to retain water within fish muscle. This would represent protein denaturation and aggregation during frozen storage. Across all cutting methods, biofloc-cultivated fish (BW, BS, BF) generally exhibited higher drip loss compared to conventional-cultivated fish (CW, CS, CF). This indicated that the cultivation method has a significant impact on drip loss, with biofloc cultivation leading to higher moisture loss over the storage period. The higher drip loss in biofloc-cultivated fish could be attributed to differences in the composition of the flesh or the way moisture is retained within the fish tissue. Fillet cuts (CF, BF) consistently showed higher drip loss compared to whole fish (CW, BW) and steak cuts (CS, BS). This suggests that the cutting method has a significant effect on drip loss, with fillet cuts being more susceptible to moisture loss compared to whole fish and steak cuts. The higher surface area exposed in fillet cuts could contribute to increased moisture evaporation over time. The interaction between cultivation and cutting methods is evident in the differing drip loss patterns observed across treatments. Conventional-cultivated whole fish had lower drip loss compared to biofloc-cultivated whole fish, this difference might vary when considering other cutting methods. During cutting or filleting, the fish muscle is exposed which increases the surface area and then subsequently the potential for water loss. Rotabakk, Melberg, & Lerfall (2017) reported that the interactions between localization and season and localization and rigor affected the drip loss significantly. The results indicated that the impact of cultivation methods on drip loss might vary depending on the cutting method used.

Table 3. Drip loss (%) of conventional and biofloc cultivated hybrid catfish during frozen storage for 3 months

Treatments	Drip loss (%) Storage period (months)			
	0	**1**	**2**	**3**
CW	2.10±0.29^{b}	2.42±0.44^{b}	3.06±1.23^{b}	3.44±0.95^{b}
CS	2.61±1.3^{b}	4.02±1.51^{b}	4.28±0.21^{b}	5.03±1.42^{b}
CF	4.78±3.70^{ab}	4.79±3.20^{b}	5.44±2.90^{b}	5.52±0.33^{b}
BW	4.22±0.10^{b}	5.08±0.28^{b}	5.29±0.62^{b}	5.37±0.64^{b}
BS	7.95±1.33^{a}	9.58±2.12^{a}	9.69±1.11^{a}	9.50±0.26^{a}
BF	7.85±1.53^{a}	9.58±2.15^{a}	8.68±0.90^{a}	8.88±1.55^{a}

* All values are mean ± standard deviation of three replicates (n = 3).
Different [a,b,c] lowercase letters within a column indicate a significant difference (P<0.05).
CW=whole conventional-cultivated hybrid catfish without the organ, CS=conventional-cultivated hybrid catfish steak, CF=conventional-cultivated hybrid catfish fillet, BW=whole biofloc-cultivated hybrid catfish without the organ and BS=biofloc-cultivated hybrid catfish steak, BF=biofloc-cultivated hybrid catfish fillet with skin

3.2 Water holding capacity (WHC)

WHC of conventional and biofloc cultivated hybrid catfish with various cuttings method during frozen storage for 3-months. The average initial WHC ranged from 82.31 to 89.82% (Table 2). According to the various cutting methods, there were no significant differences observed in WHC between conventional-cultivated (CW, CS, CF) and biofloc-cultivated fish (BW, BS, BF) at each storage period. This suggests that the cultivation method did not have a consistent impact on WHC over time, regardless of the cutting method used. The lack of significant differences indicated that both cultivation methods might have similar effects on the ability of fish tissue to retain water during storage. Consider to both conventional and biofloc cultivation methods, there were also no significant differences in WHC observed among whole fish (CW, BW), steak (CS, BS), and fillet (CF, BF) cuts at each storage period. The lack of significant differences in water holding capacity across both cultivation and cutting methods were detected (P>0.05). Jorpeland, Imsland, Stien, Bleie, & Roth (2013) found the same effect in farmed cod, where filleting method, pre-slaughter stress and storage had no significant difference in WHC except season factors. However, WHC of all frozen hybrid catfish samples increased when storage period increased. Water holding properties include drip loss and water holding capacity, two representative indicators for freshness considering the affinity between fish muscle and water (Walayat, et al., 2023). This resulted indicated the storage period influenced the WHC of frozen hybrid catfish and implied the decrease in fish freshness during frozen storage. WHC related to drip loss when the storage period increase. WHC decrease with storage time. It relates to drip loss increase when storage time increase, indicating water can release from fish structure. The physical damage cause by ice crystal to the cell membrane was the direct cause water loss (Li et al.,2023)

Table 4. Water holding capacity (%) of conventional and biofloc cultivated hybrid catfish during frozen storage for 3 months

Treatments	Water Holding Capacity (%) Storage period (months)			
	0[ns]	1[ns]	2[ns]	3[ns]
CW	89.82±1.52	80.62±2.90	74.83±2.17	74.03±2.17
CS	85.00±3.6	82.36±0.25	75.52±1.27	75.50±1.27
CF	83.65±1.82	83.59±1.64	76.62±1.44	74.22±1.58
BW	83.59±2.65	83.66±2.87	76.17±0.50	71.88±1.56
BS	83.53±2.91	83.28±4.14	72.16±1.53	70.15±3.16
BF	84.16±0.95	84.06±2.84	76.82±0.74	74.93±2.34

*All values are mean ± standard deviation of three replicates (n = 3).
Different [a,b,c] lowercase letters within a column indicate a significant difference (P<0.05).
[ns] Not significant (P>0.05).
CW=whole conventional-cultivated hybrid catfish without the organ, CS=conventional-cultivated hybrid catfish steak, CF=conventional-cultivated hybrid catfish fillet, BW=whole biofloc-cultivated

hybrid catfish without the organ and BS=biofloc-cultivated hybrid catfish steak, BF=biofloc-cultivated hybrid catfish fillet with skin

3.3 pH value

The pH value of conventional and biofloc cultivated hybrid catfish with various cuttings method during frozen storage for 3-months (Table 3). The average pH of frozen samples (month 0) ranged from 6.45-6.74. The cultivation factors had no significant effects on the pH value, whereas the fish cutting method influenced the pH value during frozen storage. For cutting methods, significant differences were observed in pH values between conventional-cultivated (CW, CS, CF) and biofloc-cultivated fish (BW, BS, BF) after 2-months storage periods (P<0.05). Biofloc-cultivated fish tended to have slightly higher pH values compared to conventionally-cultivated fish, indicating a potential difference in the acidity or alkalinity of the fish tissue. This difference could be attributed to variations in the cultivation environment or diet between conventional and biofloc systems, which might influence the metabolic processes and ultimately affect pH levels in the fish tissue. These results are in line with Gandotra, Koul, Gupta, & Gupta (2014) who reported the pH value of frozen Rohu fillets showed an increasing trend with increase in frozen period. The pH of whole ungutted aquacultured rainbow trout increase during 12 days chilling at 0-2 °C due to accumulation of alkaline compounds through autolytic activity and microbial metabolism (Ninan, Lalitha, Zynudheen, & Joseph, 2011). Increase in pH of frozen hybrid catfish samples during frozen storage might be contributed by bacterial growth that produces the basis components such as ammonia. The significant differences in pH values observed across different cultivation and cutting methods indicate an interaction between these factors in influencing pH levels in the fish tissue. Adjustments in cultivation practices or cutting methods may be necessary to achieve consistent pH levels throughout the storage period

Table 5. pH value of conventional and biofloc cultivated hybrid catfish during frozen storage for 3 months

Treatments	pH value Storage period (months)			
	0^{ns}	1^{ns}	2	3
CW	6.50±0.38	6.78±0.03	6.78±0.09^{c}	6.79±0.04^{cd}
CS	6.58±0.40	7.03±0.03	7.02±0.08^{a}	7.16±0.09^{a}
CF	6.74±0.17	7.04±0.023	7.07±0.06^{a}	7.01±0.09^{b}
BW	6.63±0.10	6.69±0.04	6.82±0.01^{c}	6.90±0.07^{d}
BS	6.74±0.28	6.96±0.04	6.96±0.03^{ab}	6.99±0.02^{b}
BF	6.45±0.21	6.91±0.13	6.88±0.05^{bc}	6.89±0.07^{bc}

*All values are mean ± standard deviation of three replicates (n = 3).

Different ^{a,b,c} lowercase letters within a column indicate a significant difference (P<0.05).

^{ns} Not significant (P>0.05).

CW=whole conventional-cultivated hybrid catfish without the organ, CS=conventional-cultivated hybrid catfish steak, CF=conventional-cultivated hybrid catfish fillet, BW=whole biofloc-cultivated hybrid catfish without the organ and BS=biofloc-cultivated hybrid catfish steak, BF=biofloc-cultivated hybrid catfish fillet with skin

3.4 Color measurement

Color parameter of conventional and biofloc cultivated hybrid catfish with various cuttings method during frozen storage for 3-months. Changes in color parameter of frozen hybrid catfish is given in Figure 2. Color characteristics ($L*$, $a*$ and $b*$) and whiteness of frozen fish from both conventional and biofloc cultivation and from various the cutting methods were not different at 0-month of frozen storage (P>0.05). During frozen storage, there was a lightly decrease in $L*$ value (lightness) with corresponding decrease in $b*$ (yellowness). Surprisingly, the $a*$ (redness) tended to increase during storage period. This might be due to the limited variation in surface color of vacuum-packaged frozen fish which may be caused by a lack of residual oxygen inside the package, then oxidation reaction of myoglobin pigment was reduced. Fresh fish sample (0 day) has pinkish white flesh and then there was progressively turned paler during storage and became yellow stained white color as the $L*$ and $b*$ increased. The whiteness of all sample decreased during storage, indicating surface color of frozen fish became darker, with a greater amount of browning and a greater percentage of discoloration. Wang et al., (2022) reported that the biochemical changes such as protein oxidation and fat oxidation and the change in the color during frozen storage, ferrous ions bound to myoglobin are easily oxidized to high iron myoglobin, which results in browning of the flesh. Sriket & La-ongnual., (2018) reported the increase in TBARS values of the basa fillets was concomitant with the increase in b* value when storage period increase. Aldehyde, ketons, and carbonyl compounds, products from the oxidations of unsaturated fatty acids, can react with free amino groups in protein. These reactions lead to discoloration. Sveinsdóttir et al., (2020) who reported the yellow colour will changes during frozen storage. A changes in colour associated with lipid oxidation and fish filleted mackerel were in all cases more yellow (Wąsowicz et al., 2004).

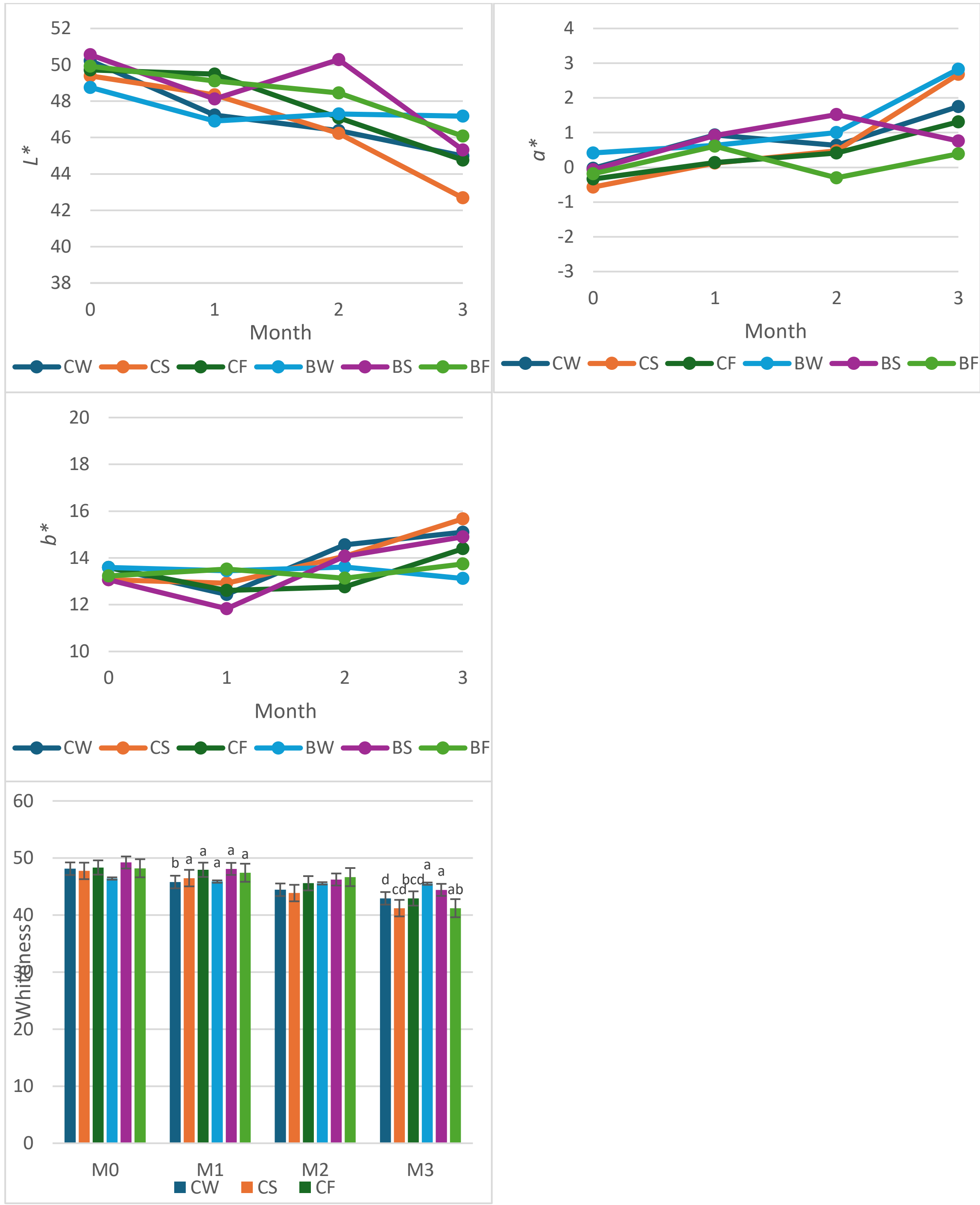

Figure 5. Color value (L*, a* and b*), whiteness of conventional and biofloc cultivated hybrid catfish during frozen storage for 3 months.

* Different ^{a,b,c} lowercase letters indicate a significant difference (P<0.05)

CW=whole conventional-cultivated hybrid catfish without the organ, CS=conventional-cultivated hybrid catfish steak, CF=conventional-cultivated hybrid catfish fillet, BW=whole biofloc-cultivated hybrid catfish without the organ and BS=biofloc-cultivated hybrid catfish steak, BF=biofloc-cultivated hybrid catfish fillet with skin.

3.5 Salt soluble protein content

Salt soluble protein (SSP) of conventional and biofloc cultivated hybrid catfish with various cuttings method during frozen storage for 3-months (Figure 3). The initial salt soluble protein (SSP) content of all frozen fish samples ranged from 1.501 to 2.364 mg/g of sample. At 0-month, the interaction between cultivation and fish cutting method was no significant difference (P>0.05) but the cultivation system influenced the content of SSP, implying the conventional cultivated fish had higher salt soluble protein or myofibrillar protein than the biofloc cultivated fish due to different feeding source. After 3-months of frozen storage period, the cutting method influenced the content of SSP. The results showed that the SSP content of frozen whole fish from both cultivation systems was lower than that of frozen fish steaks and fillets. Yan et al., (2023) found that the SSP content of Hairtail fish decreased 1 time after 4-months storage. A decreased in SSP content of Nile tilapia fillets was observe around 10 % at the end 120 days storage. (Xie et al.,2023). After 2-months storage, the interaction between cultivation and cutting methods revealed that biofloc cultivation consistently enhances protein concentrations across different fish cuts compared to conventional methods. The highest protein enhancement is observed in fillets, followed by steaks, and then whole fish. This indicates that biofloc cultivation not only improves overall fish quality but is particularly beneficial for the most commercially valuable cuts, making it a preferred method for fish farming aimed at maximizing nutritional value

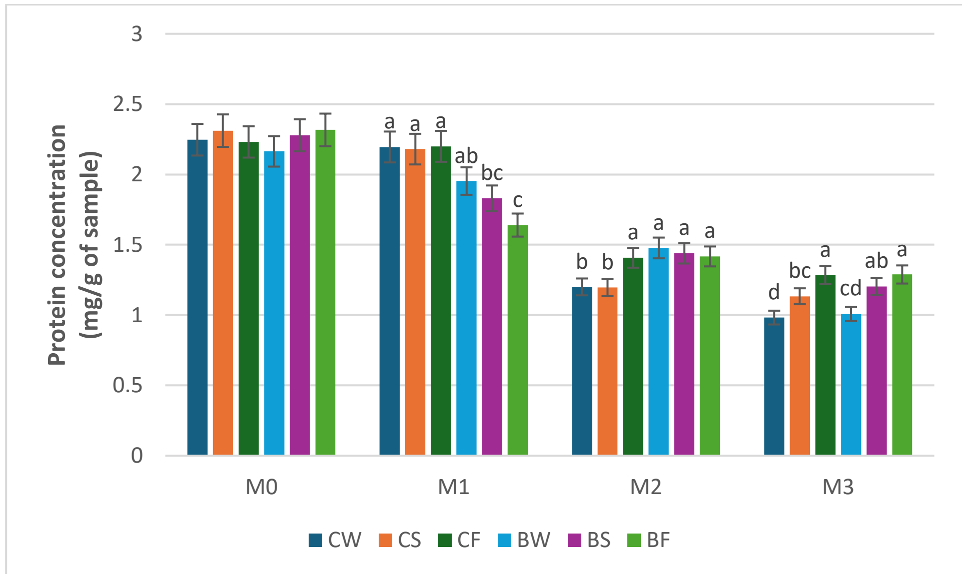

Figure 6. Salt soluble protein content of conventional and biofloc cultivated hybrid during catfish frozen storage for 3 months

*Different ^{a,b,c} lowercase letters indicate a significant difference (P<0.05)

CW=whole conventional-cultivated hybrid catfish without the organ, CS=conventional-cultivated hybrid catfish steak, CF=conventional-cultivated hybrid catfish fillet, BW=whole biofloc-cultivated hybrid catfish without the organ and BS=biofloc-cultivated hybrid catfish steak, BF=biofloc-cultivated hybrid catfish fillet with skin.

3.6 Surface hydrophobicity

Surface hydrophobicity (S_0) of conventional and biofloc cultivated hybrid catfish with various cuttings method during frozen storage for 3-months. Surface hydrophobicity (S_0) of frozen hybrid catfish samples during frozen storage is shown in Figure 4. Increases in S_0 of all frozen fish samples during frozen storage was observed. These results were caused by an exposure of hydrophobic group of amino acid due to the conformational changes in protein. The H-bonding of water on the surface hydrophobic protein was disrupted and partially exposed as increases in drip loss (Table 1) and decreases in WHC (Table 2). The significant increase in S_0 of frozen fish after a 2- months storage indicated the exposure of hydrophobic sit of protein due to protein unfolding. These results are in line with Sriket and La-ongnual (2018) who reported the S_0 of frozen Basa fillets showed an increasing trend with increase in frozen period. The S_0 of common carp frozen increased after storage for 60 days (Sun et al., 2021). The results indicated that Biofloc cultivation generally produces fish with lower surface hydrophobicity compared to conventional methods. The combined effects of cultivation and cutting methods is presented showing variations in surface hydrophobicity and other quality metrics for each combination of methods (CW, CS, CF, BW, BS, BF). Key observations included that Biofloc fillets have lower surface hydrophobicity than conventional fillets. This section emphasizes the importance of considering both cultivation and cutting methods to achieve desired fish quality attributes.

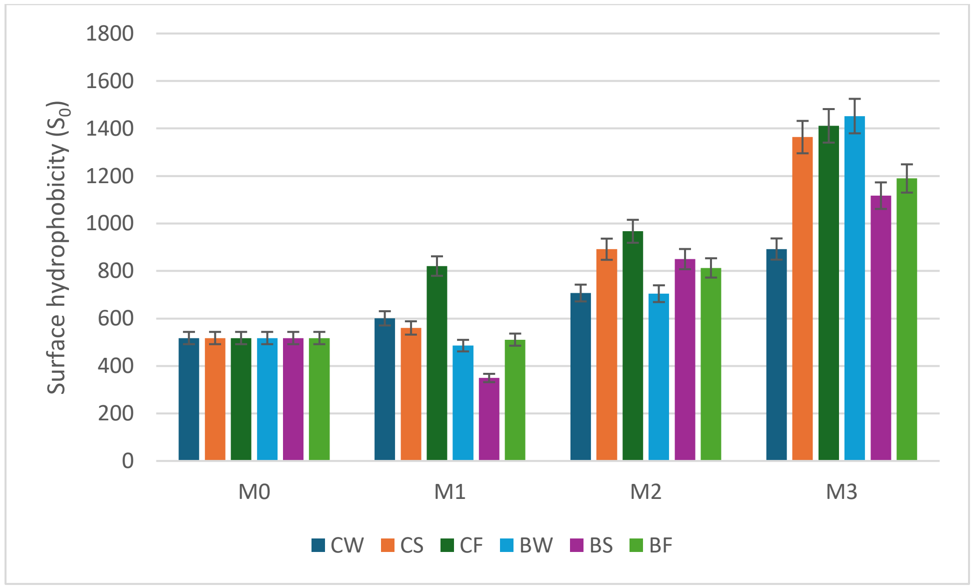

Figure 7. Surface hydrophobic of conventional and biofloc cultivated hybrid catfish during frozen storage for 3 months

*Different [a,b,c] lowercase letters indicate a significant difference ($P<0.05$)

CW=whole conventional-cultivated hybrid catfish without the organ, CS=conventional-cultivated hybrid catfish steak, CF=conventional-cultivated hybrid catfish fillet, BW=whole biofloc-cultivated hybrid catfish without the organ and BS=biofloc-cultivated hybrid catfish steak, BF=biofloc-cultivated hybrid catfish fillet with ski

3.7 Total sulfhydryl content

Total Sulfhydryl (TSH) of conventional and biofloc cultivated hybrid catfish with various cuttings method during frozen storage for 3-months. Total sulfhydryl (TSH) content of frozen fish decreased during frozen storage (Figure 5), indicating the oxidation of thiol groups. The reduction of TSH was in agreement with Wang et al. (2024) who reported that the TSH content of fish was affected by frozen storage periods. Interaction factors between the cultivation system and fish cutting method had no significant effect on TSH content (P>0.05). The decreases in TSH content indicated the formation of disulfide bonds, resulting in protein denature during frozen storage. This result would affect the texture of frozen fish. After 1- month storage, biofloc-cultivated fish consistently showed higher sulfhydryl content across all cutting methods compared to conventional-cultivated fish. This could be due to the biofloc system providing a more controlled and nutrient-rich environment, reducing oxidative stress and preserving protein sulfhydryl groups. Across both cultivation methods, whole fish generally had the highest sulfhydryl content, followed by steak and then fillet. The cutting process might expose proteins to oxidative conditions, leading to the loss of sulfhydryl groups, which could explain the lower content in steaks and fillets compared to whole fish. These results suggested that the integrity of the fish structure might influence the preservation of sulfhydryl groups.

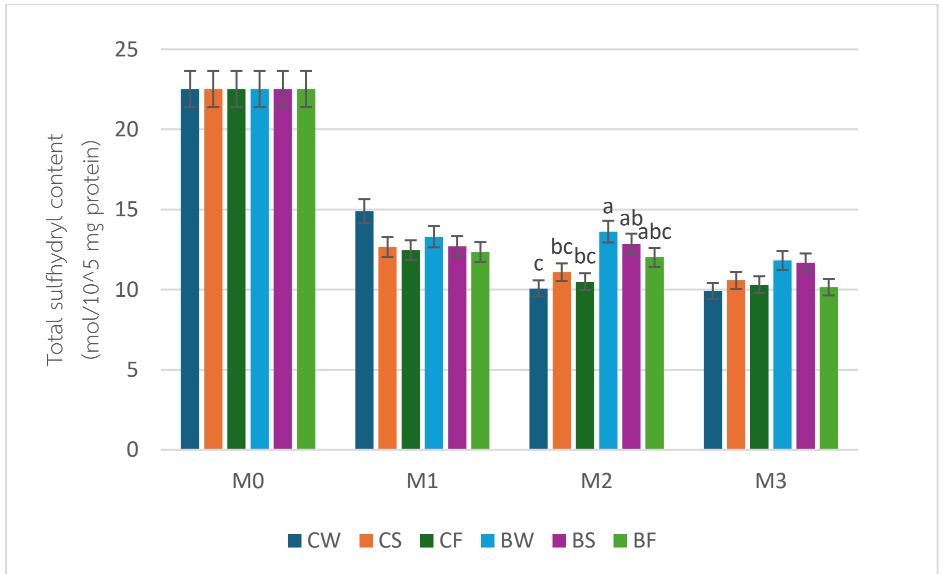

Figure 8. Total sulfhydryl content of conventional and biofloc cultivated hybrid catfish during frozen storage for 3 months

*Different [a,b,c] lowercase letters indicate a significant difference (P<0.05)

CW=whole conventional-cultivated hybrid catfish without the organ, CS=conventional-cultivated hybrid catfish steak, CF=conventional-cultivated hybrid catfish fillet, BW=whole biofloc-cultivated hybrid catfish without the organ and BS=biofloc-cultivated hybrid catfish steak, BF=biofloc-cultivated hybrid catfish fillet with skin

3.8 Texture Profile Analysis (TPA)

TPA of conventional and biofloc cultivated hybrid catfish with various cuttings method during frozen storage for 3-months. TPA results of hybrid catfish during frozen storage periods is shown in Figure 6.

In terms of hardness, conventionally cultivated fish showed higher hardness values overall compared to biofloc-cultivated fish, which exhibited lower hardness. The changes of hardness might be due to physicochemical changes of protein meat during frozen storage such as changes in salt soluble protein extraction (Figure 3), S_0 (Figure4) and TSH (Figure 5). When considering cutting methods, whole fish (CW, BW) exhibit the highest hardness values. This indicating that the structure remains more intact compared to steaks (CS, BS) and fillets (CF, BF), with fillets consistently showing the lowest hardness. This trend suggests that the structural integrity of the fish is better preserved in whole fish.

Adhesiveness varies between cultivation methods, with conventional-cultivated fish showing a more variable range of adhesiveness. Biofloc-cultivated fish tend to exhibit slightly higher adhesiveness, which might be due to the different microbial and nutrient profiles in the biofloc environment. In terms of cutting methods, whole fish (CW, BW) show higher adhesiveness values compared to steaks (CS, BS) and fillets (CF, BF). Fillets have the lowest adhesiveness values, likely due to the higher processing and more exposed surface area, which reduces adhesive properties.

Springiness is another parameter that shows distinct differences between cultivation methods. Biofloc-cultivated fish displayed higher springiness compared to conventional samples, possibly indicating better muscle structure preservation. Among the cutting methods, whole fish (CW, BW) exhibited the highest springiness, maintaining their integrity better over time. Steaks (CS, BS) had moderate springiness values, while fillets (CF, BF) showed the lowest springiness, likely due to the breakdown of muscle fibers during the process.

Gumminess tend to be higher in conventionally cultivated fish, whereas biofloc-cultivated fish showed lower gumminess, which may indicate a softer texture. Whole fish (CW, BW) and steaks (CS, BS) have higher gumminess compared to fillets (CF, BF), with fillets exhibiting the lowest gumminess values. This trend suggests that the intact muscle structure in whole fish contributes to higher gumminess.

Chewiness followed a similar pattern to gumminess. Conventional-cultivated samples exhibited higher chewiness values, while biofloc-cultivated samples showed lower chewiness, indicating a potentially more pleasant eating experience. Whole fish (CW, BW) have the highest chewiness values, maintaining their muscle structure, while steaks (CS, BS) have moderate chewiness, and fillets (CF, BF) show the lowest chewiness, likely due to the finer texture resulting from the cutting process.

Cohesiveness was higher in biofloc-cultivated fish compared to conventional-cultivated ones. Among the cutting methods, whole fish (CW, BW) exhibited the highest cohesiveness, indicating intact structural integrity. Steaks (CS, BS) show moderate cohesiveness values, while fillets (CF, BF) had the lowest cohesiveness, due to the more extensive breakdown of muscle tissues during processing.

Over the storage period from 0 to 3 months, several trends were observed. Hardness generally decreased over time for all methods, with the most significant drop in fillets. Adhesiveness fluctuated but generally decreased, especially in fillets. Springiness decreased slightly over time, with whole fish maintaining the highest values. Gumminess and chewiness both decreased over time, particularly in fillets, indicating a softer texture developing. Cohesiveness remained relatively stable but slightly decreased, particularly in fillets, suggesting a loss in structural integrity over time. The similar results could be observed in Mandarin fish (Sun et al., 2018). The loss of texture in frozen hybrid catfish samples indicated gradual softening of fish muscle. This mainly influenced by the formation of ice crystals to break-up of structure of protein and its denaturation. The change in the hardness of the samples could be primarily attributed to hydrogen bonds. The protein network was unfolding, leading to an increase in disulfide bonds and hydrophobic interactions. The softening of frozen fish was caused by the degradation and denaturation of proteins, along with lipid oxidation (Wei et al., 2021). This phenomenon is associated with the observed increase in S0 as storage time extends (Figure 4).

Both cultivation and cutting methods significantly affected the textural qualities of frozen fish. Biofloc cultivation resulted in better texture retention, with lower hardness, gumminess, and chewiness, and higher cohesiveness and springiness. Whole fish cuts also maintain better textural properties over time compared to steaks and fillets, which tend to show a decrease in these properties during frozen storage.

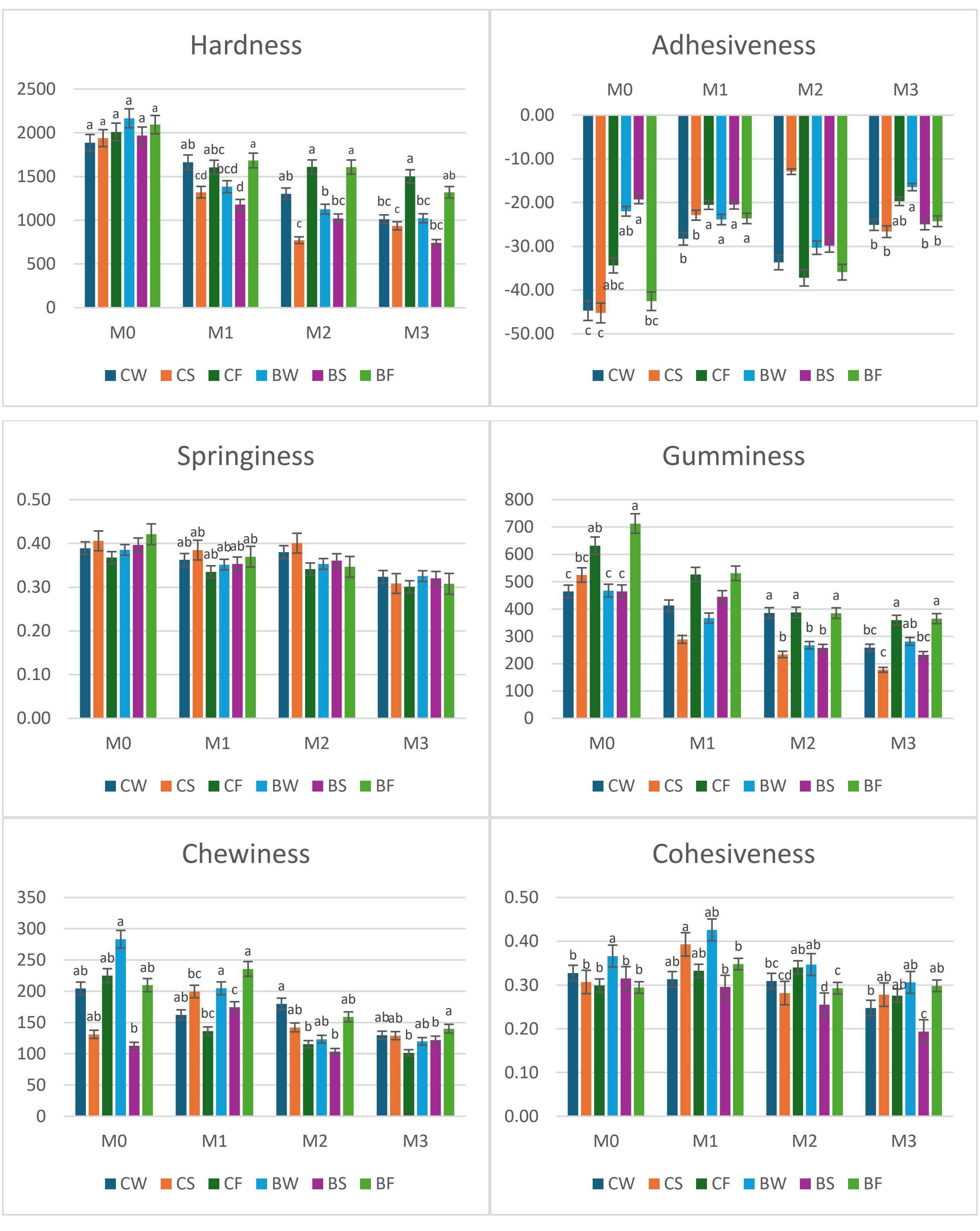

Figure 9. Texture Profile Analysis (TPA) of conventional and biofloc cultivated hybrid catfish frozen storage for 3 months

*Different [a,b,c] lowercase letters indicate a significant difference (P<0.05)
CW=whole conventional-cultivated hybrid catfish without the organ, CS=conventional-cultivated hybrid catfish steak, CF=conventional-cultivated hybrid catfish fillet, BW=whole biofloc-cultivated hybrid catfish without the organ and BS=biofloc-cultivated hybrid catfish steak, BF=biofloc-cultivated hybrid catfish fillet with skin

4. CONCLUSIONS

The research findings demonstrated that the cultivation systems and cutting methods significantly influenced the physicochemical properties of protein in hybrid catfish during three months of frozen storage. Fish cultivated using biofloc technology exhibited superior texture retention, characterized by higher cohesiveness and springiness, despite higher drip loss compared to conventionally cultivated fish. Notably, whole fish cuts maintained better textural properties over time. Conversely, fillet cuts were the most susceptible to quality degradation, showing substantial moisture loss and textural changes. The study suggests that biofloc technology is advantageous for enhancing the quality of frozen fish, but underscores the necessity for optimized cutting methods to mitigate moisture loss. Future research should focus on elucidating the biochemical mechanisms behind these quality changes and investigating lipid and protein oxidation.

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Division B

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Effects of roasting degree of Robusta coffee bean and maltodextrin concentration on quality of spray-dried powder

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ABSTRACT

Coffee is a beverage that is popular all over the world. In the southern region of Thailand, Robusta coffee is popularly coffee variety grown in this area which commonly used to produce instant coffee. Roasting is the important coffee processing step which affect to the quality of the coffee product. Spray drying is one of drying process for coffee powder production. The objective of this research was to study the effect of roasting degree (light, medium and dark) and maltodextrin concentration (0.5, 1 and 2%) on selected physicochemical properties of spray dried Robusta coffee. Moisture content, water activity and pH of coffee powder were range from 2.85-2.93%, 0.34-0.42 and 5.22-5.42, respectively. Coffee powder with higher maltodextrin concentration possessed higher lightness and lower redness. %Yield of coffee powder increased as maltodextrin concentration increased. Increasing of roasting degree exhibited higher water solubility index. Dark roasting coffee powder with addition of 2% maltodextrin showed the lowest hygroscopicity and bulk density as well as the highest water solubility index.

1. INTRODUCTION

Coffee is one of the world's leading economic crops and one of the world's most popular beverages. Coffee consumption continues to grow, primarily as a result of its unique aroma, perceived health benefits due to the presence of highly active natural antioxidants, mostly polyphenols [1]. There are two main coffee varieties that are currently grown in Thailand;

Robusta (*Coffea canephora*) and Arabica (*Coffea arabica*). Arabica is grown mainly in most provinces in the north. While, Robusta (*Coffea canephora*) is grown mostly in the provinces of southern Thailand, such as Chumphon, Surat Thani, Nakhon Si Thammarat, Krabi, Phang Nga and Ranong. Robusta coffee has a higher caffeine content and more bitter taste compared to Arabica coffee. For domestic consumption, Robusta coffee is usually produced in the form of roasted, powdered and canned coffee.

Roasting is the important step of coffee processing which is temperature dependent and affect to the quality of the coffee product. The roasting process can lead to modification and/or generation and release of different chemical compounds occur through Maillard reactions, caramelization and other chemical reactions. These reactions are responsible for the distinctive flavor, aroma and color in brewed coffee, being influenced by degree of roasting. The quality of coffee bean is significantly related to the coffee roasting degree with generally three roasting profiles: light, medium and dark roast. The coffee roasting degree can affect the flavor and consumer acceptance. In addition, roasting degree of coffee have an effect on the some physical property such as powder flowability [2].

Spray drying is a process to convert a fluid into a dried powder form by spraying the fluid into a chamber that contain stream of hot air. Spray drying is productive process used for drying coffee extract to coffee powder with good physical properties, consistent powder quality and suitable for high heat sensitivity food [3]. Spray drying has many advantages for production of coffee powder such as short drying time, high yield and economic aspects of the process with reduced moisture to extend shelf-life of product. Abrahão et al. [4] applied the spray drying technique to encapsulate of bioactive compounds extracted from espresso spent coffee. The microparticles obtained can carry and protect considerable amounts of antioxidants present in the coffee, which were extracted from residues from the preparation of the coffee drink.

In spray drying, carrier agents can be applied to alter the particle structure with decreasing adhesion and hygroscopicity, while increasing powder stability. Maltodextrin is commonly used as carrier agent for spray drying due to the advantages of low cost, good solubility, low viscosity and neutral flavor [5]. Ballesteros et al. [6] studied the effect of coating material (maltodextrin, gum arabic, mixture of these components at 1:1 ratio) on the antioxidant phenolic compounds extracted from spent coffee grounds prepared by spray drying. The use of maltodextrin was more appropriate for preserving these components with the highest retention of phenolic compounds and flavonoids and also the best antioxidant activities for the encapsulated sample.

Despite the aforementioned studies, no information on roasting degree of Robusta coffee beans and concentration of maltodextrin as carrier agent is so far available. This study investigated the effect of roasting degree of Robusta coffee and percentage of maltodextrin on the qualities of spray dried coffee powder.

2. MATERIAL AND METHODS

2.1 Materials

Green Robusta coffee beans (*Coffea canephora*) harvested in October, 2023 were purchased from coffee farmer at Krabi province. Coffee samples were roasted with three different temperatures (190 °C, 210 °C and 230 °C with roasting time 18 min) for three roasting degree: light, medium and dark (Figure 1).

Figure 1. Green Robusta coffee bean (a) light (b) medium (c) and dark (d) roasted coffee bean

2.2 Coffee extract preparation

For coffee extracts, coffee beans were ground using an electric grinder (model Electric Spice and Coffee Grinder, Epica, NY, USA). The ground coffee (1 kg) was mixed with water 10 L and extracted with Hi speed Extractor (model HXL-10E, Euro Best Technology Co., Ltd., Thailand). Then, coffee extract were evaporated with falling film evaporator. For coffee extract, the higher the roasting degree, the higher dark color of coffee extract (Figure 2). To prepare feed solution for spray drying, maltodextrin (dextrose equivalent 10 (DE 10) at different concentration (0.5, 1 and 2%, w/v) were mixed with coffee extract.

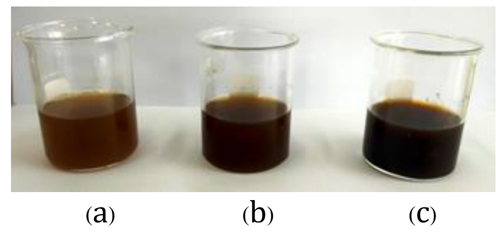

Figure 2. Robusta coffee extract of different roasting degree (a) light (b) medium (c) dark

2.3 Spray drying

Coffee powder produced by spray dryers model SDE-e EURO2 (Euro Best Technology Co., Ltd., Thailand) using inlet air temperature of 160 °C, outlet air temperature of 80 °C, blower speed of 2100 rpm under pressure of 3.5 bar with a liquid feed flow rate of 2 l/h. The nozzle type of this spray dryer is rotary nozzle.

2.4 Quality determination

2.4.1 Yield (%)

Yield (%) of coffee powder was evaluated with the method described by Ravichandran et al. [7] Calculation of %yield was given by following equation (1).

$$\%\ \text{Yield} = \frac{\text{mass of powder}}{\text{total solid in feed solution}} \times 100 \qquad (1)$$

2.4.2 Moisture content

Moisture content of the samples was determined using a gravimetric method at 105 °C in a hot air oven (AOAC, 2000). Each drying experiment was continued until a constant weight was obtained.

2.4.3 Water activity

Water activity of coffee powder was determined using water activity meter (Freund, Tokyo, Japan).

2.4.4 Color

The color measurements of coffee powder were performed using a Colorimeter (Hunter lab, Color Quest XE, USA). Color values for L* (L*=100 means white, L*=0 means black), a* [redness (+) and greenness (−)] and b* [yellowness (+) and blueness (−)] were recorded.

2.4.5 pH

Coffee powder samples were diluted with water (0.5 g of powder in 25 mL). The pH was determined with pH meter.

2.4.6 Bulk density

Approximately 2 g of coffee powder sample was transferred into a 10 mL graduated cylinder and the volume occupied by the powder was used to calculate the bulk density (equation 2).

$$\text{Bulk density (g/ml)} = \text{sample mass/bulk volume} \quad (2)$$

2.4.7 Hygroscopicity

Hygroscopicity of spray dried coffee powder was determined as described by Ravichandran et al. Approximately 0.5 g of sample was placed in a desiccator with a NaCl saturated solution (75% relative humidity at 30 °C) for 7 days, then sample was weighed. The hygroscopicity was calculated as a percentage based on the ratio of the mass of absorbed moisture to the initial mass of coffee powder (equation 3)

$$\text{Hygroscopicity (\%)} = \frac{\text{mass of coffee powder after 7 days} - \text{initial mass of coffee powder} \times 100}{\text{initial mass of coffee powder}} \quad (3)$$

2.4.8 Water solubility index (WSI)

Water solubility index (WSI) was determined using the method of Wongsa et al. with some modifications. Spray-dried coffee sample (2 g) was mixed with 20 mL of hot water (90–95 °C) and completely dissolved by gently mixing. Fine particles were removed by centrifuging at 14,006 rpm using centrifuge for 10 min. The supernatant was poured on a pre-weighed moisture can, then oven dried at 105 °C until a constant weight was obtained. WSI was calculated using the following equation (4):

$$WSI = (DM_{sup}/DM_{ini}) \times 100 \quad (4)$$

where DM_{sup} is the dried weight of supernatant
DM_{ini} is the initial weight of coffee powder

2.4.9 Data Analysis

The data obtained in this study were expressed as the mean of at least three replicate determinations and standard deviation by using the software SPSS version 23. Data were analyzed by one way analysis of variance (ANOVA) statistical differences were examined by the Duncan's multiple range test at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 %Yield, moisture content, water activity and pH of spray dried Robusta coffee

Yield is an essential parameter to determine the effectiveness of the spray drying for commercial production. The % yield range of the coffee powder was 66.23 to 92.56 (Table 1). Shofinita et al. [8] reported that the spray-drying method obtained a yield of coffee powder in the range of 71.63–87.98%, similar to the results of this study. From Table 1, it was showed that the increasing of maltodextrin resulted the higher % yield of Robusta coffee powder. Maltodextrin normally used as the carrier agent resulted in higher drying yield. This is may be due to the addition of maltodextrin increase the total soluble solid as drying carrier as well as the reduction in stickiness via encapsulation.

The initial moisture content of Robusta coffee powder were range from 2.85% to 2.93% and water activity were range from 0.34-0.42. Wongsa et al. [9] reported that the moisture content of spray-dried coffee blending of Arabica and Robusta ranged between 1.37% and 2.79%. The moisture content of spray dried food products should be lower than 5% with water activity of less than 0.6. Othman et al. [10] reported that moisture content of spray-dried coffee normally less than 11%. Moisture absorption can cause full agglomeration of the powder particles when the moisture content reaches 7% to 8%.

The pH value of coffee powder with different maltodextrin concentration was in the range of 5.22 to 5.42, respectively. The pH slightly decreased when maltodextrin concentration increased (Table 1). This was probably due to the addition of maltodextrin substitute for coffee extract quantity. This pH range of coffee powder may be due to Robusta coffee chemical composition of caffeine compounds and organic acids. The pH of Robusta coffee boiled water was 5.54 that similar to pH value of this study [11]. Similar results were reported by Freitas et al. [12] who observed that pH value for Robusta coffee with different roasting temperature (135-275 °C) and time ranged from 5.25 to 6.30. The slightly different of pH value of coffee powder prepared by different roasting degree may be due to the changes in chemical compounds during the roasting of coffee beans [13].

Table 1. %Yield, moisture content, water activity and pH of spray dried Robusta coffee

Roasting degree	Maltodextrin (% w/v)	%Yield	Moisture content (%)^{ns}	Water activity	pH
Light	0.5	66.23	2.93±0.29	^{A}0.40±0.01^{b}	^{B}5.39±0.02^{b}
	1	74.52	2.91±0.20	^{A}0.36 ±0.01^{a}	^{B}5.37±0.02^{b}
	2	87.69	2.93±0.37	^{A}0.34±0.01^{a}	^{A}5.25±0.01^{a}
Medium	0.5	66.81	2.85±0.22	^{A}0.42±0.02^{b}	^{A}5.31±0.04^{c}
	1	76.92	2.91±0.36	^{A}0.38±0.01^{a}	^{A}5.26±0.01^{b}
	2	89.33	2.92±0.28	^{AB}0.36±0.01^{a}	^{A}5.22±0.01^{a}
Dark	0.5	69.17	2.87±0.20	^{A}0.39±0.01^{b}	^{B}5.42±0.01^{b}
	1	78.67	2.92±0.32	^{A}0.37±0.01^{a}	^{B}5.40±0.02^{b}
	2	92.56	2.91±0.19	^{B}0.37±0.01^{a}	^{B}5.32±0.01^{a}

*Results are presented as means ± standard deviations (n = 3).

*Means with different small letter superscripts the same column within the same roasting degree is significantly different at $p < 0.05$.
*Means with different capital letter superscripts the same column within the same % maltodextrin is significantly different at $p < 0.05$.

3.2 Color

L*, a* and b* values of coffee powder with different roasting degree and maltodextrin concentration were in the range from 54.83 to 72.68, 2.87 to 8.47 and 23.15 to 28.01, respectively (Table 2). The results showed that color attributes of coffee powder were affected by roasting degree and maltodextrin concentration. The Robusta coffee beans with different roasting degree and %maltodextrin are shown in Figure 3. Coffee powder contained higher maltodextrin concentration tended to be brighter with higher L* values and possess a lower a* and b* values. Moreover, lower L* and higher a* and b* values of coffee powder with roasting degree increase may be due to increased formation of pigments generated by non-enzymatic Maillard browning reaction between sugars and amines as well as degradation of phospholipids, as well as thermal total phenolics oxidation resulting the color of the coffee beans darker [12].

Table 2. Color of spray dried Robusta coffee

Roasting degree	Maltodextrin (% w/v)	L*	a*	b*
Light	0.5	$^{C}66.96±0.06^{a}$	$^{A}4.17±0.11^{c}$	$^{B}28.01±0.03^{c}$
	1	$^{C}68.96±0.22^{b}$	$^{A}3.65±0.34^{b}$	$^{A}26.63±0.09^{b}$
	2	$^{C}72.68±0.14^{c}$	$^{A}2.87±0.14^{a}$	$^{A}23.15±0.13^{a}$
Medium	0.5	$^{B}60.49±0.25^{a}$	$^{B}6.46±0.07^{b}$	$^{A}26.98±0.28^{b}$
	1	$^{B}63.54±0.11^{a}$	$^{B}6.37±0.07^{b}$	$^{A}26.80±0.24^{b}$
	2	$^{B}66.56±0.27^{b}$	$^{B}5.44±0.09^{a}$	$^{A}25.21±0.30^{a}$
Dark	0.5	$^{A}54.83±0.44^{a}$	$^{C}8.47±0.23^{c}$	$^{AB}27.39±0.30^{c}$
	1	$^{A}61.42±0.14^{b}$	$^{C}6.64±0.13^{b}$	$^{A}26.72±0.20^{b}$
	2	$^{A}64.59±0.33^{c}$	$^{C}5.81±0.21^{a}$	$^{A}25.18±0.32^{a}$

*Results are presented as means ± standard deviations (n = 3).
* Means with different small letter superscripts the same column within the same roasting degree is significantly different at $p < 0.05$.
*Means with different capital letter superscripts the same column within the same % maltodextrin is significantly different at $p < 0.05$.

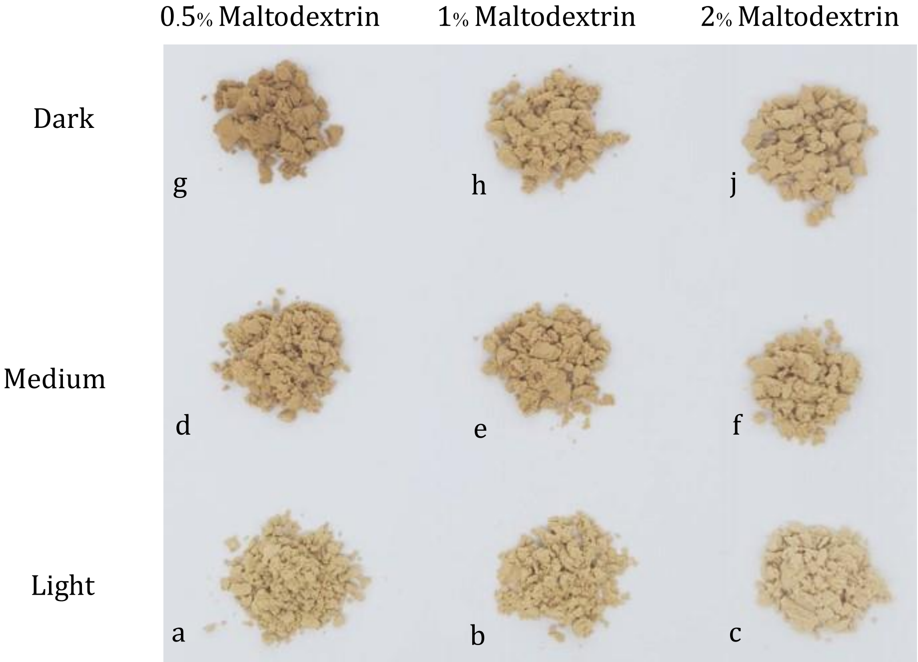

Figure 3. Spray dried Robusta coffee powder with different roasting degree (Light, Medium and Dark) and maltodextrin concentration (0.5, 1 and 2%)

3.3 Bulk density, water solubility index and hygroscopicity of spray dried Robusta coffee

Bulk density is an important physical property for transportation of a food product. An increase in the maltodextrin concentration (1 and 2%) did not significantly affect the bulk density (Table 3). This finding is consistent with the study by Mishra et al. [14].

Table 3. Bulk density, water solubility index and hygroscopicity of spray dried Robusta coffee

Roasting degree	Maltodextrin (% w/v)	Bulk density (g/ml)	Water solubility index (%)	Hygroscopicity (%)
Light	0.5	$^{C}0.35\pm0.08^{b}$	$^{A}80.35\pm0.35^{a}$	$^{C}35.12\pm0.72^{b}$
	1	$^{B}0.23\pm0.05^{a}$	$^{A}82.14\pm0.29^{a}$	$^{C}31.06\pm0.63^{a}$
	2	$^{B}0.20\pm0.04^{a}$	$^{A}85.05\pm0.19^{b}$	$^{C}30.08\pm0.65^{a}$
Medium	0.5	$^{B}0.22\pm0.02^{b}$	$^{B}82.43\pm0.25^{a}$	$^{B}29.00\pm0.74^{b}$
	1	$^{A}0.15\pm0.01^{a}$	$^{B}84.59\pm0.18^{a}$	$^{B}28.43\pm0.61^{ab}$
	2	$^{A}0.14\pm0.01^{a}$	$^{B}86.01\pm0.12^{b}$	$^{B}27.45\pm0.52^{a}$
Dark	0.5	$^{A}0.16\pm0.01^{b}$	$^{C}83.51\pm0.21^{a}$	$^{A}25.29\pm0.69^{b}$
	1	$^{A}0.14\pm0.01^{a}$	$^{C}87.41\pm0.49^{b}$	$^{A}24.51\pm0.56^{b}$
	2	$^{A}0.13\pm0.01^{a}$	$^{C}88.23\pm0.34^{c}$	$^{A}22.12\pm0.48^{a}$

*Results are presented as means ± standard deviations (n = 3).

* Means with different small letter superscripts the same column within the same roasting degree is significantly different at $p < 0.05$.

*Means with different capital letter superscripts the same column within the same % maltodextrin is significantly different at $p < 0.05$

Water solubility index (WSI) is an important property represent the ability of the powder to fully dissolve in water. WSI of coffee powder ranged from 80.35 to 88.23%. An increase in the maltodextrin concentration was significantly affect the WSI. This may be attributed to the higher content of maltodextrin with high solubility property [14]. A higher WSI was observed in the samples prepared from a dark roast coffee with 2 % maltodextrin (Table 3). When considering roasting degree, coffee powder showed higher water solubility index with the increase of this parameter. This is probably because of more water-soluble heterocyclic compounds which occur during high roasting degree [15].

Hygroscopicity is defined as the ability of a dried product to absorb water from the atmosphere, which causes the change of food quality. An increase in the maltodextrin concentration was significantly affect hygroscopicity. It was found that maltodextrin addition improved hygroscopicity of powder (Table 3). This may be because maltodextrin has relatively few hydrophilic groups and binds fewer water molecules [16].

4. CONCLUSIONS

It was found that the increasing of maltodextrin resulted the higher % yield of coffee powder. Dark roasting coffee with 0.5% maltodextrin showed the lowest lightness, however, presented the highest redness. The pH value of coffee powder was 5.22-5.42, while the moisture content and water activity was 2.85-2.93 % and 0.34-0.42, respectively. Water solubility index of coffee powder increased with increasing of roasting degree. Dark roasting coffee with 2% maltodextrin showed the lowest hygroscopicity. However, Dark roasting coffee with 2% maltodextrin resulted the highest water solubility.

5. ACKNOWLEDGEMENTS

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Optimization of the extraction method for producing *Centella asiatica* extract with antioxidant activities

BP-P030

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ABSTRACT

Centella asiatica is a valuable medicinal herb widely used in traditional Indian and Chinese systems of medicine. The aim of this study was to determine the effect of different extraction solvents and extraction methods on the constituents of C. asiatica extracts prepared from solid-liquid extraction (SLE) at room temperature for 24 h, SLE at 60°C for 2 h, SLE at 60°C for 2 h followed by microwave-assisted extraction (MAE) at 600 W 2 min, ultrasound-assisted extraction (UAE) at 60°C for 2 h, and MAE at 600 W, 2 cycles, 2 min/cycle. Evaluation was performed through antioxidant activities and antibacterial tests. The extraction condition was optimized based on total phenolic content (TPC) and antioxidant activities. The antioxidative activities were evaluated by the ferric reducing antioxidant power (FRAP) assay and 1,1-diphenyl-2-picrylhydrazyl (DPPH)-radical scavenging. The results showed that the extraction of C. asiatica using ethanol as a solvent exhibited the highest values in TPC and antioxidant activities by FRAP and DPPH (IC_{50}). In terms of extraction methods, MAE at 600 W, 2 cycles, 2 min/cycle was the most effective with TPC (96.14±9.93 mg GAE/g extract) and FRAP (1,125.73±32.50 µmol $FeSO_4$/mg extract) and it has the lowest minimum inhibitory concentration values (DPPH; IC_{50} 3.843±0.396 mg/mL). The ethanolic extract from C. asiatica prepared by MAE exhibited antimicrobial activity in agar disc diffusion assays against Staphylococcus aureus, Bacillus cereus, Escherichia coli O157:H7, and Salmonella Typhi. Clear zone of inhibition ranged from 6.72 ± 0.08 mm. to 10.97 ± 0.30 mm. at extract concentrations of 250 and 500 mg/mL. Therefore, the MAE method combined with ethanolic extract has the potential to serve as an alternative to synthetic preservatives in the food packaging, pharmaceutical, cosmetic, and food industries in the prevention of contaminated pathogens

1. INTRODUCTION

Globally, *Centella asiatica* (L.) Urban, a herbaceous creeper belonging to the Apiaceae family, is primarily found in tropical and subtropical regions [1]. Throughout history, it has been employed for various purposes such as enhancing memory, treating wounds, caring for the skin, anxiolytic, anti-cancer, anti-ulcer, antibacterial, and anti-inflammatory [2]. The therapeutic attributes attributed to *C. asiatica* encompass a diverse array of chemical components, such as volatile oils, terpenoids, and flavonoids [3]. *C. asiatica* contains a wide variety of terpenes, including but not limited to madecassoside, asiatic acid, brahmic acid, brahminoside, thankuniside, isothankunisode, centellosides, and madasiatic acid [4], according to a multiplicity of studies. *C. asiatica* is a rich source of flavonoids, including apigenin, kaempferol, quercetin, naringin, and rutin [3]. As a result, it is considered an exceptional antioxidant source [5]. The initial step in the process of isolating bioactive compounds from plant materials is extraction. The principal objective of the extraction process is to isolate bioactive compounds from the extracts that possess the greatest antioxidant potential [6]. Significant bioactive compounds present in the extract may be significantly altered in quality and quantity by a number of process parameters, such as the solvent utilized and the extraction method [7]. A variety of extraction techniques have been documented in the literature to extract bioactive compounds from medicinal plants. These techniques include ultrasound-assisted extraction, maceration, and heat reflux extraction [8]. Typically, conventional extraction techniques such as heat reflux and maceration are employed. Nonetheless, these methods have a number of drawbacks, including lengthy extraction times and the need for substantial quantities of solvents [9]. Conversely, contemporary methodologies such as microwave-assisted extraction and ultra-sound-assisted extraction are favored over their analogous counterparts on account of their superior yield, expedited processing, and reduced processing time [7]. The selection of the solvent is a critical determinant in the extraction procedure, given that the polarity of the solvent [10] can impact the extraction of a specific category of bioactive components. There exists a logical correlation between the bioactive component present in the plant extract and its antioxidant capacity. Consequently, ABTS scavenging assays are frequently utilized in order to evaluate the antioxidant capacity of particular plant extracts [7]. Although several publications have examined the extraction of triterpenoids and phenolic compounds from *C. asiatica* utilizing diverse solvents and extraction techniques, none of them have conducted a comparative analysis of the effectiveness of a single extraction method [2,11]. Assessing the extractive potential of a compound necessitates more than a single extraction technique.

The objective of this research was to determine the most effective extraction solvent and extraction technique to test the antibacterial activity of *C. asiatica* extracts against foodborne pathogens at the optimum extraction condition. This research will increase agricultural revenue while decreasing the utilization of synthetic preservative and increasing the value of *C. asiatica*.

2. MATERIAL AND METHODS

2.1 Plant preparation

C. asiatica was obtained from the Thai province of Nakhon Pathom. The leaf portion alone was utilized. Overnight, the newly harvested leaves were dried at 45°C in a hot air furnace (FD023UL-120V, Binder, Bohemia, NY, USA). By means of a food blender, the desiccated leaves were reduced to a fine powder. Until it was utilized, herb powder was sealed in an aluminum foil container and refrigerated at 4°C.

2.2 Plant extraction

2.2.1 Study the suitable extraction solvent

The sample powder was extracted through the process of maceration, employing a series of seven solvents: petroleum ether, water, ethanol, methanol, chloroform, hexane, and acetone. One day at room temperature, maceration was performed in a 100 mL conical flask containing. 4 g of sample dissolved in 120 mL of each respective solvent. Following the extraction process, the supernatant and sediment were filtered through Whatman No. 1 filter paper. The extracts were dried through vacuum evaporation at a temperature of 45°C utilizing a rotary evaporator (BUCHI, Rotavapor, Switzerland). The dry extract was stored at -20°C until analysis.

2.3 Extraction methods

2.3.1 Solid Liquid Extraction (SLE)

The procedure was performed in accordance with the modifications outlined in the protocol by Jitrangsri et al. [12]. Four grams of desiccated *C. asiatica* were incorporated into a 250 mL volumetric vessel that was sealed. Following the addition of 120 mL of ethanol at a concentration of 80% (v/v), the solution was incubated at 60°C for 2 h in a water bath. The liquid obtained after passing the specimen through a Whatman No.1 filter was dehydrated at 45°C using an evaporator. Until analysis, the dried extract was kept at a temperature of -20°C. Three replicates were performed under each condition throughout the performance of the sample.

2.3.2 Ultrasound-Assisted Extraction (UAE)

The protocol was conducted according to the protocol of Jitrangsri et al. [12] with slight modifications. Four grams of ground, desiccated *C. asiatica* were placed in a sealed 250 mL container. A solution was prepared by sonicating 120 mL of ethanol, which had an 80% (v/v) concentration, in an ultrasonic chamber (Crest®, 230D) set at 60°C for a period of 2 h. Following the passage of the sample through a Whatman No. 1, the liquid that was obtained was desiccated at a temperature of 45°C using an evaporator. Prior to analysis, the dried extract was kept at a temperature of -20°C. Three replicates of each condition were extracted.

2.3.3 Microwave-Assisted Extraction (MAE)

The experiment was assessed by the method of Mohapatra et al. [13] with some modification. Four grams of pulverized and desiccated *C. asiatica* were extracted in a 250 mL round bottom flask containing 120 mL of 80% (v/v) ethanol. Connecting it to a condenser and extracting it with a household microwave (Samsung, Model: CE76JD-B/XTL) comprised the extraction procedure. The extraction procedure was carried out for a period of 2 min at a power level of 600 W. Following that, the specimen underwent filtration utilizing Whatman No. 1, and the filtrate obtained was evaporated at a temperature of 45°C. Prior to analysis, the dried extract was kept at a temperature of -20°C.

2.4 Determination of antioxidant activity

2.4.1 Total phenolic acid (TPC)

The TPC was determined utilizing a method established by Habila et al. [14]. A solution was formed by combining 25 µL of the extract with 75 µL of water and 20 µL of Folin-Ciocalteau reagent. After allowing the mixture to attain equilibrium for 6 min, 100 µL of a 7% w/v $NaCO_3$ was added. The

concentration of TPC was determined at 765 nm subsequent to a 30-min incubation period at room temperature by employing a microplate reader (M965, Metertech, Taiwan). As the standard, Gallic acid was employed. The findings were documented in milligrams of gallic acid equivalents (GAE) per gram of extract (mg GAE/g extract).

2.4.2 Determination of 1,1-diphenyl-2-picrylhydrazyl radical scavenging assay (DPPH)

The DPPH radical scavenging assay was performed in accordance with the modifications outlined in the protocol by Williams et al. [15]. Each 50 µL of distinct extract/gallic acid concentration was mixed with 100 µL of a 200 M ethanol solution of DPPH. After an incubation period of 30 min in the absence of light at room temperature, the specimen was observed. At 517 nm, the optical density of the reaction was ascertained. The results were expressed in mg/mL as the IC_{50} value of DPPH.

2.4.3 Determination of ferric reducing antioxidant power assay (FRAP)

The FRAP assay was performed in accordance with the methodology outlined by Benzie and Strain [16], modified accordingly. Prior to utilization, the FRAP reagent was heated to 37°C. It was composed of acetate buffer (300 mM, pH 3.6), 2, 4, 6-Tris (2-pyridyl)-s-triazine (TPTZ) solution (10 mM in 40 mM HCl), and $FeCl_3 \cdot 7H_2O$ solution (20 mM). These components comprised the 10:1:1 ratio. The experiment was carried out utilizing a 96-well microplate with a flat bottom. A aliquot of 30 µL of the sample was mixed with 270 µL of FRAP reagent and incubated for 30 min at ambient temperature (37°C). Following this, the mixture was analyzed at a wavelength of 593 nm. Using a standard curve, the antioxidant activity was determined by determining the concentration of $FeSO_4$ in µmol per mg of extract.

2.5 Antibacterial activity of the extract from the C. asiatica

2.5.1 Agar disc diffusion method

The antibacterial properties of the *C. asiatica* extracts were evaluated at a concentration of 25% extract (250 mg/mL) using the agar disc diffusion method according to the CLSI guidelines from 2007. *Bacillus cereus* (TISTR 747), *Staphylococcus aureus* (TISTR 808), *Escherichia coli* O157:H7 (DMST 23156), and *Salmonella* Typhi (DMST 22842) were grown in tryptic soy broth (TSB) at a temperature of 37°C for a duration of 24 h. Each culture was diluted in water to a concentration of 0.5 MacFarland standard, which corresponds to 10^5 CFU/ mL. Ramli et al. [17] proposed the utilization of the conventional agar disk diffusion technique to assess the vulnerability of microbial strains to plant extracts. After dipping a sterile cotton swab in the newly formed bacterial culture, the organism was transferred to the suitable medium's surface. Under laminar hood flow, the medium surface was dry for three min to eliminate excess moisture. Using sterile instruments, a 6-mm. antiseptic filter paper disc was promptly positioned onto the MHA's surface. Subsequently, 10 µL of *C. asiatica* extract was pipetted onto the aforementioned paper discs at concentrations of 250 and 500 mg/mL. A positive control for microorganisms comprised a chlorhexidine compound solution with a concentration of 0.1% (w/v). Water that had been sterilized was employed as the negative control. Upon 24 h of incubation at 37°C (MHA), the circumference of the inhibition zones around the sterile disc was determined.

2.6 Statistical analysis

The analyses were conducted for each extraction in triplicate, and the outcomes are displayed as the mean value with a standard deviation of the mean (SD). The researchers performed a one-way

analysis of variance (ANOVA) and then utilized Duncan's multiple comparison tests to ascertain whether there were statistically significant differences between values with $p<0.05$. For this objective, SPSS 17.0 for Windows (SPSS Inc., IBM Corp., IL, USA) was utilized.

3. RESULTS AND DISCUSSION

To ascertain the stability of free radicals or transition metals via electron or hydrogen atom transfer, the samples were analyzed utilizing the assays outlined in Table 1: TPC, FRAP, and DPPH (IC_{50}).

3.1 Effect of different extract solvents

Table 1 displays the extractive values of specific bioactive compounds derived from *C. asiatica* through solid liquid extraction and seven distinct solvents.

Table 1 The antioxidative properties of the *C. asiatica* extract sample were evaluated using various extraction solvents

Extraction Solvent Type	Total Phenolic Content (mg GAE/g extract)	Ferric reducing antioxidant power (µmol $FeSO_4$/mg extract)	IC_{50} of DPPH (mg/mL)
water	18.98±0.08^{c}	144.47±2.47^{c}	8.90±0.48^{c}
ethanol	37.48±0.41^{a}	260.91±0.58^{a}	9.04±0.69^{c}
methanol	32.59±0.12^{a}	242.88±0.88^{a}	8.57±0.75^{c}
chloroform	19.09±0.96^{c}	93.40±0.97^{d}	19.72±0.61^{a}
hexane	26.79±0.62^{b}	186.15±0.42^{b}	8.25±0.09^{c}
acetone	28.51±0.16^{b}	242.61±1.36^{a}	15.87±1.16^{b}
Petroleum ether	5.73±0.02^{d}	34.80±1.08^{d}	14.34±0.58^{b}

*Value is the mean ± standard deviation (n=3)

^{a,b,c,d} within the column with the different letter were significantly different ($p<0.05$)

3.1.1 Total phenolic content (TPC)

The TPC concentrations in seven solvent types varied between 5.73 and 37.48 mg GAE/g extract. A notable distinction ($p<0.05$) was observed among the samples that underwent extraction using distinct solvents. The greatest TPC values were obtained with ethanol and methanol extracts, at 32.59 and 37.48 mg GAE/g extract, respectively. Due to their comparable polarity, ethanol and methanol exhibited similar behavior. In contrast, the utilization of petroleum ether for extraction resulted in the lowest TPC concentration. This is because petroleum ether has a lower polarity compared to ethanol and methanol. The results demonstrated that the TPC's extraction capability followed the order of the solvent's polarity. Phenolic chemicals belonged to a polar group. Hence, the inclusion of a polar solvent such as ethanol or methanol augmented the polarity, in accordance with the principle of solubility. In order to enhance the effectiveness of extraction, it is necessary for the solvent and solute to possess either identical polarity (following the principle of "like dissolves like") or comparable solubility values. This finding aligns with the research conducted by Murga et al. [18] on the supercritical carbon dioxide extraction of phenolic compounds from grape seeds using ethanol and methanol. Based on the experiment's results, both solvents yielded a substantial amount

of phenol extract. Ethanol exhibited considerably higher efficiency compared to methanol, acetone, hexane, chloroform, water, and petroleum ether for TPC extraction of *C. asiatica* ($p < 0.05$; Table 1). The TPC of crude extracts exhibited variation depending on the kind of solvent employed. The results of our study align with the findings of Nurlaily et al., 2012, who observed that the ethanolic extract of *C. asiatica* had a higher recovery of phenolic compounds (17.76 g/100g) compared to the methanolic extract (15.52 g/100g) and water extract (13.16 g/100g).

3.1.2 Ferric reducing antioxidant power assay (FRAP assay)

The findings indicated that the *C. asiatica* leaf extract, when utilizing ethanol and methanol as solvents, demonstrated the highest FRAP values. The obtained values for both solvents 242.88 and 260.91 µmol $FeSO_4$/mg extract, were not statistically significant. Amongst other herbal preparations, the edible flower tea displayed the highest overall reduction capacity of 36.143 µmol $FeSO_4$/mg extract [19]. Hence, the extract of *C. asiatica* can be regarded as one of the herbal substances abundant in antioxidants. Nevertheless, ethanol is a chemical that is not harmful and can be readily isolated from extracts. Furthermore, the acquired extract exhibited superior antioxidation characteristics compared to the methanol extract. Furthermore, it was deemed suitable for human consumption. Consequently, ethanol is a preferable choice as a solvent over methanol.

3.1.3 DPPH radical scavenging activity

The DPPH radicals are commonly employed to evaluate the efficacy of scavenging free radicals due to the simplicity of the reaction. The DPPH assay was used to determine the relative antioxidant activity, which is given in Table 1 and expressed as IC_{50}. The extract with the lowest IC_{50} value demonstrates the highest efficacy in acting as a DPPH scavenger. The extract from *C. asiatica* samples exhibited the most potent radical scavenging properties, with an IC_{50} value ranging from 8.25 to 19.72 mg/mL. Using water, ethanol, methanol, and hexane to extract the dried powder of *C. asiatica* provided the lowest group of IC_{50} values (8.90, 9.04, 8.57, and 8.25 mg/mL) of the extract. As indicated by the tiny values, it exhibited a commendable antioxidant capacity. The crude extract obtained from the desiccated powder of *C. asiatica* leaves contained both polar and non-polar substances. The findings of the analysis revealed that the type of solvent employed had a significantly impact on the antioxidative properties of the extract. Crude extracts of *C. asiatica* produced with methanol and ethanol exhibited the highest TPC on average. Likewise, it was observed that DPPH and FRAP exhibited commendable antioxidant capabilities. In consideration of food safety, ethanol was selected as the appropriate solvent to investigate further the optimal extraction procedure for *C. asiatica* extract used in pharmaceuticals or food products [20].

3.2 Effect of different extract methods

Table 2 displays the extractive content of specific bioactive compounds derived from *C. asiatica* through the implementation of five distinct extraction methods

Table 2 Determination of the total phenolic content and antioxidant activities of *C. asiatica* extract using various extraction methods

Method Extraction	Total Phenolic Content (mg GAE/ g extract)	Ferric reducing antioxidant power (µmol $FeSO_4$/ mg extract)	IC_{50} of DPPH (mg/mL)
SLE at 60°C for 2 h	70.04±21.67[c]	799.22±147.91[c]	7.278±0.847[a]
SLE at 60°C for 2 h followed by MAE at 600 W 2 min	95.76±11.97[a]	1,078.65±13.88[a]	5.371±0.162[c]
MAE 600 W, 2 cycles, 2 min/cycle	96.14±9.93[a]	1,125.73±32.50[a]	3.843±0.396[d]
UAE at 60°C for 2 h	94.83±5.41[a]	1,004.22±38.35[a]	5.717±0.244[c]
SLE at room temperature	85.34±11.06[b]	812.85±57.90[b]	4.296±0.248[b]

*Value is the mean ± standard deviation (n=3)

[a,b,c,d] within the column with the different letter were significantly different ($p<0.05$)

The range of values ascertained by TPC and FRAP was as follows: 70.04–96.14 mg GAE/g extract and 799.22–1,125.73 µmol $FeSO_4$/mg extract, respectively. MAE at 600 W, 2 cycles, and 2 min/ cycle produced the highest FRAP and TPC. By utilizing microwave radiation, MAE induces moisture within plant cells to evaporate and applies pressure to the cell walls by increasing their temperature. As a consequence of the bioactive components released from the ruptured cells, the increased production of phytocomponents is caused by the cell wall erupting under the influence of the generated pressure [9]. Prior research has documented that MAE exhibits a higher capacity for extracting bioactive constituents from a variety of medicinal plants [21]. Ethanol constituted the second most effective method in the UAE. The UAE facilitates the extraction process through the utilization of ultrasonic sound energy, which induces acoustic cavitation. This causes the degradation of cell walls and the subsequent liberation of bioactive compounds [8]. Consistent with prior investigations, our findings validate the efficacy of MAE and UAE as extraction methods for bioactive compounds [22, 23]. It was found that alternative conventional methods, including maceration at ambient temperature, were substandard. Previous research has indicated that maceration, when used as an extraction technique, exhibits reduced efficacy in the elimination of compounds [24]. A protracted process, maceration exposes the extract to detrimental elements such as light and oxygen. This exposure leads to chemical degradation and a subsequent reduction in the extract's antioxidant capacity [25]. The MAE extraction method, which utilized 600 W of power, 2 cycles, and 2 min/cycle, demonstrated the most significant antioxidant activity, as evidenced by the DPPH IC_{50} values of 3.843±0.396 mg/mL, the lowest minimum inhibitory concentrations. Low IC_{50} values, as stated by Ray et al. [26], serve as an indicator of substantial antioxidant activity. Musa et al. [10] established a logical correlation between the bioactive component present in the plant extract and its antioxidant activity. Phenolic compounds, which are predominant antioxidants in plant extracts, serve as chelators of metals, radical scavengers, reducing agents, and donors of hydrogen; this elucidates their antioxidant characteristics [7]. The findings of our study align with previous research, which has also found that both MAE and UAE are effective techniques for extracting bioactive chemicals [22,23]. MAE exhibited a much greater antioxidant capacity of the extract compared to UAE and SLE. It may be inferred that

utilizing MAE in conjunction with an extraction solvent containing 80% ethanol concentration appears to be a more suitable extraction condition. With respect to energy conservation and extraction methods, MAE emerged as the most efficient, yielding the greatest antioxidant activity and facilitating extraction in less than the other methods. Subsequently, ethanol demonstrated the greatest total phenolic and antioxidant activity (FRAP and DPPH;IC_{50}), which most likely contributes to *C. asiatica*'s antimicrobial properties.

3.3 Antimicrobial susceptibility testing of the C. asiatica extracts

The antimicrobial susceptibility of the *C. asiatica* extracts was assessed using the agar disc diffusion assay according to the CLSI guidelines from 2007. The test involved analyzing the crude extract sample produced from the ideal extraction condition. The extraction was done using a 1:30 (w/w) ratio of *C. asiatica* to solvent, with a microwave extraction strength of 600 W, 2 cycles, 2 min/cycle. This particular condition resulted in the highest TPC, FRAP, and the lowest IC_{50} for DPPH. The purpose of the analysis was to assess the antimicrobial susceptibility.

Table 3 The average diameter (mm.) of the inhibitory zones of *C. asiatica* extracts at concentrations of 25% and 50% (250 and 500 mg/mL), as well as the antibiotic and distilled water, were tested against *Bacillus cereus, Staphylococcus aureus, Escherichia coli* O157:H7 and *Salmonella* Typhi

Bacteria strain	25% extract	50 % extract	0.1% (w/v) chlorhexidine	Control (H_2O)
Gram-positive				
Bacillus cereus	8.89 ± 0.16^{b}	10.97 ± 0.30^{a}	10.20 ± 0.12^{a}	-
Staphylococcus aureus	8.05 ± 0.59^{b}	10.05 ± 0.26^{a}	10.18 ± 0.06^{a}	-
Gram-negative				
Escherichia coli O157:H7	6.72 ± 0.08^{b}	8.39 ± 0.14^{a}	8.50 ± 0.04^{a}	-
Salmonella Typhi	7.76 ± 0.18^{b}	8.12 ± 0.38^{a}	8.80 ± 0.02^{a}	-

*Absence of inhibition zone detection; Diameter of inhibition zone (mm.); The means and standard deviations of all three replicates were used to represent the data. For values that differ significantly at a significance level of $p < 0.05$, various superscripts were appended to each vertical.
^{a,b} within the row with the different letter were significantly different ($p<0.05$).

The antimicrobial efficacy of extracts from *C. asiatica* plants was evaluated by measuring the zone of inhibition of microbial growth, as presented in Table 3. The antibacterial efficacy of *C. asiatica* was assessed against all tested cultures using extract doses of 250 and 500 mg/mL. The *C. asiatica* extract obtained using MAE shown inhibitory effects on all four bacteria, regardless of the extraction condition. The average inhibition zone diameters of the *C. asiatica* extract solution at 250 and 500 mg/mL were 8.89 ± 0.16 and 10.97 ± 0.30 mm. against *B. cereus*, 8.05 ± 0.59 and 10.05 ± 0.26 against *S. aureus*, 6.72 ± 0.08 and 8.39 ± 0.14 against *E. coli* O157:H7, and 7.76 ± 0.18 and 8.12 ± 0.38 mm. against *S.* Typhi. There was no observation of water control zones. The study observed that the inhibition zones of *S. aureus* and *B. cereus* were significantly greater in size compared to those of *E. coli* O157: H7 and *S.* Typhi, which are Gram-negative bacteria. When comparing the effects of 0.1% chlorhexidine on *B. cereus*, *S. aureus*, *E. coli* O157:H7, and *S.* Typhi, the average diameters of the inhibition zones were 10.20 ± 0.12 mm., 10.18 ± 0.06 mm., 8.50 ± 0.04 mm., and 8.80 ± 0.02 mm., respectively. The antimicrobial capability of *C. asiatica* was dependent the concentration of extract

and the microbial strains tested. Results revealed that the crude extract obtained from the optimal extraction condition at 1:30 (w/w) *C. asiatica*: solvent ratio using microwave extraction power of 600 W, 2 cycles, and 2 min/cycle could potentially be an alternative for synthetic preservatives against foodborne pathogens. Therefore, future research will concentrate on utilizing an ethanolic extract of *C. asiatica* as a natural preservative in edible plastic film for fresh-cut fruits, vegetables, and ready-to-eat food. Subsequent to customer health concerns, increased will be undertaken to enhance and refine Thai herb preservatives. Customers also prefer to see the nation's natural resources utilized to their fullest capacity.

4. CONCLUSIONS

The solvents and extraction methods utilized during the extraction procedure had a substantial impact on the antioxidant capacity exhibited by *C. asiatica*. Based on the findings, it was determined that MAE with 80% ethanol at 600 W, 2 cycles, and 2 min/cycle was exceptionally effective at extracting bioactive compounds and antioxidant capacity from *C. asiatica*. Additionally, the extract exhibited potential antibacterial activity against pathogenic pathogens (*B. cereus*, *S. aureus*, *E. coli* O157:H7, *S.* Typhi) at concentrations of 25% and 50%. Based on the findings, it can be inferred that the *C. asiatica* extract possesses considerable promise as an inherent antioxidant and antibacterial agent. This implies that the extract may find application in the treatment of diverse microbial control.

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Encapsulation of Betacyanin from Red Dragon Fruit Peels Using Maltodextrin and Pectin: Impact on Physicochemical Properties

BP-O040

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ABSTRACT

The red dragon fruit peels are often considered as agricultural waste but contain a functional pigment known as betacyanin. This study aimed to evaluate the physicochemical properties of encapsulated betacyanin with a combination of maltodextrin (MD) and pectin (PE) as wall materials. All samples were analyzed for physical and chemical properties, including moisture content, water activity, bulk and tapped density, and flowability, betacyanin content (BTC), total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (DPPH and FRAP). Encapsulation significantly improved the yield and reduced the water activity ($p < 0.05$). The use of a combination of wall materials resulted in a higher yield compared to the use of a single wall material. The increasing concentrations of PE lead to high encapsulation efficiency (EE) and BTC. Combination of 37% MD + 3% PE exhibited the highest value of BTC, TPC, TPC, DPPH, and FRAP (85.18 mg/100 g, 116.78 mg GAE/100 g, 94.84 mg QE/100 g, 69.84 µmol TE/100 g, 743.02 µmol Fe^{2+}/100 g, respectively). This study demonstrated that an encapsulation betacyanin using a combination of wall materials (MD + PE) showed higher values of chemical properties than a single wall material.

1. INTRODUCTION

Dragon fruit (*Hylocereus* sp.), also known as pitahaya or pitaya, has expanded globally due to commercial demand and has grown extensively in over than 20 tropical and subtropical countries, including Indonesia, Myanmar, Thailand, Vietnam, Taiwan, and Southern China [1]. Red dragon fruit (*Hylocereus polyrhizus*) has gained significant attention as a natural food colorant source in recent years. The vibrant color is the result of an abundant pigmentation compound, known as betacyanin, which is responsible for the red-purple pigment [2].

Furthermore, red dragon fruit peels are typically discarded after consumption of inner flesh, despite being a by-product of the fruit juice industry and classified as agricultural waste. The red dragon fruit peel contains a higher betacyanin level than the pulp (35.12 ± 0.01 and 30.15 ± 0.03 mg/g sample, respectively) [3].

Betacyanin is a nitrogen-containing compound consisting of betalamic acid as the chromophore core that is condensed with *cyclo*-DOPA (dioxyphenylalanine) [4]. It belongs to the betalain group, which also includes betaxanthin, a yellow-orange pigment. Betacyanin is well known for its functional health benefits, such as regulating the glycemic response, mitigating diabetes, and modulating the immune system [5]. However, this compound is very susceptible to degradation. Several influencing factors that can decrease betacyanin are the absence of matric, high water activity, low extent of glycosylation and acylation, pH < 3, pH > 7, high temperature, presence of light, high concentration of oxygen, free radicals, metal cations, and degrading enzymes [6].

Encapsulation is one of the suitable methods for stabilizing betacyanin by protecting against degradation factors and improving retention during storage [7]. Freeze-drying is one of the physical encapsulation methods and involves converting the ice into vapor after rapid freezing under high vacuum conditions. Thermally unstable compounds, such as betacyanin, are more likely to be dried using this technology [8]. Maltodextrin (MD) belongs to modified starch and has become the most widely used wall material from the polysaccharides group due to low bulk density and high solubility at high solid content [9]. Pectin is a natural anionic biopolymer consisting of heteropolysaccharides of D-galacturonic acids linked by α-1,4 glycosidic bonds. Their ability to interact with other food macromolecules and the mechanical and physical resistances (thermal stability) are some promising factors that make this polysaccharide a potential wall material for the encapsulation of bioactive compounds [10]. Encapsulated anthocyanin with pectin and whey protein could increase its heat stability and encapsulation efficiency along with strong antioxidant activity [11]. Previous research has demonstrated that using a combination of two wall materials has proven to be more satisfactory compared to using a single wall material activity [12].

However, there is a lack of comprehensive research exploring the utilization of a combination of maltodextrin and pectin as betacyanin encapsulation wall materials. Therefore, this study aims to evaluate the impact of the combination of maltodextrin and pectin as encapsulation wall materials on the physicochemical properties of the freeze-dried betacyanin extract of red dragon fruit peels.

2. MATERIAL AND METHODS

2.1 Materials

Red dragon fruit (*Hylocereus polyrhizus*) with 100% maturity (45 days after blooming) was obtained from Pathum Thani Province, Thailand (Kenny Dragon Fruit Farm). Maltodextrin DE 10-12 (Chemipan Co. Ltd, Bangkok, Thailand) and low methoxyl pectin (Louis Francois, Croissy-Beaubourg, France) were used. All of the chemicals and reagents used in this research were of analytical grade.

2.2 Preparation of Betacyanin Extract (BTE) from Red Dragon Fruit Peels

This extraction procedure was used an Ultrasound-Assisted Extraction (UAE) method [13,14]. Red dragon fruits were peeled to obtain only the skin/peel. The fruit peels were diced into 2 x 2 cm size and dried at 42 °C using a hot air oven (Guangdong IKE, WRH-100B, Guangdong, China) for 24 hours. The dried dragon fruit peels were then ground and passed through a 20-mesh filter. The

dragon fruit peel powders were collected in a vacuum-sealed aluminum laminated pouch and stored at -18 °C freezer before extraction. The red dragon fruit peel powders were mixed in a 1:50 ratio with 50% aqueous ethanol (v/v) and stirred with a magnetic stirrer at 200 rpm for 2 hours at room temperature. Further extraction was carried out using an UAE (Sonics VCX 500 – 750, Connecticut, USA) with a 22 mm diameter probe tip (24 kHz, 400 W) for 5 minutes at 30% amplitude. The BTE was filtered using Whatman paper No.1. The excess solvent was removed using a Rotary Evaporator at 40 °C to concentrate the BTE. Finally, the concentrated BTE was stored in a dark bottle at 4 °C for further analysis.

2.3 Preparation of Betacyanin Encapsulation

The procedure followed the method developed by Stoica et al. (2022) [15] and Putri et al. (2021) [16] with some modifications. In this study, maltodextrin (MD) and maltodextrin + pectin (MP) were used as wall materials for the encapsulation of betacyanin. A preliminary study showed that the maximum concentration of PE was 3%; otherwise, it would become too viscous. Therefore, to prepare the combination of MP, MD was dissolved in distilled water at concentrations (w/v) of 39%, 38%, and 37%, together with 1%, 2%, and 3% concentrations (w/v) of PE, respectively (see Table 1). The wall material solutions were mixed and dissolved in distilled water (100 mL) at 40 °C for 3 hours using a magnetic stirrer (400 rpm) to achieve a 40% concentration (w/v) of the solution. The solutions were then stored at 4 °C overnight for total hydration. BTE was added to the wall material solution in a 1: 3 (BTE : Wall material) proportion and mixed at 25 °C for 2 hours using a magnetic stirrer (400 rpm). The dispersions were then frozen at -20 °C overnight and lyophilized at -42 °C under a pressure of 0.10 mBar for 48 hours (Thermo Fisher, Supermodulyo-230, Massachusetts, USA). Lastly, the freeze-dried powders were packed in a vacuum aluminum laminated pouch and stored at 4 °C for further analysis.

2.4 Encapsulation Efficiency (EE) and Drying Yield

EE was determined by analyzing the betacyanin content in the core (CBC) and surface (SBC) [17]. For CBC,100 mg of the sample was diluted in 1 mL of ethanol: acetic acid: water mixture (50:8:42 v/v/v), vortexed for 1 minute, and centrifuged (Hettich, Universal 320 R, Kirchlengern, Germany) for 2 min at 14,000xg (supernatant separated). For SBC, 100 mg of the sample was diluted in 1 mL of ethanol: methanol mixture (1:1, v/v), vortexed for 1 minute, and centrifuged for 2 min at 3018.6 xg (supernatant separated). The reaction mixture was prepared by mixing 230 µL of Mcllvaine buffer (pH 6.5) and 20 µL of the sample in a 96-well microplate [16,17]. The absorbance was measured at 535 nm. The BTC was determined based on Equation 1.

$$BTC\ (mg/100g) = \frac{A \times DF \times MW \times V \times 100}{\varepsilon \times W \times L} \quad (1)$$

where A was the absorbance at 535 nm, MW was the molecular weight of betanin (550 g/mol), V was the volume of the extract, DF was the dilution factor, E was the molar extinction coefficient of betanin (65,000/M/cm), L was the path length (0.286 cm), and W was the weight of the extract (g). Furthermore, the EE was determined using the equation (see Equation 2).

$$\%EE = \frac{(CBC - SBC)}{CBC} \times 100\% \quad (2)$$

The drying yield was determined by the ratio of powder obtained after freeze-drying with total solid content in the encapsulation mixture.

2.5 Physicochemical Properties of Encapsulated Betacyanin Powder

The moisture content was measured using a halogen moisture analyzer (Mettler Toledo, HB43-S Halogen, Greifensee, Switzerland) by placing 0.5 g of samples on the instrument. Water activity (aw) was determined using an aw meter (Aqualab 4TE, Pullman, USA) at 25 °C.

The bulk density was determined by placing a measured amount of powder in a 10 mL measuring cylinder. The total volume occupied by the powder was then measured to calculate the bulk density (ρB), expressed as weight per unit volume (g/mL). To assess the tapped density (ρT), the same amount of powder was tapped for minutes in the measuring cylinder. The tapped density was recorded as weight per unit volume (g/mL). The flowability was assessed as the Hausner ratio (HR) and estimated by the ratio of bulk density (ρB) and tapped density (ρT).

2.6 Bioactive Compounds and Antioxidant Activity of Encapsulated Betacyanin Powder

The betacyanin content (BTC) in the BTE and encapsulated betacyanin powders was determined using a spectrophotometric method on a 96-well microplate as previously described in Section 2.4. Analysis of total phenolic content (TPC) and total flavonoid content (TFC) was determined using the spectrophotometric method on a 96-well microplate [17]. The TPC used the Folin-Ciocalteu method and the TFC used the reaction mixture of $AlCl_3$. The absorbance was measured at 750 nm (TPC) and 430 nm (TFC). The results were expressed as gallic acid equivalents (GAE) per 100 g of encapsulated powder for TPC and quercetin equivalents (QE) per 100 g of encapsulated powder for TFC. The analysis was carried out in triplicate.

The antioxidant activity of the betacyanin extract and encapsulated powder was determined using ferric-reducing antioxidant power (FRAP) [18], and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [2]. For the FRAP method, the absorbance was measured at 620 nm after incubation of the plate for 5 min at 37 °C. For the DPPH method, after 30 minutes of incubation in the dark, the absorbance was measured at 515 nm. The results were expressed as µmol Fe^{2+}/100 g of encapsulated powders for FRAP and as µmol Trolox equivalent (TE)/100 g of encapsulated powders for DPPH. Those analyses were conducted in triplicate.

2.7 Statistical Analysis

The study followed the Completely Randomized Design (CRD) method to conduct the experiments. All data were presented as the mean ± standard error of the three replicates. Statistical analysis involved the use of one-way analysis of variance (ANOVA) and Duncan test with SPSS for Windows version 16.0. The significance level for testing differences was set at 0.05.

3. RESULTS AND DISCUSSION

3.1 Encapsulation Efficiency and Drying Yield

Encapsulation efficiency (EE) is defined as the percentage of core material (bioactive compounds) incorporated into microcapsules relative to the total amount of core material added during the encapsulation process [19]. The smaller number of bioactive compounds on the surface of the microcapsule indicates the higher encapsulation efficiency. The MP3 treatment produced the highest EE (90.87%) as shown in Table 1. The incorporation of pectin results in the impartation of a polyanionic characteristic to the wall materials due to the composition of glucuronic acid and galacturonic acid. Consequently, the polyanionic matrix will facilitate a strong electrostatic interaction with cationic betacyanin. Therefore, it resulted in a high EE [20].

Table 1. Wall materials formulation, encapsulation efficiency (EE) and drying yield

Treatment	Wall Materials (%)		EE (%)	Yield (%)
	MD	PE		
BTE	-	-	ND	22.94 ± 0.23^{c}
MD	40	-	90.51 ± 0.11^{a}	74.62 ± 0.45^{b}
MP1	39	1	90.64 ± 0.10^{a}	76.14 ± 1.47^{ab}
MP2	38	2	90.60 ± 0.06^{a}	77.45 ± 1.79^{a}
MP3	37	3	90.87 ± 0.38^{a}	76.52 ± 1.53^{ab}

*Columns with different letters indicate significant differences ($p < 0.05$); BTE: Betacyanin extract; MD: Maltodextrin; PE: Pectin; MP: Maltodextrin + Pectin; ND: Not determined

The yield of the encapsulated betacyanin powder was significantly different ($p < 0.05$) ranging from 74.62 – 77.45% (Table 1). Encapsulation significantly improved BTE yield (22.94%) by increasing the total solid of powders ($p < 0.05$). The lowest yield was obtained from the MD treatment. According to the findings, the use of a combination of wall materials resulted in a higher yield compared to the use of a single wall material. A previous study showed that the yield of freeze-dried propolis increased when combinations of wall materials were used [21]. The increase in maltodextrin concentration has a positive effect on drying yield due to an increase in glass transition temperature (Tg) in the surface layer and a reduction in the stickiness of the dried product [22].

3.2 Physicochemical Properties of Encapsulated Betacyanin Powder

Encapsulated powders exhibited an increasing trend in moisture content ($p > 0.05$) and water activity ($p < 0.05$) as increasing PE concentration as shown in Table 2. These results are consistent with Yang et al. (2022) [23], suggesting that PE may possess a greater water-holding capacity compared to MD. The moisture content remained below 5%, ensuring improved stability of the powdered product [12]. BTE had the highest a_w at 0.26. High a_w values can enhance both chemical reactions and microbial growth, which can affect the stability of betacyanin. Low moisture content and water activity are essential to prevent agglomeration and caking, which can cause issues such as wet powders, degradation of bioactive compounds, and impediments to flowability and dispersion, to extend the shelf life of encapsulated powders [24]. The a_w values of the encapsulated powders ranged from 0.17 to 0.24, which is within a safe range to inhibit microbial growth and enzymatic and

non-enzymatic degradation [23]. Therefore, these findings underscore the significance of the encapsulation process in protecting betacyanin from degradation.

Table 2. Physicochemical properties of encapsulated betacyanin powder

Treatment	Moisture content (%)	Water activity (aw)	Bulk density	Tapped density	Hausner ratio
MD	2.61 ± 0.50^{a}	0.17 ± 0.01^{d}	0.43 ± 0.01^{b}	0.65 ± 0.00^{b}	1.52 ± 0.01^{a}
MP1	2.66 ± 0.79^{a}	0.20 ± 0.02^{c}	0.43 ± 0.00^{b}	0.66 ± 0.01^{ab}	1.53 ± 0.02^{a}
MP2	2.94 ± 0.81^{a}	0.23 ± 0.01^{b}	0.43 ± 0.01^{b}	0.66 ± 0.00^{ab}	1.54 ± 0.00^{a}
MP3	3.01 ± 0.85^{a}	0.24 ± 0.00^{a}	0.46 ± 0.01^{a}	0.67 ± 0.00^{a}	1.46 ± 0.01^{b}

*Columns with different letters indicate significant differences ($p < 0.05$); BTE: Betacyanin extract; MD: Maltodextrin; MP: Maltodextrin + Pectin

The bulk and tapped density are crucial parameters in encapsulated powder materials to determine the storage and handling of the materials during processing and transportation. In terms of packing, the tapped density plays an important role in determining the maximum packing density of the powder sample. Both parameters provide insight into the flow properties of powders. MP3 (37% MD + 3% PE) had the highest bulk density ($p < 0.05$) and tapped density (see Table 2). The heavier the material, the more likely it is to fill the gaps between the particles, covering a minimum space and leading to higher values of bulk density [25]. Low methoxyl pectin had the highest molecular weight (283 – 562 kDa)[26], followed by MD DE 10 – 12 (1.62 kDa) [27].

The flowability of the encapsulated powders was determined by the Hausner ratio in which MP3 had significant differences ($p < 0.05$) (see Table 2). All treatments were categorized as very poor flowability (1.46 – 1.59) [28]. A high value of the bulk density and a low value of the tapped density led to good flowability properties (low value of Hausner ratio). In addition, the flowability of powders increases as particle size increases, due to an increase in gravitational forces that become dominant compared to the forces [29]. This is attributed to the highly heterogeneous shape of the encapsulated powders using the freeze-drying method.

3.3 Bioactive Compounds and Antioxidant Activity of Encapsulated Betacyanin Powder

There were no significant differences in BTC between all treatments ($p \geq 0.05$) with values ranging from 81.85 – 85.18 mg/100 g (see Table 3). In general, MP treatments exhibited higher BTC compared to MD with the highest value obtained by MP3. Similar results also showed that a higher BTC was obtained from the encapsulated powder that used MD, dragon fruit PE and cellulose as wall material [30]. The TPC values of the encapsulated powders ranged from 91.31 - 116.78 mg GAE/100 g of dry powders and were partially different ($p < 0.05$). The highest TPC among the treatments was obtained by MP3. The lowest TPC value was observed in MP2, which may be attributed to the strong interactions between PE and phenolic compounds [31]. These interactions probably inhibited the reaction between the Folin reagent and the phenolic compounds. Meanwhile, at high concentrations, it allows the formation of a thick coating, thus creating stable microcapsules that preserve more phenolic compounds [23]. The TFC of the encapsulated powders showed a significant difference between all treatments ($p < 0.05$) and ranged from 79.13 – 94.84 mg QE/ 100 g dry powders. The TFC was positively correlated with an increase in the concentration of PE. Pectin interactions with phenolic acids are stronger than with flavonoids, due to the carboxylic groups [31]. Therefore, the

TFC of the encapsulated powders originated primarily from the betacyanin extract of the red dragon fruit peels with less influence from the natural flavonoid of pectin.

Table 3. Bioactive compounds and antioxidant activity of encapsulated betacyanin powders

Treatment	BTC (mg/100 g)	TPC (mg GAE /100 g)	TFC (mg QE /100 g)	DPPH (µmol TE /100 g)	FRAP (µmol Fe^{2+} /100 g)
MD	81.85 ± 0.27^{a}	110.65 ± 5.46^{a}	89.54 ± 0.93^{b}	71.22 ± 9.35^{a}	714.44 ± 7.64^{ab}
MP1	82.96 ± 0.93^{a}	105.63 ± 3.68^{ab}	79.13 ± 2.10^{d}	60.83 ± 5.69^{a}	658.25 ± 14.30^{c}
MP2	82.59 ± 0.56^{a}	91.31 ± 8.07^{b}	86.58 ± 1.07^{c}	68.98 ± 3.09^{a}	679.21 ± 22.62^{bc}
MP3	85.18 ± 3.51^{a}	116.78 ± 11.19^{a}	94.84 ± 1.01^{a}	69.84 ± 2.05^{a}	743.02 ± 28.10^{a}

*Columns with different letters indicate significant differences ($p < 0.05$); BTE: Betacyanin extract; MD: Maltodextrin; MP: Maltodextrin + Pectin

The encapsulated powders had antioxidant activity (AA) using the DPPH method, ranging from 60.83 – 71.22 µmol TE/100 g of dry powders with no significant differences ($p \geq 0.05$) (see Table 3). On the other hand, when evaluated using the FRAP method, the AA values ranged from 658.25 – 743.02 µmol Fe^{2+}/100 g of dry powders and significant differences ($p < 0.05$). The highest value was obtained by MP3 treatments, but MP1 and MP2 exhibited a lower value than MD treatment. The TFC exhibited a strong positive correlation with antioxidant activity using the FRAP ($R = 0.873$) and DPPH ($R = 0.641$) method. Polygalacturonic acid in the structure of pectin may be complex with certain phenolic compounds through Ca^{2+} ions, and the porosity of pectin compounds may also contribute to the entrapment of phenolic compounds. Therefore, it can reduce its release, affecting its bioactivity as an antioxidant [32]. Furthermore, the ramification (carboxymethylcellulose, hydroxyethylcellulose, hydroxypropylmethylcellulose, and methylcellulose) of pectin affects its antioxidant activity [31].

4. CONCLUSIONS

The encapsulation process using a combination of MD and PE as wall materials successfully altered the physicochemical properties of betacyanin. However, the composition and concentration of wall materials are not equally effective in preserving betacyanin. In general, MP treatments (MP1, MP2, and MP3) increased EE and drying yield. Meanwhile, increasing the concentration of PE leads to an increase in moisture content and water activity. Treatment with MP3 stood out with the best bioactive compounds and antioxidant properties. The combination of wall materials showed better results than the single wall material. Encapsulated betacyanin powders could be a promising option for the food industry to use natural food colorants to develop functional foods. More studies are needed to explore different factors, including the use of different storage and processing conditions, to better understand the stability of encapsulated betacyanin extracts.

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Effect of different conditions of ultrasound-assisted extraction (UAE) on antioxidant property of kratom leaf (*Mitragyna speciosa* Korth.)

BP-P047

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ABSTRACT

In recent decades, the extraction of plant material has been carried out in various ways, including ultrasound-assisted extraction (UAE). The UAE presents a more effective, discerning, and eco-friendly approach to extracting bioactive compounds from botanical sources. This study assessed the impact of various UAE parameters on total phenolic compound (TPC), total flavonoid content (TFC), total tannin content (TTC), mitragynine and antioxidant activity measured through 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and ferric reducing antioxidant power (FRAP) assays, in kratom leaf. The extraction variables considered were ethanol concentration (80% and 90% v/v) and duration (10, 20 and 30 min), maintaining a consistent ratio of kratom powder (g) to ethanol solvent (ml) at 1:40 (w/v) under UAE. The results indicated that increasing ethanol concentration led to higher values of antioxidant activities, TPC, TTC and mitragynine but not for TFC. Optimal conditions for the highest TPC (326.09±3.68 mg GAE/g db.), antioxidant activities via DPPH (1976.54±9.16 µM TE/100 g db.), FRAP (1992.91±5.03 µM TE/100 g db.), TTC (342.95±3.63 mg TAE/g db.) and mitragynine content (97.56±0.84 mg/g db.) were observed at an ethanol concentration of 90% v/v and an extraction duration of 10 min. Conversely, the best conditions for TFC (287.26±5.45 mg QE/g db.) reported at ethanol concentration of 80% v/v and extraction duration of 30 min. Therefore, this research could provide important knowledge for the future extraction method to obtain more bioactive compounds and antioxidant activity from kratom leaf.

1. INTRODUCTION

Kratom is a perennial plant with the scientific name *Mitragyna speciosa* Korth. It is classified in the *Rubiaceae* family. It is native to the humid tropics and is mostly found in Southeast Asian countries especially Thailand, Indonesia, and Malaysia. Kratom is a plant that has been with Thai society for a long time. It is often used as a Thai folk medicine to help relieve body aches and relieve stomach pain, antidiabetic, blood pressure-lowering also as a stimulant to increase work efficiency, endurance, tolerance for manual labourers [11]. Currently, kratom has been removed from the status of a harmful drug. This makes kratom widely used, both in the form of chewing fresh kratom leaf and brewing as a tea. Kratom leaves are recognized to be high in alkaloids, flavonoids, phenolic compounds, antioxidant activity and mitragynine. Mitragynine is an interesting alkaloid that can be used as a substitute for opium or morphine in the treatment of drug addicts [1]. Previously, the extraction of bioactive compounds of kratom had been carried out in conventional extraction such as soaking and maceration which had limitation including low extraction yield and poor extraction efficiency. Ultrasound-assisted extraction (UAE) can be considered as a non-conventional green technology which overcomes these limitations. From a previously research of Orio et al [14]. on the extraction mitragynine from kratom using various methods, it was found that ultrasound extraction yielded a higher amount of mitragynine and took less time to extract than classical method (maceration).

Ultrasound-assisted extraction (UAE) is labeled as a green extraction process with higher bioactive compound yield. **It** uses high-frequency sound waves (20 to 100kHz) in combination with organic solvents or water to extract biologically active compounds. This process causes gas formation and collapse/implosion of bubbles, the gas bubble expands, it pulls out the substances inside the material and dissolves them in the solvent causing the phenomenon of cavitation. UAE is a simple operation, inexpensive, reduces the processing time, and is efficient alternative to extracting bioactive compounds from plant materials [10]. This study evaluated the effects of different UAE conditions including ethanol concentration (80 and 90% v/v) and extraction time (10, 20 and 30 min) on antioxidant property (TPC, TFC, TTC, mitragynine and antioxidant activity by DPPH and FRAP assays) of kratom leaf.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

All the chemicals, reagents, and solvents used in this experiment were analytical grade. The materials and chemicals used in this research were; kratom leaf (Phatthalung, Thailand), 2,2-diphenyl-1-picryl-hydrazyl (DPPH) (Sigma, Swizerland),2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ) (Sigma Aldrich, USA), 6-hydroxy-2,5,7,8-tretamethylchroman-2-carboxylic acid (Trolox) (Sigma, Swizerland), aluminum chloride (KemAus, Australia), ethanol (DEPTAL-AX, Thailand), ferric chloride (Fisher Scientific, UK), Folin-Ciocalteu reagent (Loba, india), gallic acid (Fluka, Spain), glacial acetic acid (ANaPURE, New Zealand), hydrochloric acid (ANaPURE, Newzealand), tannic acid (Himedia, India), quercetin (Sigma-Aldrich, Germany), Standard mitragynine (Chromadex, USA) and sodium carbonate (Na_2CO_3) (KemAus™, Australia).

2.2 Kratom leaf sample preparation

The leaves of Kratom were collected from a kratom farm (Phatthalung, Thailand) and transported to Department of Food Technology, Faculty of Science, Chulalongkorn University. In the laboratory, the leaves were rinsed under water to remove any dirt and dust, washed, and then dried in a hot air oven (Memmert, DO 6062, Germany) at 60°C until moisture content < 5% dry basis (db.). The dried kratom leaves were powdered using a 50-mesh sieve, vacuum-packed in an aluminum-laminated foil bag and kept in a freezer at -20°C for UAE experiment.

2.3 Ultrasound-assisted extraction (UAE)

For extraction of kratom leaf, 5 g of sample was mixed with 200 mL of solvent in a 500 mL flask. The extraction variables considered were ethanol concentration (80% and 90% v/v) and time (10, 20, and 30 min). Then, the UAE was performed with an ultrasound bath (P70H Ultrasonic processor, Elma, Germany) with a 220 W power, 37 kHz. The samples were then centrifuged (Hettich Zentrifugen, 320r, Germany) at 4,500 rpm for 15 min at room temperature and the supernatant was filtered through a Whatman No.1 filter paper, then evaporated under vacuum condition at 40°C with a rotary evaporator (Oil bath B-485, BÜCHI, Switzerland). The final sample volume was then adjusted to 10 mL with 95% ethanol (kratom leaf extract) and kept in amber vial at -20°C before the further analysis.

2.4 Characterization of Kratom Extract

2.4.1 Determination of total phenolic compound

Total phenolic compound was determined following to Folin-Ciocalteu method previously described by Waterhouse [19]. Briefly, 100 µL of kratom leaf extract was transferred into a test tube, followed by the addition of 1.4 mL of distilled water and 1.5 mL of 10% v/v Folin-Ciocalteu reagent, respectively. The mixture was then vortexed for 15 sec and allowed to sit at room temperature for 5 min. Thereafter, saturated sodium carbonate solution (0.5 mL) was added and incubated for 1 h at room temperature in a dark place. Gallic acid standard (0-200 mg/L) was prepared in the same manner. Then, the absorbance of kratom leaf extract and gallic acid were read at 765 nm using spectrophotometer (GENE-SYSTM 20 Visible, Thermo Fisher Scientifc, USA). Total phenolic compound was reported as mg gallic acid equivalents/g dry basis (mg GAE/g db.).

2.4.2 Determination of total tannin content

Total tannin content was determined following to Folin-Ciocalteu method previously described by Hanifah et al. [22] with the same protocol as total phenolic compound determination except the absorbance was measured at 725 nm with tannic acid as a standard (0-200 mg/L). Total tannin content was reported as mg tannic acid equivalents/g dry basis (mg TAE/g db.).

2.4.3 Determination of total flavonoid content

The analysis of total flavonoid content (TFC) was conducted by aluminum chloride

colorimetric method according to the method of Maisuthisakul, Suttajit, and Pongsawatmanit [15], 1 mL of kratom leaf extract was pipetted and mixed with aluminum chloride solution (2% w/v, 1 mL), and incubated for 30 min at room temperature. Absorbance was measured at the wavelength of 430 nm using spectrophotometer (GENE-SYSTM 20 Visible, Thermo Fisher Scientifc, USA). The similar protocol was used to prepare a quercetin standard (0-200 mg/L). Finally, total flavonoid content of kratom leaf extract was expressed as mg quercetin equivalents/ g dry basis (mg QE/g db.).

2.4.4 DPPH radical scavenging activity

The analysis of antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method was conducted from method of Brand-Williams et al. [5]. Briefly, 100 μL of kratom leaf extract were mixed with 1 mL of DPPH ethanol solution ($6x10^{-5}$ M), vortex-mixed, and incubated in darkness at room temperature for 30 min. Absorbance of DPPH daily solution and kratom leaf extract were measured at 515 nm using spectrophotometer (GENE-SYSTM 20 Visible, Thermo Fisher Scientific, USA). Trolox (0-800 μM) was used to prepare standard curve. Antioxidant activity by DPPH assay was reported as μM trolox equivalents/ 100 g (μM TE/100 g db.) using the following equation.

$$A_{diff} = A_{initial} - A_{final} \quad (1)$$

where A_{diff} is the difference of the absorbance between DPPH and sample, $A_{initial}$ is absorbance of DPPH, and A_{final} is absorbance of sample.

2.4.5 Ferric reducing antioxidant power (FRAP)

Antioxidant activity by ferric reducing antioxidant power (FRAP) assay was performed according to the method of Benzie and Strain [3]. Briefly, the FRAP solution was prepared by use 25 mL of acetate buffer (pH 3.6) add 2.5 ml of ferric chloride and 2.5 ml of TPTZ then mixed thoroughly with vortex mixer and preheated at 37°C for 10 min until the color was changed from brown to orange brown. Then, 2.850 mL of FRAP solution and 150 μL of kratom leaf extract were vortex-mixed and left in the dark at room temperature for 30 min. Absorbance of FRAP solution and kratom leaf extract were measured at 593 nm using spectrophotometer (GENE-SYSTM 20 Visible, Thermo Fisher Scientific, USA). Trolox (0-800 μM) was used to prepare standard curve. Antioxidant activity by FRAP assay was calculated according to the following equation and the results were reported as μM trolox equivalents/100 g (μM TE/100 g db.).

$$A_{diff} = A_{final} - A_{initial} \quad (2)$$

where $A_{initial}$ is absorbance of FRAP, A_{final} is absorbance of sample and A_{diff} is difference of the absorbance between FRAP and sample.

2.4.6 Determination of mitragynine content by HPLC

High performance Liquid Chromatography (HPLC) method modified from Fazila et al. [21] was conducted using the Dionex Ultimate 3000 Ultra High-performance Liquid Chromatography system

(Thermo Fisher Scientifc, Massachusetts, USA) equipped with a Kinetex core-shell C18 column (250mm × 4.6 mm internal diameter, 5 µm) (Kinetex™ core-shell technology, India). Before injection, a kratom leaf extract (50 µL) was mixed with 10 mL of absolute methanol. Then, the mixture was filtered via a 0.22-µm nylon filter. During the analysis, the temperature was retained at 25°C at a flow rate of 1 mL/min. Solvent A (water mixed with ammonium acetate) pH6 adjusted by glacial acetic acid and solvent B (acetonitrile) were used as the mobile phase. Beginning at ratio 35:65 of solvent B, the gradient elution program was set for 1.0 min. The injection volume was set at 10 µL. Mitragynine contents were determined using the ultra-violet spectroscopy with a High-performance Liquid Chromatography (HPLC) measuring the absorbance of kratom leaf extract and standard mitragynine (Chromadex, USA) at UV detection of 225 nm. Mitragynine content was reported as mg/g using the mitragynine standard curve (concentration 0-1000 mg/L).

2.5 Statistical analysis

The completely randomized design (CRD) was used in this study with three replicates. The significant difference among mean values was analysed by analysis of variance (ANOVA), followed by Duncan's new multiple range test (SPSS version 23). Statistical significance was defined at a 95% confidence level.

3. RESULTS AND DISCUSION

3.1 Effects of UAE conditions on TPC and TTC

The results of TPC and TTC from the ethanol extraction of 80 and 90% concentration ethanol at 10, 20, 30 min from kratom leaf are shown in Figures 1 and 2, respectively. The optimal condition for the highest TPC (326.09±3.68 mg GAE/g db.) and TTC (342.95±3.63 mg TAE/g db.) were 90% of ethanol concentration at 10 min of extraction time, maintaining a consistent ratio of kratom powder (g) to ethanol solvent (ml) at 1:40 (w/v) as the previously researched by Karunakaran et al. [8] and mentioned that high percentage of ethanol concentration increased the yield of phenolic compound extraction. Similarly to total tannin content, previous research by Hilary et al. [23] has shown that a high percentage of ethanol-water solvents is effective for extracting tannins. This is because the extraction process depends on the solvent's ability to dissolve biologically active substances from plant cells. Each type of solvent has a different polarity, affecting its ability to dissolve specific substances unequally [7]. As the extraction time increases, both TPC and TTC decrease. This phenomenon can be explained by Fick's law of diffusion, where TPC and TTC values increase until the sample becomes saturated, after which the diffusion of compounds ceases [4].

3.2 Effects of UAE conditions on TFC

The best conditions for TFC (287.26±5.45 mg QE/g db.) was at ethanol concentration of 80% v/v and extraction duration of 30 min (Figure 3). It was found that ethanol is a good polar solvent for extracting flavonoids from plants and the solvent mixture (water-ethanol) could improve the effectiveness of the extraction and increase surface area for the solvent solid contact. The longer extraction time make the solvent mixture (water-ethanol) infiltrate to solid (kratom leaf powder) therefore more flavonoid compound diffuse into the extracting solvent [20].

3.3 Effects of UAE conditions on antioxidant activities

DPPH assay is used for the determination of the antioxidant activity of the substances. Valacchi et al. [18] declared that DPPH antioxidant analysis is based on the mechanism of the reaction by providing hydrogen to the free radicals. Antioxidant activity by DPPH assay of kratom leaf extract ranged from 1318.54±9.03 to 1976.54±9.16 µM TE/100 g db. In general, increasing ethanol concentration from 80% to 90% resulted in an increase in antioxidant activity by DPPH assay. However, when the duration time increased from 10 to 30 min, the antioxidant activity decreased. Optimal conditions for the highest DPPH assay (1976.54±9.16 µM TE/100 g db.) was observed at an ethanol concentration of 90% v/v with extraction duration of 10 min (Figure 4). According to the results, antioxidant activity directly correlates with the total phenolic content [17]. When examining the effect of extraction time, prolonged extraction leads to a decrease in antioxidant activity. This decline is due to the sensitivity of bioactive compounds to heat and the cavitation force from ultrasonication, which can degrade these compounds and reduce antioxidant activity [2,6].

In the extraction at 10 min and 30 min, the antioxidant activity by FRAP assay increased with increasing ethanol concentration from 80% to 90% (v/v). This might be due to high phenolic content found in 90% ethanol concentration and then the antioxidant activity contributed to those phenolic compounds [12]. Optimal conditions for the highest FRAP assays (1992.91±5.03 µM TE/100 g db.) were observed at an ethanol concentration of 90% v/v with extraction duration of 10 min (Figure 5). The increase in antioxidant activity during increasing extraction period could be because of the diffusion of bioactive compounds from plant cells to solvents. However, if the extraction time took too long, bioactive compounds would be broken and then antioxidant activity would be reduced [9].

3.4 Effect of UAE conditions on mitragynine

Different extraction conditions (ethanol concentration and time) affected the mitragynine content (Figure 6). The value of mitragynine from 90% ethanol concentration condition were higher than 80% ethanol concentration. High percent of ethanol as solvents can increase the extraction efficiency of mitragynine. In fact, ethanol is less polar than water. The increase of alkaloid is consistent with the decrease of polarity [8]. Regarding the extraction time factor, extraction time is inversely related to mitragynine content because prolonged extraction can exceed the saturation point. Once saturation is reached, the extraction process ceases. Additionally, the increased heat during extended extraction can degrade mitragynine. These results are consistent with the previous research by Rusydan et al. [16]. The optimal condition for the highest mitragynine (97.56±0.84 mg/g db) extraction were 90% of ethanol concentration and 10 min of extraction time.

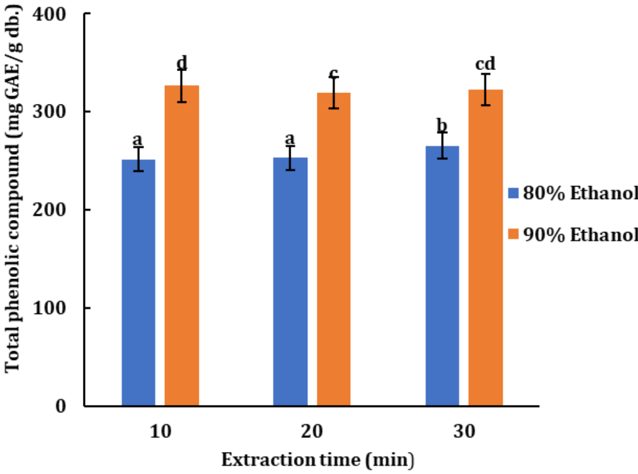

Figure 1. The effect of ethanol concentration (80% and 90%) and extraction time (10, 20 and 30 min) on total phenolic compound of kratom leaf. a-d Different letter indicates a significant difference (P≤0.05)

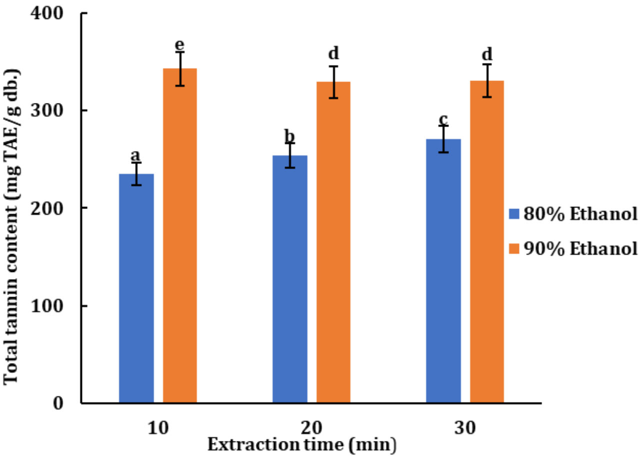

Figure 2. The effect of ethanol concentration (80% and 90%) and extraction time (10, 20 and 30 min) on total tannin content of kratom leaf. a-e Different letter indicates a significant difference (P≤0.05)

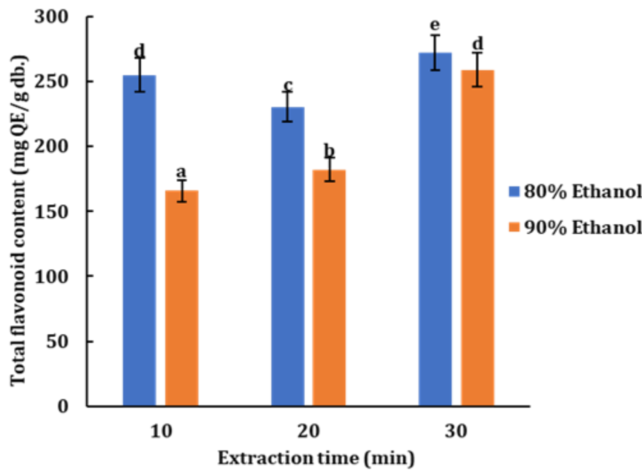

Figure 3. The effect of ethanol concentration (80% and 90%) and extraction time (10, 20 and 30 min) on total flavonoid content of kratom leaf. a-e Different letter indicates a significant difference ($P \leq 0.05$)

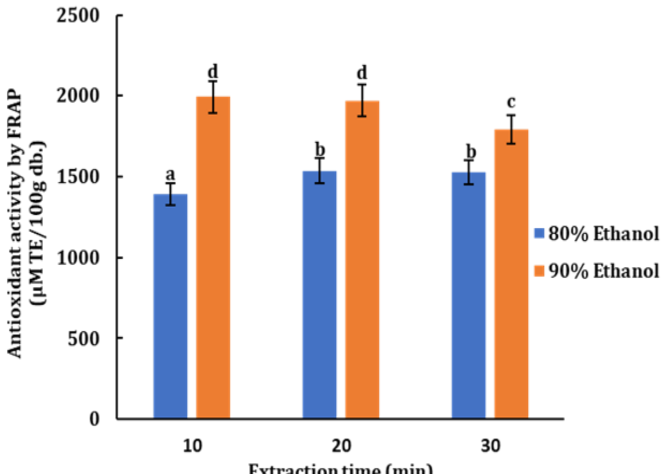

Figure 4. The effect of ethanol concentration (80% and 90%) and extraction time (10, 20 and 30 min) on antioxidant activity by DPPH assay of kratom leaf. a-e Different letter indicates a significant difference ($P \leq 0.05$)

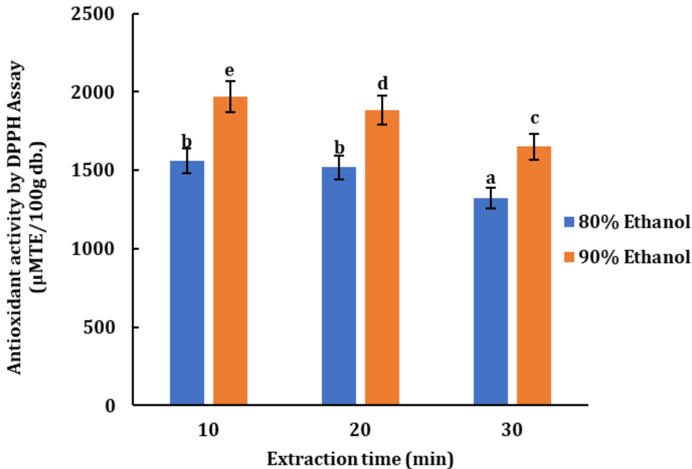

Figure 5. The effect of ethanol concentration (80% and 90%) and extraction time (10, 20 and 30 min) on antioxidant activity by FRAP assay of kratom leaf. a-d Different letter indicates a significant difference (P≤0.05)

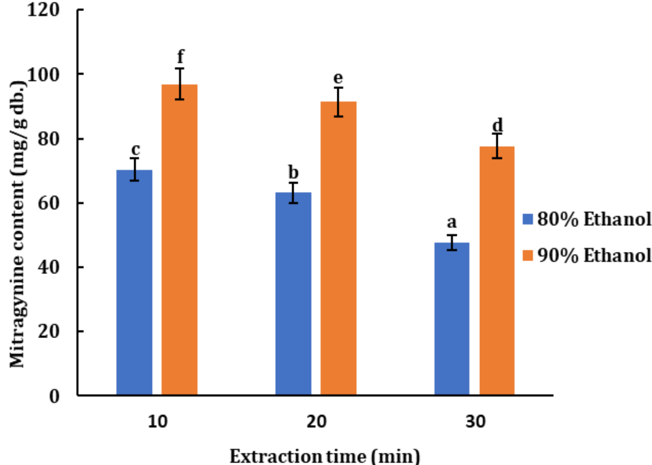

Figure 6. The effect of ethanol concentration (80% and 90%) and extraction time (10, 20 and 30 min) on mitragynine of kratom leaf. a-f Different letter indicates a significant difference (P≤0.05)

4. CONCLUSIONS

The extraction condition (extraction time and ethanol concentration) significantly affected the amounts of bioactive compound and antioxidant activity of kratom leaf. The extraction condition of 90% ethanol and 10 min yielded highest total phenolic compound, total tannin content and mitragyine content and improved antioxidant activity by DPPH and FRAP methods. On the other hand, the extraction conditions of 80% ethanol and 30 min yielded highest total flavonoid compound.

In summary, this research could provide important knowledge for the future UAE method to obtain more bioactive compound and antioxidant activity from kratom leaf.

5. ACKNOWLEDGEMENTS

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Effect of Brine Pretreatment on Physical Properties of Sous-vide Spent Laying Hen Breast

BP-P067

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Keywords

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ABSTRACT

Spent laying hen is a chicken that reach the end of their egg-laying cycle. Its meat is tough, while it is rich with protein and other nutrients. This research observed the effect of brine pretreatment on properties of sous-vide spent laying hen's breast. The breast was injected with a brine solution at the levels of 0, 5, 10, and 15% by weight before sous vide cooking at 60°C for 1.5 hr. The sous-vide yield, sous-vide loss, color parameters (L*, a*, b*), water holding capacity (WHC) and texture of samples were measured. It was found that brine solution affects sous-vide yield, sous-vide loss, color parameters, WHC, springiness and chewiness of chicken breast ($p \leq 0.05$). However, there was no significant difference in hardness of samples ($p > 0.05$). The results indicated that chicken breast injected with 10% of brine solution showed the highest value in sous-vide yield percentage, lightness and WHC. In contrast, its showed the lowest value in sous-vide loss percentage, redness, springiness and chewiness, compared to other conditions. This experiment could provide the benefits in the market value of spent laying hen and increase the consumer acceptance.

1. INTRODUCTION

Spent laying hen (*Gallus gallus domesticus*) is the chicken that reaches the end of laying cycles. It has less juiciness and tenderness due to high degrees of cross linkages and hence it is limited in economic values (1). In general, spent laying hen is distributed to food processing for rendering into meatball, emulsion sausage or jerky (2). In the western society, they treated the spent laying hen as a by-product disposed by burial, incineration, composting (as fertilizers), rendered to animal feed and pet food, which either create little market value or cause animal welfare and environmental concerns (3). However, spent laying hen is rich in fat and cholesterol content (1), while it is limited in its textural properties. Possibility to improve the texture of spent laying hen and recall back in consumption by human is desired.

In meat and meat qualities researches, both traditional and innovative techniques have been studied to improve textural properties and nutritional value as well as flavor and consumer acceptance of the meat and meat products (4,5). Brining solution has been proved to increase those mentioned properties (6). The injection of sodium chloride into the meat increasing the ionic strength of muscle tissue, denatures the proteins and changing their molecular structure (4). Extraction and solubilization of this muscle protein contribute to meat particle binding, fat emulsification, and water-holding capacity, and thus, it reduces cook losses and improves quality and texture (6). Lopez et al. (5) found that NaCl concentration up to 1.0% increased moisture retention in chicken breast meat during the cooking process. Moreover, using low cooking temperature could maintain juiciness, optimize flavor, color and maximize nutrient levels, while reducing the level of lipid deterioration and protein damage compared with traditional cooking methods (7). Sous-vide cooking is a pasteurization method that placed in heat-stable vacuumized pouches and containers at low temperature between 49 and 71°C for long time and then cooled to 0–3 °C for refrigerated storage (8). There are maximizes the transformation of collagen to gelatin in meat materials, which can significantly enhance the tenderness of meat product, particular in meat having high content of connective tissue. In addition, Sous-vide can minimize shrinkage and moisture loss of the meat (9). Kerdpiboon et al. (10) found that chicken breasts sous-vide cooked at 60°C for 3 to 5 h. had the lowest hardness and chewiness, but the highest value in springiness. The objective of this research is to study the effect of brining treatment on properties of sous-vide spent laying hen.

2. MATERIAL AND METHODS

2.1 Meat preparation

Spent laying hen breasts were purchased from Bangpra market in Chonburi, Thailand. The chicken breast was cut, removed the skin, trimmed and weighed approximate 70 g of each breast. The breast was packed in plastic bag and stored overnight at a refrigerated temperature of 4°C before used.

2.2 Brine solution preparation

The brine solution was prepared by mixing the 15 g of NaCl, 10 g of $Na_5P_3O_{10}$ and 7 g of sugar. Then dissolved all ingredients in 1 L of water. The brine was stored at refrigerator before used in the experiment.

2.3 Brine injection treatment and sous-vide process

The samples from 2.1 were injected with brine solution at levels of 0, 5(0.05% NaCl), 10(0.11% NaCl) and 15%(0.16% NaCl) by weight of breast. The samples were vacuumed packed in vacuum bags (Nylon/LLDPE) and then stored at 4°C for 24 hr before sous-vide cooking in a water bath at 60°C for 1.5 hr. Then, samples cooled at 4°C for 30 min.

2.4 Properties determination

2.4.1 Sous-vide yield and loss

The sous-vide yield and sous vide loss were calculated from the mass difference before and after cooking (10). Percentage of Sous-vide yield and Sous-vide loss were calculated using equations (1) and (2), respectively.

$$\%\text{Sous-vide yield} = \frac{\text{weight of sample after Sous-vide cooking}}{\text{weight of sample before Sous-vide cooking}} \times 100 \quad (1)$$

$$\%\text{Sous-vide loss} = 100 - \%\text{Sous-vide yield} \quad (2)$$

2.4.2 Color measurement

The color of sample was measured using a colorimeter (R-400, Konica Minolta, Japan) equipped with a standard illuminant D65 and presented in terms of L*(lightness), a*(redness) and b*(yellowness). At each condition, chicken breast color was measured at 1 area of inner and 3 areas of outer meat. Each treatment was measured in 5 replicate samples.

2.4.3 Texture profile analysis

Sample after cooking was cut into 1.5x1.5x1.5 cm. in vertical of muscle fibers. Texture was measured using a texture analyzer (TA-XT plus, England) and compressed with P/50. Texture parameters for hardness, chewiness and springiness were following the method described by Roldan et al. (11). Test settings were modified as follow; pretest speed of 1 mm/s, test speed of 2 mm/s, posttest speed of 2 mm/s, 20% strain, relaxation time of 5 and force of 5 g.

2.4.4 Water holding capacity

The water holding capacity of samples were measured using Filter Paper Press Method (FPPM) (12). Samples after cooking were minced. The 0.3 g of minced sample was put on filter paper and compressed for 5 min. Water holding capacity was calculated from area of the ring of fluid as described in equation (3).

$$\text{Area of the ring of fluid} = A_1 - A_2 \quad (3)$$

Where A_1 is area from center point to outer edge of ring and A_2 is sample placement area.

2.5 Statistical analyses

The effect of brine concentration was analysed by using one-way analysis of variance (ANOVA) with SPSS software. A significance difference test at 95% confidence level ($P \leq 0.05$) was applied to test differences of evaluated parameters. The Duncan's test was used to determine the differences between samples.

3 RESULTS AND DISCUSSION

3.1 *Sous-vide yield and sous-vide loss*

Results of sous-vide yield and sous-vide loss was shown in Figure 1. It was found that injection of brine solution into chicken breasts at level of 0-10% weight of sample increased sous-vide yield and decrease sous-vide loss. However, after injected the 15% of brine solution, the sous-vide yield of sample was decreased, whereas sous-vide loss increased. Sodium chloride in brine solution can increased the ionic strength of muscle tissue and induce meat absorbed more water (4). Moreover, the concentration and optimization of salt solution affected cooking yield and cooking loss of meat (13) and this might be reduced sous-vide cooking yield of chicken breast. The higher injection levels the muscle fibers could not hold the excess water injected into meat. In addition, higher concentration of NaCl may cause the stronger protein-protein bonds. Addition of high concentration of salt increased the ionic strength. The salt ions are removed water from around the protein cause protein aggregation and dehydration (14,15).

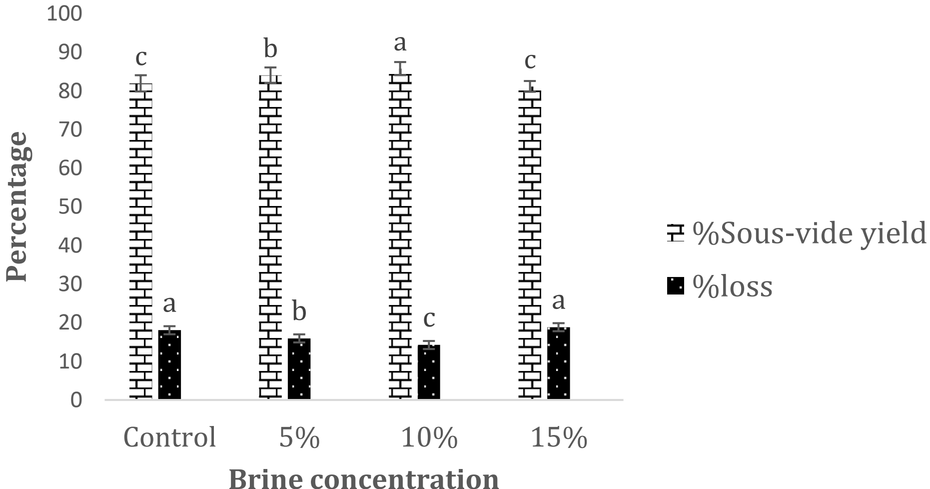

Figure 1. Percentage of sous-vide yield and loss of spent laying hen's breast affected by brine solution with different concentration (Sous-vide at 60°C for 1.5 hr).

3.2 *Color analysis*

In table 1, they showed a slight difference in the color measurements of inner and outer chicken breasts. In the inner, lightness (L*) value was in the range of 82.11-84.40. The chicken breast was injected with brine solution at 10% had the highest lightness compared with other conditions while redness(a*) and yellowness(b*) value were not significantly different (P>0.05). In the outer of chicken breast, redness (a*) was in the range of 1.99-2.82 and yellowness (b*) was in the range of 9.68-10.78, respectively. The highest value of redness and yellowness were observed when injection with brine solution at 0% (control). The large of amount water retained in the meat, causing greater light reflectance and scattering which increased lightness. Bae et al. (16) reported that ground chicken breasts mixed with 0% NaCl had the highest redness and yellowness value and tended to decrease with increasing NaCl concentration due to the NaCl may reduce the redness of cooked products by promoting oxidative conditions and denaturing myoglobin.

Table 1. Color of inner and outer spent laying hen breast as affected by brine solution with different concentration (Sous-vide at 60°C for 1.5 hr)

Treatment	0% Brine	5% Brine	10% Brine	15% Brine
Inner				
L*	82.11±1.08^{b}	83.44±2.49^{ab}	84.40±1.09^{a}	82.84±0.92^{b}
a*^{ns}	3.16±0.89	3.16±1.07	2.57±2.40	2.54±0.57
b*^{ns}	7.63±1.11	7.31±0.81	7.00±1.10	6.91±0.50
Outer				
L*^{ns}	79.90±1.22	80.71±1.30	80.86±0.91	80.56±0.90
a*	2.82±0.79^{a}	2.70±0.90^{ab}	2.00±0.47^{b}	2.28±0.82^{ab}
b*	10.78±1.46^{a}	9.68±0.65^{b}	9.91±0.62^{b}	10.41±0.60^{ab}

*Values within a column not followed by the same superscript indicate significant difference ($p \leq 0.05$). Values are presented as mean ±SD (*n*=*5*)

3.3 Water holding capacity (WHC)

The sample injected with a 10% brine solution exhibited the highest WHC while the sample injected with a 15% brine solution has the lowest WHC when compared to the other conditions (Table 2). The increase of brine concentration, which is increased ionic strength, caused the swelling of muscle fiber, increased extractability and solubility of myofibrillar protein and increased the water holding capacity loss (17). Thorarinsdottie et al. (14) stated that at higher salt concentration, proteins had probably denatured that led to less WHC and dehydration of the muscle. Graiver et al. (6) reported that meat was immersed in NaCl 5 g/l causing the essential structure of the myofibrils appeared to be intact. Fibers immersed in NaCl 140g/l showed a swelling while NaCl 330 g/l produced fragmented and dehydrated fibers with a granular appearance.

Ozuna et al. (18) reported that dehydration of meat sample treated in saturated brine (280 Kg/m^3), which was a high brine concentration, increased the osmotic effect due to the salting out phenomenon produced by the insolubilization of proteins that resulted decrease the WHC. On the other hand, the hydration of meat sample treated at the lowest brine concentration (50 Kg/m^3) was not affected the osmotic pressure and the salting in phenomenon.

3.4 Texture profile analysis

The sample that was injected with a brine solution at a concentration of 10% exhibited the lowest value of hardness and chewiness ($p \leq 0.05$). However, there was no significant difference in springiness ($p > 0.05$) (Table 2). The hardness and chewiness were affected by the amount of water in meat. Salt and sodium tripolyphosphate increased the water holding capacity. As a result, the lower force was used to compressed on the meat when compared to chicken breasts without salt and sodium tripolyphosphate in brine solution (19).

Table 2. Water holding capacity and texture of spent laying hen breast as affected by brine solution with different concentration (Sous-vide at 60°C for 1.5 hr)

Treatment	Water holding capacity (%)	Hardness (N)	Springiness^{ns}	Chewiness (N)
0% Brine	14.90±2.79^{b}	4011.28±303.26^{b}	0.53±0.02	1334.06±203.64^{a}
5% Brine	16.33±2.67^{ab}	4193.56±524.90^{ab}	0.51±0.03	1214.16±215.71^{ab}
10% Brine	17.49±1.92^{a}	2962.28±497.38^{c}	0.53±0.05	1133.45±81.01^{b}
15% Brine	12.34±2.28^{c}	4588.12±697.08^{a}	0.55±0.03	1374.67±277.71^{a}

*Values within a column not followed by the same superscript indicate significant difference ($p \leq 0.05$). Values are presented as mean ±SD (*n=10*)

4 CONCLUSIONS

The concentration of brine solution affected the physical properties of sous-vide cooked brined chicken breast. The appropriate of brine concentration could retain the water content of chicken breasts, resulting in increased the sous-vide yield percentage, lightness and WHC. While sous-vide loss percentage, redness, yellowness, hardness, and chewiness were decreased. The chicken breast injection with a brine solution of 10% by weight of sample produced chicken meat with the highest sous-vide yield, lightness value, WHC and the lowest sous-vide loss, redness value, hardness and chewiness. The data from this research can be use to improved the tenderness and juiciness of spent laying hen's breast, which is increased the value of chicken meat.

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Effect of Particle Size Classification on Pasting Properties of Cassava Starch

BP-O136

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ABSTRACT

This study examined how different methods of classifying cassava starch by particle size influence its pasting properties. Cassava starch samples were separated by three methods, including gravitational sedimentation (SD), centrifugation (CT), and dry-sieving (SV). Morphology analysis revealed no significant differences in granule shape across all samples. However, particle size separation methods significantly affected pasting behavior. Increasing SD time decreased median granule size and led to increased peak, minimum, breakdown, final, and setback viscosities, but decreased pasting temperature. Increasing CT time did not significantly affect particle size and most pasting properties. Lastly, reducing the sieving aperture for the SV method resulted in finer starch particle size, exhibiting higher paste viscosities. These findings support the established link between particle size and starch functionality. Reduced particle size increases surface area, enhancing water interaction during gelatinization and resulting in higher viscosities.

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the major economic crops in Thailand. The roots of tapioca are the main source of starch. Starch comprises two main components: amylose and amylopectin [**1**]. Cassava starch is an important raw material across diverse industrial sectors, encompassing food, paper, packaging, pharmaceuticals, and textiles [**2**].

It is used as a stabilizer and thickener in the food industry, as outlined by the US Code of Federal Regulations. The most significant functionalities of starch in practical applications relate to its pasting properties, including viscosity, texture, paste transparency, shear resistance, the tendency to retrograde [**3**], and the palatability of starch-based food products [**4**]. The study by Bala et al. [**5**] found that the properties of flour or powder are influenced by its constituent composition, such as particle surface properties or particle size. Furthermore, the flour functionality can be elucidated by examining its particle size distribution. Consistency in particle size distribution contributes to enhancing functional properties in product development.

This study aims to use a more sustainable technique to separate cassava starch granule sizes to obtain samples with different functional properties. The methods used for separation include gravitational sedimentation, peeler centrifugation, and dry sieving. The separated starch granules were then examined for morphological characteristics, particle size distribution, and pasting properties. This knowledge can be valuable to the cassava starch industry as it enables the selection of specific size fractions to achieve desired functionalities in various applications.

2. MATERIAL AND METHODS

2.1 Samples Preparation

In the production process, samples including starch slurry, high-moisture starch, and cassava starch were collected and analyzed. The starch slurry underwent gravitational sedimentation, with samples collected from a separation funnel at 20-minute intervals over a total duration of 120 minutes. These samples were compared to control samples, labeled as SD-control, SD-20, SD-40, SD-60, SD-80, and SD-120, respectively. To ensure complete settling, the slurry was agitated, followed by moisture reduction at 60°C for 6 hours using a dehydrator (IKE, China). High-moisture starch was obtained using a peeler centrifuge. Samples were collected at different intervals—0, 30, 60, and 90 seconds—and labeled as CT-0, CT-30, CT-60, and CT-90. These samples then underwent moisture reduction in a hot air oven (Memmert, Germany) at 60°C for 5 hours. In the final stage, starch intended for packaging was processed via a dry-sieving method using a vibratory sieve shaker (AS200 control, Retsch, Germany). The sieve shaker employed screens with mesh sizes of 200, 325, and 500 (corresponding to 77, 44, and 25 microns, respectively). The resulting samples were categorized based on particle size: 25-44 microns (SV-25), 44-74 microns (SV-44), and sizes larger than 74 microns (SV-74). All prepared samples were sealed in zip lock bags for subsequent experiments and were compared to a control sample (SV-control).

2.2 Granule Morphology and Size Analysis

The starch sample, immersed in 50% glycerol, was examined under normal and polarized light using an Olympus BX51 microscope (Melville, NY, USA). Particle sizes were measured using the ImageJ program, and their size distribution was analyzed using IBM SPSS Statistics software, Version 29.0.0.

2.3 Pasting Properties

The pasting properties of the starch sample were assessed using a Rapid Visco Analyzer (Model RVA-4, Newport Scientific, Australia). Three grams of dry flour were dissolved in 25 mL of deionized water in the test cup. The samples were heated and sheared according to the RVA's standard profile 2. Key parameters including peak viscosity (**PV**), trough viscosity (minimum viscosity,

MV), final viscosity (**FV**), and pasting temperature (**PT**) were recorded. Additionally, breakdown (**BD**) and setback (**SB**) were calculated using the Thermocline software supplied with the instrument.

2.4 Statistical Analysis

The experiment was conducted using a Completely Randomized Design (CRD). Data analysis was performed using one-way analysis of variance (ANOVA) and Duncan's new multiple range test at a 95% confidence level, utilizing IBM SPSS Statistics software, Version 29.0.0.0.

3. RESULTS AND DISCUSSION

3.1 Granule Morphology and Size Analysis

Figure 1 compares the morphology of starch granules in sedimented and control starch samples. **Figures 2** and **3** show the starch samples collected from the peeler centrifugal machine at different times and the dry-sieved starch samples, respectively. The morphology of the starch granules in all samples was not significantly different. Cassava starch granules were mostly irregular and truncated in shape, with a few round and oval-shaped granules also observed. These results are consistent with previous studies [**6, 7, 8, 9**].

Gravitational sedimentation can reduce the median particle size from the control (9 microns), with the size decreasing further with increasing time. In contrast, the size of the granules in the samples collected at different times did not change with collection time, with a median particle size between 8.2 and 9.9 microns. Furthermore, sieving the samples reduced the median particle size for the sieved samples (ranging from 7.7 to 9.6 microns) compared to the un-sieved samples (10.5 microns). Notably, samples that did not pass through the 200-mesh sieve exhibited a broader size distribution compared to other samples. Across all starch samples, the average granule size ranged between 5 and 22 µm, consistent with findings from prior studies [**6, 7, 8, 9**].

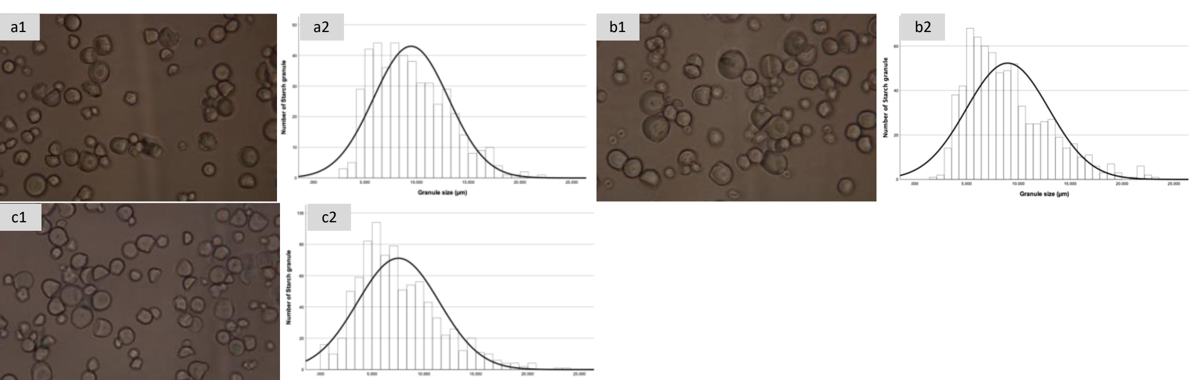

Figure 1. Control starch (**a**), sedimented starch sample at 20 min (**b**), and 80 min (**c**) viewed by Light microscopy and analyze size distribution (**1 and 2 respectively**)

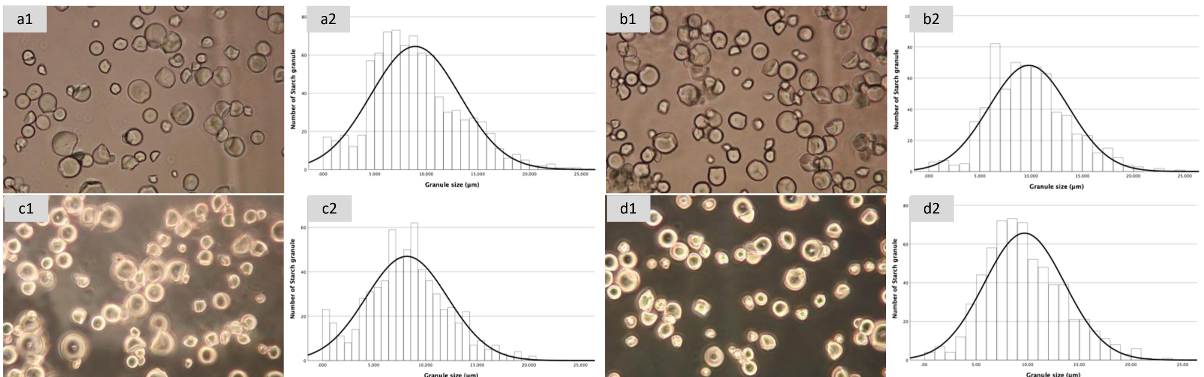

Figure 2. Granule morphology (**1**) and granule size distribution (**2**) of samples collected at 0, 30, 60, and 90 minutes from peeler centrifugal machine (**a, b, c, and d respectively**)

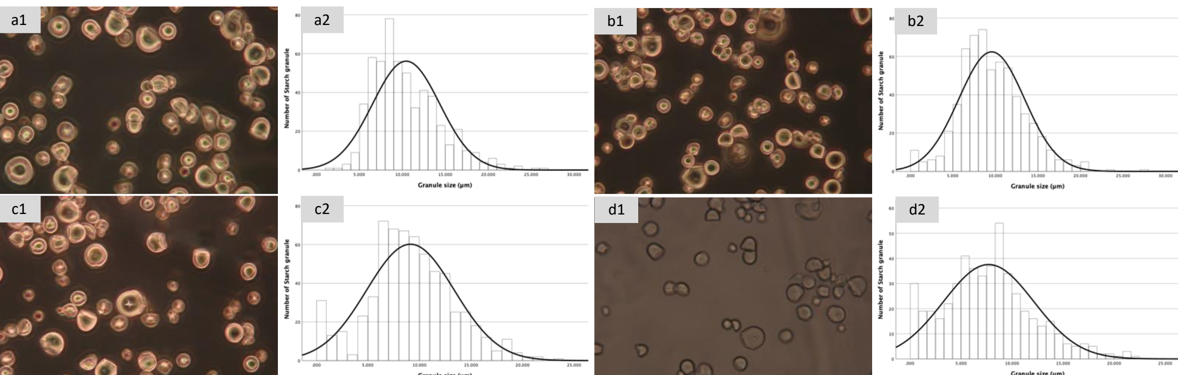

Figure 3. Control sample (**a**) compared to samples sieved with 25, 44, and 74-micron sieves size: size 25 - 44 microns (**b**), 44 - 74 microns (**c**), and size larger than 74 microns (**d**). Granule morphology and granule size distribution are shown in Figures (**1**) and (**2**) respectively.

3.2 Pasting Properties

Table 1 presents the pasting properties of cassava starch following separation by gravitational sedimentation, denoted as **SD**. Notably, the peak, minimum, breakdown, final, and setback viscosities of the sedimented samples significantly exceeded those of the control ($P < 0.05$) 0.05). Conversely, the pasting temperature exhibited a significant decrease ($P < 0.05$) in the sedimented samples. This outcome mirrors findings from the study by Anuntagool & Soonthonsun [**10**]. Additionally, sieving, which segregates granules by size, impacts the flow characteristics of the paste (e.g., peak, minimum viscosities) while leaving pasting temperature unaffected. Regarding samples collected from the peeler centrifuge (**CT**) at different centrifugation times (0 and 90 seconds), notable distinctions were observed in breakdown, final, and setback viscosities ($P < 0.05$), ranging from 2539 to 2642 cP, 2789 to 2908 cP, and 1037 to 1146 cP, respectively. However, peak viscosity, minimum viscosity, and pasting temperature did not exhibit significant differences ($P \geq 0.05$). Furthermore, Table 1 presents the pasting properties of cassava starch after size separation through dry-sieving with screens of 25, 44, and 74 microns (denoted as SV-25, SV-44, and SV-74, respectively). All pasting properties exhibited significantly higher values for sieved cassava starch compared to non-sieved

cassava starch. Notably, the smallest particle size range (25 - 44 microns) demonstrated the highest peak, minimum, breakdown, final, and setback viscosities. This finding is in line with the research of Watanabe et al. [**11**], which highlights the impact of granule size separation on paste properties.

Jane et al. [**12**] and Liao, Tattiyakul, & Rao [**13**] discussed the multifaceted nature of starch flow properties, identifying key determinants such as starch characteristics (including size, size distribution, concentration, shape, and stability), starch composition (encompassing factors like the quantity and type of amylose and amylopectin, as well as the presence of lipid complex amylose chains and phosphorus content), and interactions between internal components. Additionally, Bala et al. [**14**] and Agnes, Felix, & Ugochukwu [**15**] emphasized the pivotal role of particle composition and surface properties in determining starch behavior, highlighting how reducing particle size increases the surface area-to-volume ratio, directly affecting starch functional properties. Moreover, the rate of water absorption during processing is significantly influenced by particle size, with finer particles exhibiting a greater propensity for hydration compared to larger counterparts, a phenomenon supported by Hatcher et al. [**16**] and Hou et al. [**17**]. Tian et al. [**18**] further suggested that smaller starch granules possess enhanced solubility, leading to increased water absorption capacity, with positive implications for flour performance across diverse food processing applications. In the food industry, particularly in products like sauces, starch serves as a keystone in achieving desired thickening and swelling properties, which are directly linked to the pasting characteristics inherent to the starch itself [**19**].

Table 1. The pasting properties of starch samples separating by sedimentation time (SD-Control, 20, 40, 60, 80, and 120 minutes), centrifugation time (CT-0, 30, 60, and 90 seconds), and sieving size (SV-Control, 77, 44 and 25 microns)

Sample	PV (cP)	MV (cP)	BD (cP)	FV (cP)	SB (cP)	PT (ºC)
SD-Control	4181^{d}±75	1677^{d}±33	2509^{e}±35	2695^{c}±3	1018^{c}±35	71.95^{a}±0.07
SD-20	4336^{c}±40	1720^{cd}±24	2616^{bc}±16	2894^{b}±49	1174^{ab}±25	71.68^{ab}±0.24
SD-40	4383^{abc}±44	1703^{d}±26	2679^{ab}±18	2839^{b}±7	1135^{ab}±33	71.40^{ab}±0.07
SD-60	4375^{bc}±25	1763^{bc}±15	2611^{bc}±40	2904^{b}±71	1140^{ab}±56	71.60^{ab}±0.14
SD-80	4488^{a}±46	1791^{b}±23	2697^{a}±23	3027^{a}±8	1236^{a}±16	71.35^{ab}±0.49
SD-100	4325^{c}±10	1729^{cd}±8	2596^{c}±18	2840^{b}±85	1111^{bc}±93	71.20^{b}±0.42
SD-120	4477^{ab}±32	1847^{a}±12	2630^{abc}±44	3090^{a}±36	1243^{a}±48	71.60^{ab}±0.21
CT-0	4319±18	1779±30	2539^{b}±12	2871^{b}±14	1091^{ab}±45	71.75±0.00
CT-30	4333±33	1780±24	2553^{b}±8	2817^{a}±19	1037^{a}±43	71.57±0.31
CT-60	4386±49	1743±28	2642^{a}±22	2789^{a}±13	1045^{ab}±40	71.30±0.07
CT-90	4364±16	1761±4	2603^{a}±20	2908^{b}±6	1146^{b}±9	71.50±0.28
SV-Control	4222^{b}±23	1629^{b}±16	2593^{b}±7	2883^{b}±11	1254^{a}±26	70.75±0.14
SV-74	4234^{b}±1	1672^{ab}±20	2562^{b}±18	2858^{b}±37	1186^{b}±18	70.68±0.10
SV-44	4233^{b}±66	1629^{b}±32	2604^{b}±34	2841^{b}±11	1212^{ab}±21	70.68±0.03
SV-25	4398^{a}±23	1698^{a}±9	2700^{a}±14	2957^{a}±19	1259^{a}±10	70.83±0.24

*The values are presented as the mean ± standard deviations (n = 3)

*a, b, c ... Mean ± standard deviation values in the same column superscripted with different letters are significantly different (P < 0.05)

*Samples separated by different methods were analyzed separately.

4. CONCLUSIONS

This study explores the potential application of gravitational sedimentation (SD), peeler centrifugation (CT), and dry sieving (SV) as means to fractionate cassava starch with distinct properties. While the employed separation methods did not significantly alter the overall morphology of the starch granules, they effectively fractionated smaller starch granules, resulting in a significant increase in peak, minimum, breakdown, final, and setback viscosities of the starch. Concurrently, these methods lowered the pasting temperature. These findings provide valuable insights into customizing cassava starch properties through size separation. However, future research should focus on investigating separation techniques that can achieve desired particle size distributions tailored to specific functional applications. Further exploration into the effects of separation methods on crystallinity, swelling, solubility, and amylopectin/amylose content within the granules could enhance our understanding and pave the way for tailoring cassava starch functionalities in various industrial applications.

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Effect of Moisture Content on Cyanide Reduction in Tapioca Flour by Non-thermal Plasma

BP-P140

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Tapioca flour

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ABSTRACT

This study investigated the effectiveness of non-thermal plasma (NTP) treatment in reducing cyanide content in tapioca flour and its impact on pasting properties. Dry-milled tapioca flour with varying moisture levels (5%, 10%, and 15%) was treated with NTP at 12 kV for 10 minutes. Results demonstrated a significant reduction (44.32% reduction) in cyanide content following NTP treatment, with the highest efficiency achieved at 15% moisture content. NTP treatment also affected the pasting properties of the flour. Compared to untreated samples, NTP-treated flour exhibited lower gelatinization temperatures and shorter peak viscosity times, suggesting a potentially weaker flour structure. While viscosity decreased at high moisture content, NTP-treated flour was found to have a higher retrogradation value. Importantly, NTP treatment did not completely eliminate cyanide. Future research should explore non-enclosed plasma for potentially improved cyanide removal efficiency

1. INTRODUCTION

Tapioca is an economically important crop in Thailand, cultivated extensively throughout the country due to its drought tolerance, making it popular among farmers. It is widely utilized in various industries, particularly the food industry, as tapioca roots store nutrients in the form of flour, making it a good source of carbohydrates. However, tapioca contains cyanogenic glycosides, which are toxic substances hazardous to humans and animals [**1**].

Cyanogenic glycosides accumulate in tapioca in the form of cyanogenic glucosides or bound cyanide. Tapioca contains two types of cyanogenic glucosides, linamarin and lotaustralin, in a ratio of approximately 20:1. When tapioca tissue is damaged, the enzyme linamarase is released, which hydrolyzes cyanogenic glucosides.

Linamarin is then broken down into glucose and acetone cyanohydrin. Cyanohydrin can spontaneously decompose in neutral to alkaline conditions (pH>5) to form hydrogen cyanide, a toxin [**2**]. Total cyanogenic content in tapioca can be measured by extracting cyanogenic compounds from samples using phosphoric acid/alcohol and enzymatically hydrolyzing them. The resulting color is measured using pyridine/pyrazolone solution, and the absorbance value is used to calculate the total cyanogenic content [**3**].

In industrial tapioca flour production, wet milling is commonly used to extract flour from tapioca roots and reduce the cyanogenic glycoside content present as cyanogenic glucosides. Water is mixed with tapioca during milling to facilitate the process, requiring a substantial amount of water, ranging from 7,400 to 363,000 liters per 1000 kilograms of flour. Another method is dry milling, which doesn't involve adding water into the system. Instead, heat is used during baking to reduce cyanogenic glycoside content. However, this might not be sufficient to reduce cyanogenic glycoside levels to a safe point for consumers [**4**].

Plasma is a state of matter where gases receive enough energy to turn their molecules into ions. This ionized state implies the presence of at least one electron removed from the molecule. Plasma is considered the fourth state of matter because of its distinct characteristics differentiating it from other states. Plasma consists of both positively and negatively charged particles in proportions that result in a neutral net charge, giving it electrical conductivity. Plasma can be produced by subjecting gases to high-energy fields. When sufficient energy is transmitted to free electrons, they collide with atoms, causing electron ejection. This process is called ionization, which occurs rapidly, resulting in a significant increase in free electrons, leading to gas breakdown and eventually plasma formation [**5**].

Currently, plasma is widely used in various fields including material science, food industry, and environmental applications. Research by Velsero et al [**6**]. investigated the use of non-thermal discharge plasma for cyanide removal from water containing 1 milligram per liter of cyanide. They found that cyanide could be eliminated up to 99% within 3 minutes by generating high-frequency electricity over the water surface, leading to plasma formation. This plasma dissociates gases into radicals such as hydrogen peroxide, molecular oxygen, and hydrogen, as well as hydroxyl, hydroperoxyl, hydrogen, oxygen, and radicals, effectively decomposing organic compounds.

Moreover, Gao et al [**7**]. studied the effect of using non-thermal discharge plasma on the chemical and physical properties and degradation of flour. They found that flour treated with plasma had improved water solubility, along with visible cracks and fissures on the flour surface, enhancing its degradation efficiency. Additionally, Thaweewong [**8**]. investigated the effects of electrical voltage and time on cyanide removal in tapioca flour and found that cyanide levels could be reduced by up to 39.47% within 10 minutes at 20 kilovolts. However, this reduction might not meet the regulatory standards for safe consumption.

This research aims to develop an appropriate condition to enhance the efficiency of cyanide reduction in tapioca flour using non-thermal plasma, by considering the moisture content of the flour treated with plasma.

2. MATERIAL AND METHODS

2.1 *Materials*

The raw bitter tapioca roots used for the experiment were obtained from Thanawat Quality Flour Co., Ltd., Kamphangphet Province, Thailand. Helium gas and Argon gas (purity 99.995%) was purchased from Thonburi Wattana Ltd., Bangkok, Thailand.

2.2 *Preparation of dry-milled tapioca flour*

Modified from the method of Thaweewong [**8**], tapioca flour was washed and peeled. Then, it was sliced into thin pieces using a slicer and dried in a hot air oven at 60 °C for 24 hours. After that, it was ground using a grinder. The obtained sample was measured for moisture content and analyzed for cyanide content. The resulting flour was packed in a sealed zipper bag and stored in a desiccator.

2.3 *Effect of moisture content in flour on cyanide levels in plasma-treated tapioca flour*

This study investigates the impact of different moisture levels (5%, 10%, and 15%) on cyanide levels in nonthermal plasma (NTP)-treated tapioca flour. Initially, tapioca flour is prepared with moisture levels of 5%, 10%, and 15%. The flour is then placed in a desiccator containing distilled water to allow moisture absorption until the desired level is reached. If the moisture content is insufficient, distilled water is sprayed and mixed until a uniform consistency is achieved. Once flour samples with varying moisture content are obtained, they are spread into plates to a thickness of 2 millimeters without a lid. The plates are then placed in zip-lock bags, filled with helium gas, sealed tightly, and placed in the reactor chamber of the plasma generator. The machine is operated at 12 kilovolts for 10 minutes. Samples are collected from the zip-lock bags and analyzed for cyanide content.

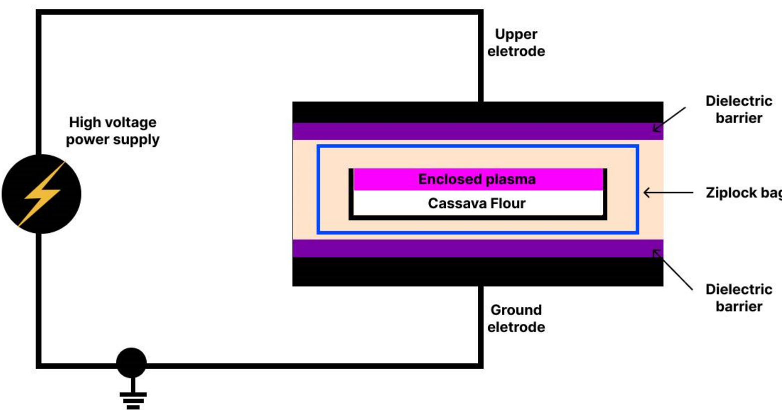

Figure 1. Schematic drawing of nonthermal dielectric barrier discharge plasma system.

2.4 *Analysis of free cyanide content*

Adapted from the method by O'Brien et al. [**3**], 2 grams of flour are weighed and mixed with the extraction solution (0.1 M phosphoric acid with 25% ethanol) in a volume of 10 milliliters. The

mixture is left to soak for 24 hours, then filtered through filter paper in a cold-water bath. A clear supernatant of the extract (0.1 milliliters) is obtained and mixed with 0.4 milliliters of 0.1 M pH 7.0 phosphate buffer solution. Enzyme linamarase solution (0.1 milliliters) is added and mixed, then incubated in a water bath at 30 degrees Celsius for 15 minutes. The sample tube is then immersed in a cold-water bath for 10 minutes. Next, 0.6 milliliters of 0.2 M NaOH solution is added and mixed, followed by continuous immersion of the tube in the cold-water bath. Subsequently, 2.8 milliliters of 0.1 M pH 6.0 phosphate buffer solution is added and mixed, followed by immersion of the tube in the cold-water bath. Then, 0.2 milliliters of Chloramine T solution is added and mixed, and the tube is incubated in cold water for 5 minutes. Afterward, 0.8 milliliters of pyridine/pyrazolone solution is added and mixed, and the sample is incubated at room temperature for 90 minutes before measuring the absorbance value at a wavelength of 620 nanometers.

$$\text{Cyanide content (mg/kg)} = \frac{(B+D)\times C\times Dilution\times 100}{S\times A\times(100-M)}$$

Where A is the sample weight (g), B is the volume of the extraction solution and water in the sample (ml), C is the absorbance value at 620 nm (OD sample – OD blank), D is the volume of water in the sample, M is the percentage of moisture, and S is the slope of the standard graph.

2.5 Pasting properties

Using the Rapid Visco Analyser (RVA-4, Newport Scientific Instrument & Engineer, Australia) and standard profile 1 following Jungtheerapanich et al., [**9**]. Pasting profiles of the flour samples were measured from suspensions of native and treated flour (14% w/w) under shearing and heating.

3. RESULTS AND DISCUSSION

3.1 Effect of moisture content in flour on cyanide levels in NTP-treated tapioca flour

Treating dry milled tapioca flour with NTP tends to reduce the amount of cyanide, with maximum efficiency achieved when the flour moisture content is at 15%. The reduction in cyanide occurs as the water within the flour sample breaks down into free radicals upon receiving plasma. These radicals, such as hydrogen peroxide, molecular oxygen, hydrogen, hydroxyl, hydroperoxyl, oxygen, and radical [**6**], all have the potential to degrade cyanide. As the moisture content in the flour increases, the number of free radicals generated also increases. Consequently, this enhances the ability to eliminate cyanide more effectively.

Table1. Effect of Moisture Content in flour on Cyanide Levels in NTP-Treated Tapioca Flour.

Moisture (%)	NTP Treatment	Cyanide Content Dry weight (ppm)	Percent reduction (%)
5	Treat	110.48	36.23
5	Untreat	173.25	-
10	Treat	114.14	37.11
10	Untreat	181.52	-
15	Treat	99.85	44.32
15	Untreat	179.33	-

3.2 Pasting properties

Table 2 shows the pasting properties of NTP-treated tapioca flour at different moisture contents compared to dry-ground tapioca flour. It was found that NTP-treated flour had significantly lower gelatinization temperatures ($p \leq 0.05$) than normal tapioca flour. This is thought to be due to depolymerization, as the free radicals generated during NTP treatment have higher energy and can easily break down glycosidic bonds [**10**]. As the flour molecules become smaller, the temperature required for gelatinization decreases. When considering the NTP-treated samples, it was found that the gelatinization temperature decreased significantly ($p \leq 0.05$) as the moisture content of the flour increased. This is because higher moisture content can lead to the generation of more free radicals. In terms of peak viscosity time, NTP-treated tapioca flour had a shorter peak viscosity time. This suggests that NTP-treated tapioca flour is less resistant to heat and shear stress. This may be due to the plasma surface etching mechanism. The peak viscosity of tapioca flour decreases after NTP treatment, but the difference is not statistically significant. At higher initial moisture contents, viscosity decreased significantly ($p \leq 0.05$). This decrease in viscosity may be due to cross-linking reactions between flour chains [**11**]. When considering trough viscosity, it was found that at 5% moisture content, trough viscosity decreased. However, at higher initial moisture contents, NTP-treated flour had higher trough viscosity, resulting in higher breakdown values. When considering final viscosity, it was found that final viscosity increased significantly ($p \leq 0.05$) with increasing initial moisture content. as well as the setback value. This suggests that NTP treatment does affect the retrogradation of tapioca flour, NTP-treated flour was found to have a higher retrogradation value.

Based on this information, we can conclude that when the cyanide content in the flour decreases, it results in a reduction in pasting temperature, peak time, and peak viscosity. However, it increases trough viscosity, breakdown, final viscosity and setback values, leading to a higher retrogradation value.

Table 2. Pasting properties of dry-milled tapioca flour and NTP-treated tapioca flour

Sample (mc% - NTP treat)	Pasting temperature (°C)	Peak time (min)	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)
5 - treat	$74.40^{a}\pm0.12$	$4.13^{ab}\pm0.04$	$2865^{b}\pm34.07$	$1197^{a}\pm7.37$	$1138^{a}\pm7.37$	$1725^{a}\pm32.02$	$531^{b}\pm9.50$
5 - non treat	$74.45^{b}\pm0.02$	$4.20^{b}\pm0.00$	$2875^{b}\pm25.65$	$1418^{b}\pm6.00$	$1425^{b}\pm6.00$	$1930^{b}\pm32.93$	$512^{a}\pm15.28$
10 - treat	$74.30^{a}\pm0.02$	$4.13^{a}\pm0.00$	$2868^{b}\pm38.15$	$1606^{c}\pm15.52$	$1560^{c}\pm16.52$	$2295^{c}\pm37.07$	$691^{b}\pm14.57$
10 - non treat	$74.40^{b}\pm0.03$	$4.20^{b}\pm0.01$	$2871^{b}\pm17.21$	$1415^{b}\pm11.23$	$1458^{b}\pm11.23$	$1942^{b}\pm16.25$	$513^{a}\pm20.22$
15 - treat	$74.20^{a}\pm0.10$	$4.13^{b}\pm0.06$	$2836^{a}\pm41.76$	$1957^{d}\pm24.66$	$2689^{d}\pm24.66$	$2667^{d}\pm10.58$	$712^{c}\pm15.04$
15 - non treat	$74.45^{b}\pm0.00$	$4.20^{b}\pm0.00$	$2874^{b}\pm17.89$	$1420^{b}\pm20.79$	$1447^{b}\pm20.7$	$1936^{b}\pm11.53$	$510^{a}\pm17.56$

*The values are presented as the mean ± standard deviations (n = 3)
*a, b, c … Mean ± standard deviation value in the same column superscripted with different letters are significantly different ($P < 0.05$)

4. CONCLUSIONS

NTP treatment effectively reduces cyanide content in tapioca flour, with the highest efficiency achieved at 15% moisture content. This reduction is likely due to moisture-generated free radicals, like hydrogen peroxide, which break down cyanide during the treatment. Higher moisture content increases free radical generation, enhancing cyanide removal. However, NTP treatment also alters the pasting properties of tapioca flour. Compared to untreated flour, NTP-treated flour has a lower gelatinization temperature and shorter peak viscosity time, indicating a weakened flour structure. While it may decrease viscosity at high moisture, possibly due to cross-linking, NTP-treated flour was found to have a higher retrogradation value. Importantly, NTP treatment did not completely eliminate cyanide. Future research should explore non-enclosed plasma for potentially improved removal efficiency.

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Egg white powder production and characterization: A foam mat drying approach

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Keyword

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ABSTRACT

Egg white powder is typically produced by using a spray drying method. Foam-mat drying, a cost-effective alternative to conventional drying for producing food powders, is considerably cheaper than freeze and spray drying. The objective of this study was to investigate the appropriate conditions of foam-mat drying to achieve the ideal physiochemical properties of egg white powder. Experiments were conducted using different stabilizers (xanthan gum and gum arabic) to optimize foam properties and stability. The hot air drying was conducted at 70°C. This article discusses the effect of stabilizers on the physical and functional properties of egg white foam and powder. The use of 1.0% xanthan gum was the most suitable for the production of egg white powder using foam mat drying. However, the gel appearance of samples containing xanthan gum and gum arabic exhibited lower quality compared to the commercial and control samples. The favorable functional properties, including binding, solubility, foaming, and gelling, identified in this study, therefore render foam mat powder a versatile ingredient in the food industry.

1. INTRODUCTION

Foam-mat drying is a process in which liquid food is formed into stable foam by incorporating or compressing air into the liquid until stable foam is achieved, followed by the drying process. The widely used foaming agents include egg albumin, whey protein concentrate, and soy protein isolate because their globular protein structures contribute to surface activity [1]. The capability of a protein molecule to build the foam depends on the intermolecular-bonds, cohesiveness, and elasticity in the three-dimensional structure of the protein molecule. The capability also correlates with the hydrophobicity of protein and the potential conformational rearrangement to create an elastic film. However, the resulting foam may lack stability during drying.

In this case, stabilizers such as gums, maltodextrin, modified starch, pectin, sorbitol syrup, xylitol, maltitol, mannitol, carboxymethyl cellulose, hydroxypropyl cellulose, microcrystalline cellulose (cellulose gel), sodium/potassium alginate, etc. may be used to enhance the foam stability. Foam-mat drying is a cost-effective alternative to conventional methods for producing food powders, providing considerably cheaper than freeze drying and spray drying. This process is suitable for large-scale production of food powders due to its effectiveness with viscous and sticky food as well as its straightforward production process. Foaming the product preserves its nutritional quality, facilitates reconstitution and increases its surface area, resulting in faster drying at lower temperatures [2,3,4,5]. The purpose of adding foaming agents and stabilizers during processing is to coat flavor components, increase total solids, enhance the volume, speed up the drying process and prevent material damage caused by heat [6]. The drying performance of foam microstructure relies on its encapsulant composition and whipping process, which in turn dictate its physical properties [7]. The phenomenon convinces the role of a polysaccharide in coating the foaming agent surface and increasing its elasticity to trap air within the bubbles [8].

Egg whites are considered a good source of protein, containing over 40 different proteins. [9] reported the protein composition of egg whites (% dry matter), which includes ovalbumin (54%), ovo-transferrin (conalbumin, 12–13%), ovo-mucoid (11%), lysozyme (3.4–3.5%), ovo-globulins G2 and G3 (2 %) and ovo-mucin (1.5–3%). They provide essential amino acids crucial for the human body and serve as a cost-effective source of leucine, an essential amino acid important for the process of tissue repair, growth and blood sugar regulation [10]. Egg whites are commonly used in various foods such as bakeries and meat products. Egg white protein, which contains more than 80% dry matter [11], plays an important role in foaming and gelling properties. Moreover, dehydrated egg white products offer many advantages including extended shelf life, reduced storage and transportation costs and specific functional properties. The industrial-scale production of egg white powder involves separating the egg yolks, pasteurizing to destroy pathogens and transforming the liquid into powder using the spray drying process. The egg white powder is suitable for people seeking high quality protein in large quantities such as athletes, the elderly, cancer patients, and those with kidney disease.

Numerous studies explored the production of powdered food protein using the foam-mat method. Factors influencing powder properties include the type of foaming agents and stabilizers as well as conditions and methods for foaming and drying. For example, maltodextrin concentration of 15% was found to be suitable for producing milk powder, resulting in a yield value of 19.40%, solubility of 71.08%, water content of 3.73% and protein content of 10.26%. These properties were comparable to those achieved with 6% gum Arabic [12]. Foam-mat drying has been successfully applied to various dairy products including milk [13], yogurt [14] and cream [15]. However, there is a lack of published information on the production of egg white powder using the foam mat method.

The objective of this study was to investigate the impact of different stabilizers on foam-mat drying to achieve the desired physiochemical properties of egg white powder including the expansion and stability of egg white foam, as well as the solubility, foaming and gelling properties of egg white powder.

2. MATERIAL AND METHODS

2.1 Material and Chemicals

Fresh hen eggs (0–7days old, jumbo size) were purchased from Mahasarakham province in Thailand. Xanthan gum food grade obtained from Chemrich (Bangkok, Thailand) and gum arabic food grade from Chemipan (Bangkok, Thailand) were used in this study.

2.2 Sample preparation

Egg whites were separated from whole eggs followed by pasteurization using a modified method described by [16] at 60°C for 4 min. Foaming agents including xanthan gum or gum arabic at 0.5 and 1 % by weight were added in egg white. The ingredients were mixed using a hand mixer (PHILIPS HR3741, 450 watt) at speed 3 for 5 min to create a foam. The egg white foam was transferred to stainless tray and dried at 70 °C for 4.25-4.5 h until the moisture content was below 0.02 g water/g solid (dry basis, d.b.), Subsequently dried foam was blender and filtered through an 80-mesh sieve to obtain powder. The sample without foaming and stabilizer agents was served as a control.

2.3 Characterization

2.3.1 Foam density, foam capacity and re-foaming capacity

The density of egg white foam was determined by measuring the volume and weight of foam. The initial volume of egg white before whipping was used to assess the foam capacity. The re-foaming capacity of the egg white powder was measured using a modified method [17] . Briefly, 0.5 g of egg white powder was dissolved in 50 ml of deionized (DI) water, then whipped with a hand mixer (PHILIPS HR3741, 450 watt) at speed 3 for 5 min to create a foam, which was then transfer to a cylinder for volume measurement. Foam density and foam capacity were calculated using following equations:[18]

$$Foam\ density = \frac{foam\ weight\ (g)}{foam\ volume\ (ml)} \quad (1)$$

$$Foam, Re - foaming\ capacity = \frac{foam\ volume\ (ml) - sample\ volume\ (ml)}{sample\ volume\ (ml)} \times 100 \quad (2)$$

2.3.2 Foam stability

To assess foam stability, the volume of foam was measured in a 200 ml beaker every hour over a 3-hour period at room temperature. Foam stability was calculated using the following equation: [18]

$$Foam\ stability = \frac{foam\ volume\ (final\ )\ (ml)}{foam\ volume\ (initial)\ (ml)} \times 100 \quad (3)$$

2.3.3 Moisture content and Water activity (a_w)

The moisture content was determined according to the standard AOAC method (AOAC, 2000)[19]. Briefly, 5 g of egg white powder was dried in a hot air oven at 105 °C until a constant weight was achieved. The moisture content (MC, g water/g solid, d.b.) of egg white powder was calculated using equation (4). Water activity (a_w) was measured using a water activity meter AQUA LAB model Series 3 TE (Decagon Devices Inc., Pullman WA, USA).

$$Moisture\ content = \frac{W0 - W1}{W0} \times 100 \quad (4)$$

Where W0: weight of initial sample (g)
W1: weight of final sample (g)

2.3.4 Color

The samples were analyzed for color using a Chroma meter CR-400 (Konica Minolta Inc., Osaka, Japan) following the manufacturing procedure. Egg white powder (2g) was placed in a sample cup. Color results were expressed in CIE L*a*b*.

The Whiteness index was calculated using equation (5)[20]

$$Whiteness\ Index = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (5)$$

Where L*, a* and b* are lightness to darkness, redness to greenness, and yellowness to blueness, respectively.

2.3.5 Water solubility index and Water adsorption index

The water solubility index and water adsorption index were determined using a modified method described by Zeng et al. (2023) [21]. Briefly, 0.2 g egg white powder was dissolved in 20 ml of DI water and stirred at 25 °C for 30 min. The mixture was then centrifuged at 1000 × g for 10 min to separate the supernatant from the sediments. Subsequently, the supernatant and the precipitate were dried at 105 °C until a stable weight was achieved. The weight of the supernatant and the precipitates were used to determine the water solubility index and water adsorption index following equations (6) and (7), respectively [21,22].

$$Water\ solubility\ index\ (\%) = \frac{weight\ of\ solid\ in\ suppernatant\ (g)}{weight\ of\ sample \times (1-MC)\ (g)} \times 100 \quad (6)$$

$$Water\ absorption\ index\ (\frac{g}{g}) = \frac{weight\ of\ solid\ in\ precipitant\ (g)}{weight\ of\ sample \times (1-MC)\ (g)} \quad (7)$$

Where MC =Moisture content (% wet basis)

2.3.6 Water holding capacity and Water binding capacity

The water holding capacity and water binding capacity were determined using a modified method described by Zeng et al. (2023) [21]. Briefly, two portions, each consisting of 1 g of egg white powder, were dissolved in 15 ml of DI water. The first portion was centrifuged at 7000 × g for 10 min and the second portion was stored at 25 °C for 24 h. The water was removed from the samples through filter paper. The weight of the sample after water removal was used to calculate water holding capacity and water binding capacity following equations (8) and (9), respectively.

$$Water\ holding\ capacity = \frac{M2 - M0\ (g)}{M0\ (g)} \quad (8)$$

$$Water\ binding\ capacity = \frac{M1 - M0\ (g)}{M0\ (g)} \quad (9)$$

Where M0: weight of initial sample, M1: weight of sample after 10 min and M2: weight of sample after 24 h

2.3.7 Gelation experiments

The gelation of egg white powder was tested using a modified method [23]. Briefly, 2 g of egg white powder was combined with 20 ml of DI water in a glass bottle. The mixture was stirred for 5 minutes before closing the bottle cap. Subsequently, the mixture was heated at 70 °C for 30 min in a water bath and then allowed to cool down at room temperature. The gels were photographed after gel setting.

2.3.8 Yield

The product yield (%) was calculated based on the weight of dried foam and wet foam. The yield calculation was determined using the following formula:

$$Yield = \frac{weight\ of\ dried\ sample\ (g)}{weight\ of\ wet\ sample\ (g)} \times 100 \quad (10)$$

2.3.9 Determination of Protein

The crude protein content of egg white powder was determined using the Kjeldahl method (AOAC, 2000) [19]. In brief, 0.3 g of egg white powder along with Kjeldahl catalyst and concentrated sulfuric acid (H_2SO_4) was placed in a tube. The samples underwent digestion, followed by distillation and titration with 0.1N hydrochloric acid according to the standard procedure.

$$Protein\ content = \frac{(A-B)xNxFx1.4007}{W} \times 100 \quad (11)$$

Where A is amount of titrant (HCl) for the sample (ml), B is amount of titrant (HCl) for blank (ml), N is normality of HCl (N), F is specific protein factor (6.25) and W is weight of sample (g)

2.3.10 Statistical analysis

The experiment was performed in triplicate and the reported data are presented as mean ± standard deviation. Analysis of variance (ANOVA) and Duncan's new multiple range test were used to assess the statistical significance at a significant level $p<0.05$ using IBM SPSS statistic software.

3 RESULTS AND DISCUSSION

The preliminary experiment found that the maximum expansion of egg white occurred within 5 minutes of whipping. The effect of stabilizer types on the properties of egg white foam is shown in **Table 1**.

Table 1 The effect of foaming stabilizer types on the characteristics of egg white foams

Sample	Foam capacity (%)	Foam Density (g/ml) ns	Foam stability (%)
Control	250.00±0.00^{a}	0.26±0.04	80.00±0.00^{b}
0.5% xanthan gum	250.00±0.00^{a}	0.26±0.04	100.00±0.00^{a}
1.0% xanthan gum	250.00±0.00^{a}	0.26±0.04	100.00±0.00^{a}
0.5% gum arabic	225.67±1.15^{b}	0.28±0.06	78.33±2.36^{b}
1.0% gum arabic	226.67±2.89^{b}	0.24±0.04	78.75±1.77^{b}

*Difference superscript letters (a,b) in the same column indicate a significant difference ($p<0.05$).

Significant differences were observed in the foam capacity and stability values ($p<0.05$), with the highest values found in the sample containing 0.5% and 1.0% xanthan gum. While the addition of xanthan gum did not increase foam expansion compared to the control, it improve stability. The volume of foam remained unchanged even after standing for more than 3 h, indicating very high stability of egg white foam (100%), particularly in the xanthan gum treatment. Conversely, both the control and the gum arabic treatment exhibited a significant decrease in foam stability. The viscosity of the mixtures contributed to this evidence when compared with the use of xanthan gum. It is observed that even 30% gum arabic solutions have lower viscosity than 1% xanthan gum at low shear rates [24] . In addition, while gum arabic exhibited Newtonian behaviour, with viscosity being shear rate independent [9][24], xanthan gum displayed non-Newtonian shear thinning characteristics. This discrepancy is explained by the fact that the latter are linear molecules and have a much larger hydrodynamic volume, allowing for intermolecular entanglements to occur at much lower concentrations than for the highly compact, branched gum arabic molecules.

Xanthan gum, an anionic polysaccharide having a cellulose backbone with a trisaccharide side-chain, was produced from *Xanthomonas camprestis*. Its low viscosity under the high shear condition makes it easy to pour and mix [25]. Xanthan gum plays a role as a surfactant, which is linked to air and water interface as a viscoelastic film to avoid mechanical or thermal damages [7]. The role of xanthan gum as a stabilizer in the encapsulant formula has also been reported to improve the foam stability of soy protein isolate. Additionally, it can be used as a thickener, stabilizer, and foam enhancer. Although whey protein isolate (WPI) has high solubility to support overrun, it also requires xanthan gum addition to stabilize its capacity because WPI protein is also susceptible to temperature [26].

However, the foam density was not significantly different ($p>0.05$). They were in the range of 0.24-0.28 g/ml, which were lower than those of protein foams, yogurt [14], shrimp [27] and cheese[15]. Generally, foam capacity, density, and stability are important factors in reducing the drying time. A higher overrun indicates that more air has been trapped in the foam. Foams with higher density resulted in prolonged drying time, which could lead to inferior powder quality due to thermal

degradation [4]. However, the properties of all egg white foams in this report are quite similar, the drying time was close (4.25-4.5 hours).

Table 2 The effect of foaming stabilizer types on moisture content, water activity and yield of egg white powder.

Sample	Moisture content (%) ns	Water activity (a_w) ns	Yield (%) ns
Control	10.31±0.53	0.37±0.07	1.51±1.29
0.5% xanthan gum	9.01±0.62	0.33±0.01	3.05±1.14
1.0% xanthan gum	8.85±1.28	0.33±0.13	4.11±1.71
0.5% gum arabic	8.81±0.21	0.29±0.06	2.59±0.96
1.0% gum arabic	9.89±0.19	0.25±0.08	3.21±1.14

* ns represents not significantly different ($p \geq 0.05$).

There was no significant difference observed in the moisture content, water activity, and yield of egg white powder (**Table 2**). The addition of stabilizer did not contribute to an increase in powder volume due to the low proportion added. Powder volume tends to increase proportionally with the amount of filling added, with different addition in the range of 2% to 6% resulting in varying amounts of dry powder [28].

Table 3 The effect of foaming stabilizer types on color parameters of egg white powder

Sample	L*	a*	b*	Whiteness index
Control	70.44±0.24^c	-2.58±0.05^c	16.73±0.13^a	65.94±0.24^c
0.5% xanthan gum	71.95±0.43^b	-2.51±0.01^b	13.10±0.15^d	68.92±0.43^b
1.0% xanthan gum	72.90±0.06^a	-1.75±0.01^a	11.96±0.01^e	70.33±0.06^a
0.5% gum arabic	70.13±0.33^c	-2.92±0.06^e	16.24±0.16^b	65.88±0.33^c
1.0%gum arabic	69.62±0.10^d	-2.77±0.03^d	15.83±0.04^c	65.63±0.10^c

*Difference superscript letters (a-d) in the same column indicate significant differences ($p<0.05$).

The effect of foaming stabilizer types on the color parameters of egg white powder is shown in **Table 3**. The highest lightness and whiteness values were found in the sample with 1.0% xanthan gum. In addition, the a* and b* coordinates were low indicating a low intensity of color. The high stability of foam using xanthan gum contributes to its stability during drying. After drying, the foam with xanthan gum showed less collapse compared to other samples, especially the control. This is possibly due to the advantages of xanthan gum solutions over other polysaccharides including its remarkably high viscosity at low concentrations and its viscosity being unaffected by temperatures

in the range of 0–100 °C [29]. The dried foam (Fig 1) has a porous structure, which makes it easy to grind.

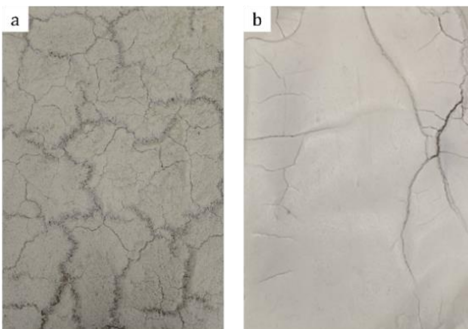

Figure 1. The control dried foam(a) and 1% xanthan gum dried foam (b).

Table 4. The effect of foaming stabilizer types on reconstitution and solubility of egg white powder

Sample	WHC (g/g)	WBC (g/g)	WAI (g/g)	WSI (%)
Control	2.31±0.64c	4.62±0.13b	0.65±0.03a	43.37±4.15d
0.5% xanthan gum	3.81±0.12b	3.82±0.13d	0.48±0.01d	58.85±0.63a
1.0% xanthan gum	4.88±0.19a	5.07±0.27a	0.53±0.01b	56.28±1.51b
0.5% gum arabic	3.43±0.31b	4.01±0.14c	0.50±0.01c	56.84±1.21b
1.0% gum arabic	3.20±0.07b	3.25±0.09d	0.53±0.01b	54.56±1.08c

Difference superscript letter (a-d) in the same column indicate significant differences ($p<0.05$). WHC: Water holding capacity, WBC: Water binding capacity, WSI: Water solubility index and WAI: Water adsorption index.

When improving powder properties through drying, reconstitution properties (WHC, WBC, WAI) should also be considered to ensure that the powder is easily dissolvable and dispersible. The effect of different foaming stabilizers on the reconstitution and solubility of egg white powder is shown in Table 3. The sample with 1.0% xanthan gum showed the highest water holding capacity and water binding capacity values. In addition, adding a stabilizer contributes to a higher water solubility index and a lower water absorption index. The control sample had the lowest reconstitution properties. The wettability of the dried powder was decreased by heat crystallization. The hydrogen bonds that occur in gums-containing ingredients may promote the reconstitution of the powder. According to a study by [30], galactomannans were characterized as substances with poor reconstitution properties due to low hydrophilization of their surfaces.

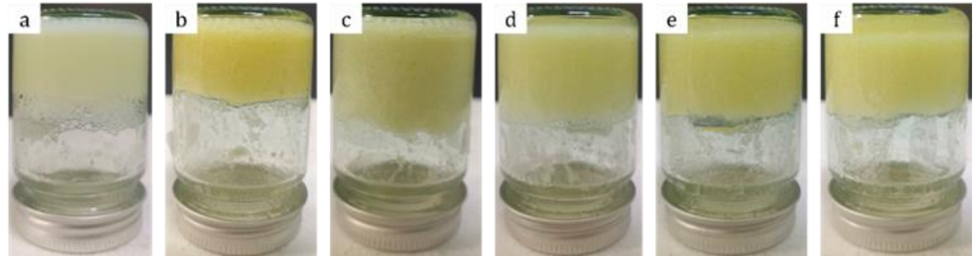

Figure 2. Gel appearance of albumin commercial (a), control (b), 0.5% xanthan gum (c), 1.0% xanthan gum (d), 0.5% gum arabic (e) and 1.0% gum arabic (f)

Table 5. The effect of foaming stabilizer types on protein content and some functional properties of egg white powder

Sample	Protein (%)	Re-foaming capacity (%)	Gelling appearance
Albumin commercial*	89.42±1.30^{a}	250	Homogeneous, opaque texture and highly stable
Control	89.17±0.37^{a}	50	Homogeneous, opaque texture and highly stable
0.5% xanthan gum	87.46±0.12^{b}	150	Air bubble texture and moderately stable
1.0% xanthan gum	86.89±0.86^{b}	230	Air bubble texture and highly stable
0.5% gum arabic	86.10±1.12^{b}	200	Slightly separated texture and less stable
1.0% gum arabic	86.26±0.72^{b}	200	Slightly separated texture and less stable

*Difference superscript letter (a,b) in the same column indicate significant differences ($p<0.05$).
*Spray dried powder

The protein content of the egg white powders was in the range of 86% to 89%. The effect of different foaming stabilizers on the protein content of egg white powder is shown in Table 4. Adding stabilizers contribute to a slightly lower protein concentration. The functional properties of egg white albumin such as foaming, emulsifying, binding, and gelling make albumin powder a versatile ingredient in the food industry. Some functional properties of egg white powder are shown in **Table 4**. The re-foaming capacity was promoted when the stabilizers were used. The foaming capacity of the obtained powders was close to that of a commercial sample except for the control and 0.5% xanthan gum sample. However, using a low concentration of xanthan gum at 0.125% was found to be suitable for production using the freeze-drying method as reported by [31]. The protein content in dried egg white (albumin powder) does affect its foaming properties. Higher protein content generally correlates with better foaming ability because proteins, particularly albumin, are responsible for forming and stabilizing air bubbles in foam. Protein molecules unfold and interact with air bubbles, creating a stronger foam structure. In addition, the presence of additives or stabilizers in the egg white powder formulation can impact its foaming properties. The results showed that using 1.0% xanthan gum or 0.5% and 1.0% gum arabic was suitable for the production of egg white powder using foam mat drying at 70°C. Generally, the denaturation temperature of egg albumin, the main protein

in egg whites, is around 70°C. Therefore, the production of egg white albumin is typically conducted by spray drying. Interestingly, foam mat drying at 70°C did not destroy the functional properties of egg white powder. According to a study by [32], the protein content was not influenced by the drying temperature (40-80°C). However, a temperature of 60°C was recommended for drying whey in a foam mat. The gel without stabilizers (Fig. 2) exhibits an opaque texture, resembling commercial examples. However, upon adding xanthan gum at 0.5% and 1%, the texture becomes less refined, with noticeable air bubbles, though moderately stable. Increasing xanthan gum to 1% enhances stability. In contrast, gum arabic addition at both 0.5% and 1% leads to instability, causing layer separation exceeding that observed with xanthan gum.

4 CONCLUSIONS

The addition of a stabilizer is crucial for enhancing the drying process. Research findings indicate that incorporating xanthan gum during foaming significantly helps maintain foam volume throughout the drying process, resulting in high quality of egg white powder compared to the commercial product. The results showed that using xanthan gum or gum arabic was proper in the production of egg white powder using foam mat drying at 70 °C. The incorporation of 1.0% xanthan gum yields egg white powder of excellent quality, with good reconstitution and functional properties. Additionally, gum arabic has been deemed suitable for egg white powder production based on its favorable functional attributes. The favorable functional properties, including binding, solubility, foaming, and gelling, identified in this study, therefore render foam mat powder a versatile ingredient in the food industry.

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Effect of Plant Proteins Mixing Ratios on Free-SH/S-S Bond Change and Functional Properties of High Moisture Meat Analogues via Extrusion

BP-O204

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ABSTRACT

This study was aimed to obtain an effect of mixing ratio of pumpkin seed protein concentrate (PPC) and rice protein isolate (RPI) (100:0, 85:15, 65:35, 45:55, 0:100) to produce a high moisture meat analogue (HMMA) with high disulfide bond formation by using twin screw extruder. The chemical compositions of PPC and RPI were evaluated, and the moisture content, Free-SH, S–S bonds, WHC, OHC of raw materials, and of HMMA at different PPC:RPI mixing ratios were analyzed. The results showed that RPI had a higher content of protein (84.32%) compared to PPC (65.10%). The moisture content of HMMA varied from 56.40% to 58.62% ($p>0.05$). The content of Free-SH of all PPC:RPI mixing ratios increased significantly after extrusion compared to raw material ($p\geq0.05$). However, different ratios of PPC:RPI did not affect Free-SH content of HMMA ($p>0.05$). The S–S bond content also significantly increased in HMMA from 33.09 μmol/g in 100:0 of PPC:RPI ratio to 50.79 μmol/g in 0:100 of PPC:RPI ratio caused by the oxidation of Free-SH and the SH/S–S bond interchange reaction. However, the formation of S–S bonds is observed in HMMA of other ratios depended on the PPC:RPI mixing ratio ($p>0.05$). The WHC and OHC of HMMA were also improved compared to the raw material ($p\geq0.05$). After extrusion, the highest WHC was the 85:15 of PPC:RPI ratio ($p\geq0.05$) among others, but lower in OHC compared to the 65:35 of PPC:RPI ratio ($p\geq0.05$). The study suggests that the mixing ratios of PPC and RPI are sought to closely mimic of real meat as both sources offer desirable sulfur-containing amino acids crucial for the formation of disulfide bonding and it can be used to develop HMMA with enhanced structural and functional properties through high moisture extrusion process.

1. INTRODUCTION

The production of plant-based meat analogues has gained significant attention in recent years due to environmental concerns and health awareness, caused the increasing demand for sustainable and healthy food alternatives [1,2]. Plant proteins are the primary ingredients of meat analogues due to they are sustainable and be able to imitate the characteristic of meat structure by forming newly chemical interaction under high moisture extrusion [3]. High moisture extrusion process is one of the most highly promising method among the various techniques available for producing plant-based meat analogues with texturized structures that closely resemble animal meat [4]. During various stages of high moisture extrusion process, the original forms of plant proteins, hydrated water content up to 40%, are unfolded globular structure by different temperatures of barrel, shearing force, and pressure of the specific extrusion parameters [5]. After unfolding, the amino acids side chains of plant proteins exposed the hydrophobic, hydrophilic, and free sulfhydryl group as the functional and reactive groups which enclosed inside the structure of native protein [6,7,8]. Then, the unfolded proteins rearranged its structure in alignment by cross-linking between hydrophobic interaction, hydrogen bonds and disulfide bonds to develop fibrous structure similar to real animal meat [9]. However, disulfide bonds, the chemical bonds formed by the oxidation between two sulfur atoms within the protein molecules, are considered as main bonding in the development meat-like structure of plant-based meat analogues [10,11,12,13,14].

There are several sources of plant proteins play an interested role for meat analogue included pumpkin seed protein, rice protein, mung bean protein, soy protein, pea protein, vital wheat gluten, mycoproteins, etc. [15,16,17,18]. Pumpkin seeds contain high concentration of crude protein, approximately 35%, based on pumpkin varieties [19]. Pumpkin seed protein exhibits a desirable sulfur-containing amino acids, such as methionine and cysteine, the amino acids crucial for disulfide bond formation [20,21]. It is a new source of plant-based protein containing the essential amino acids profile meet the nutritional requirements set by the Food and Agriculture Organization (FAO) and World Health Organization (WHO) for pre-school children, whereas adults at minimum amount of amino acids requirement [22]. Moreover, the functional properties of pumpkin seed proteins, i.e., solubility, emulsifying, foaming, water and oil holding capacity, reported to be comparable with protein isolated from soybean [20]. Another interesting source of plant protein is rice protein, which is a highly promising supplement for utilization in many formulations of plant-based food due to it has low price of production and large amount of availability [23]. The major fractions of protein in rice consist of albumin (5–10%), globulin (7–17%), glutelin (75–81%), and prolamin (3–6%) [24]. Rice protein is also a good source of cysteine and methionine, a sulfur-containing amino acids in its globulin structure which can be a potential source of disulfide bond formation [25]. In term of the nutritional values, rice protein is a favorable for daily consumption, especially infants and elderly because it has anti-hypertensive, anti-obesity, anti-cancer, and antioxidants properties [26].

A recent work on high moisture extrusion of pea protein isolates combined together with different percentages of L-cysteine to produce high moisture meat analogue, they found that the formation of disulfide bonds during the extrusion process obtained from the oxidation of sulfhydryl group improved the structural and functional properties of the final extrudate [27]. Regarding to the fibrous structure formation of high moisture meat analogue, the disulfide bonds had strengthened the structure in extruded pea protein concentrate after high moisture extrusion [28]. Depend on the specific type of plant proteins, however, the application only one source of sulfur-containing amino acids from plant proteins were mostly not provide enough disulfide bond formation after extrusion process [28]. Therefore, it is necessary to carefully combine two or more than two sources of sulfur-containing amino acids from plant proteins in order to obtain optimum disulfide bond formation which is crucial for the structure of meat analogue [17,29]. At present study, pea protein (PP) based complexes under high moisture extrusion to produce the extrudate have been studied in several

aspects [29]. It has demonstrated that the extruded pea protein-based complexes provided higher disulfide bonds after extrusion process compared to pea protein extruded alone leading the extruded pea protein-based complexes had the highest degree of fibrous structure [29]. Moreover, it is suggested that the combination of plant proteins under high shear rate, high pressure, and high temperatures conditions of the extrusion process, the chain of both proteins simply unfold. Then, the unfolded chains expose sulfhydryl groups that undergo oxidation to create disulfide bonds, thereby facilitating the formation of fiber network structures of high moisture meat analogue [11].

Thus, this study is aimed to find an optimum ratio between pumpkin seed protein and rice protein to produce high moisture meat analogue using high moisture extrusion process to obtain a high disulfide bond. The findings from this study offer significant insights that can contribute to the development of high moisture meat analogue products.

2. MATERIAL AND METHODS

2.1 Plant-based proteins

The plant-based proteins used for high moisture meat analogue (HMMA) were pumpkin seed protein concentrate (PPC) and rice protein isolate (RPI). The pumpkin (*Cucurbita pepo* L.) seed protein with 60% of protein content, was obtained from Max Global Marketing Co., Ltd. (Bangkok City, Thailand). The rice (*Oryza sativa.*) protein with 80% of protein content, was provided by Richtek Ltd (Liaoning Province, China). All of other analytical grades chemical reagents were used in this experiment.

2.2 High moisture extrusion process

All of the experimental units were conducted using a co-rotation type twin-screw extruder (CTE-D22L32, CHAREON TUT CO., LTD, Thailand). The twin-screw extruder with a gearbox designed for food extrusion consists of sample feeder, water feeder, co-rotating screws, and a short concentric slit die equipped with die head at the last section of the extruder barrel. The barrel of the extruder composed of seven sections, containing a single feeding zone located at the beginning of the barrel, and other zones were separated into mixing zone, heating zone, and die zone. The temperatures in each zone were set differently which ranging between 25–150 °C. The schematic of the twin screw extruder illustrated in Figure 1.

PPC and RPI were pre-mixed well in five different ratios (100:0, 85:15, 65:35, 45:55, 0:100) according to our preliminary experiments. The pre-mixed plant proteins powder was fed via screw feeder at 6.40 rpm/min and the distilled water was fed at the rate of 21 mL/min to obtained the high moisture meat analogue (HMMA) at a final moisture content of approximately 50–60% (wb). The HMMA was collected from the die of the extruder and cooled down at room temperature once the extrusion process had achieved a stable and constant state. After cooling, HMMA were double sealed in aluminum foil bags and immediately kept in the refrigerator at 4 °C for further analysis.

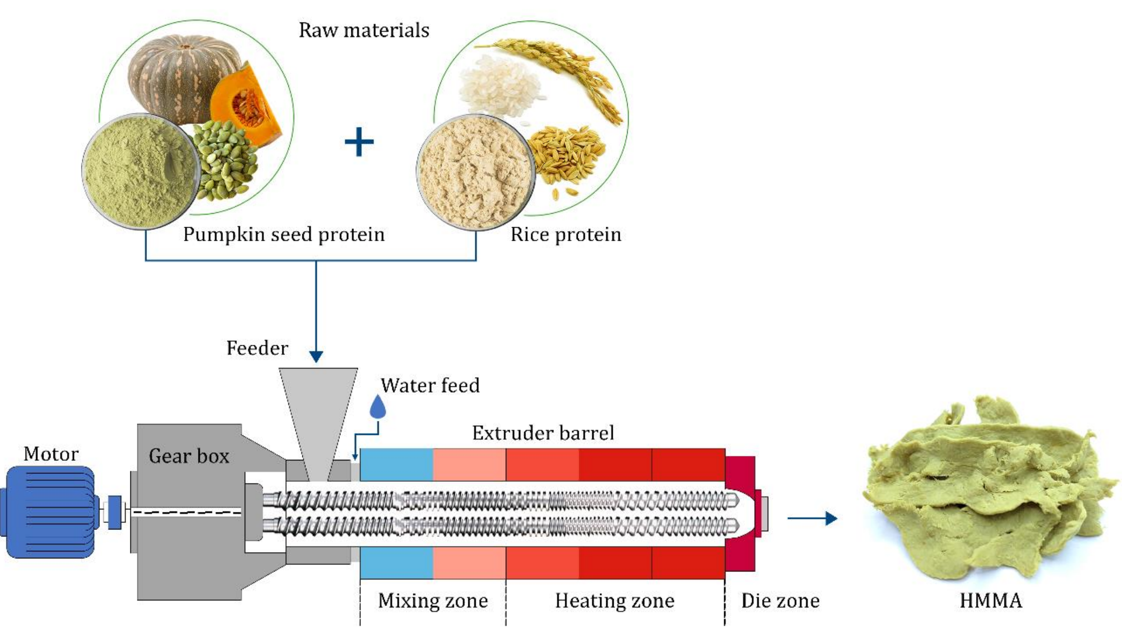

Figure 1. The schematic diagram of the twin screw extruder

2.3 Proximate analysis

Moisture content analysis was determined by following the established procedures outlined in AOAC 934.01 [30]. The determination of crude protein content was carried out the guidelines in AOAC 990.03 using a protein analyzer and the Dumas combustion method [31]. The crude fat content was analyzed by solvent extraction with petroleum ether according to the outlined in AOAC 920.39 [32]. The crude fiber content was evaluated using the method of AOAC 962.09 [33]. The ash content was analysis using a muffle furnace by burning the sample at 550 °C for 3 hours, following the method in AOAC 942.05 [34]. All analysis was performed in triplicate for each sample, and the carbohydrate content was calculated from 100% – (moisture + protein + fat + fiber + ash) [35].

2.4 Free sulfhydryl group and disulfide bonds of raw materials and HMMA

The free sulfhydryl groups (Free-SH) and disulfide bonds (S–S bonds) were analysed following the method described by Beveridge, Toma, and Nakai (1974) with slightly modification [36]. For free-SH content determination, 30 mg of PPC powder or RPI powder, or fresh ground HMMA samples were carefully weighted and added to 10 mL of Tris-glycine buffer. Then, adding 0.05 mL of Ellman's reagent (5,5′-dithio-bis-2-nitrobenzoic acid or DTNB) to the dispersions. The mixture was placed in the dark place and allowed to incubate at room temperature for 1 h. After that, the sample mixture was centrifuged at 5000g for 5 min. The supernatants were obtained and measured using UV-spectrometer at wavelength 412 nm. The Free-SH content was calculated based on equation (1).

$$Free - SH\ content\ (\mu mol/g) = \frac{73.53 \times A412 \times D}{C} \quad (1)$$

For S–S bonds content determination by Beveridge, Toma, and Nakai (1974) with a minor modification, 3 mg of PPC powder or RPI powder, or fresh ground HMMA samples were carefully

weighted and dissolve in to 5 mL of Tris-glycine buffer. Then, the sample was mixed well using a vortex mixer before adding 50 μL of 2-mercaptoethanol. The dispersions were mixed again and allowed for incubation in the dark place at 25 °C for 1 h. Next, 10 mL of 12 % trichloroacetic acid (TCA) was added. The mixture was centrifuged at 5000g for 15 min. After centrifugation, the supernatant was discarded and replaced by 10 mL of 12 % trichloroacetic acid (TCA) to clean the precipitate. The dispersions were mixed again and re-centrifuged at 5000g for 15 min. The precipitates were collected and re-dissolved in 10 mL of Tris-glycine buffer. After that, 100 μL of Ellman's reagent (5,5′-dithio-bis-2-nitrobenzoic acid or DTNB) was added into the mixture and mixed enough. The samples were measured at the absorbance of 412 nm using UV-spectrometer. The content of S–S bonds was calculated according to the equation (2).

$$S - S\ bonds\ content\ (\mu mol/g) = \left(\frac{73.53 \times A412 \times D}{C} - Free - SH\right) / 2 \quad (2)$$

Where:
73.53 was a coefficient derived from $10^6/(1.36 \times 10^4)$
1.36×10^4 was the molar absorptivity
A412 was the absorbance value at 412 nm
D was the dilution factor
C was the protein concentration (mg/mL)

2.5 Water and oil holding capacity

Water and Oil-holding capacity of pre-mixed powder of pumpkin seed protein concentrate (PPC), rice protein isolate (RPI), and HMMA were determined according to the procedure of Peng et al. (2022) with a minor modification [27]. To determine water holding capacity (WHC), 1 g pre-mixed powder of PPC, RPI, and the HMMA were weighted and put into a pre-weighted of 50 mL centrifuge tube. Then, 20 mL of distilled water was added and homogenized using a vortex mixer. The dispersions were vortex 15s every 5 min for 30 min before they were centrifuged 3500 rpm/min at 25 °C for 30 min. After centrifugation, the supernatant was discarded and the tube containing precipitate was wiped using tissue, then record the weight of samples after hydration. To determine oil holding capacity, the same procedure as above mention was applied. However, only 10 mL of soybean oil was used to replace the 20 mL of distilled water. Each of sample was determined five replications. The content of WHC and OHC were calculated according to the equation (3).

$$WHC\ or\ OHC\ (\%) = \frac{Wet\ sample\ weight - Initial\ sample\ weight}{Initial\ sample\ weight} \quad (3)$$

2.6 Statistical Analysis

The experimental procedure was designed as randomized complete block design (RCBD) with three replications. The data were reported as the mean values accompanied by their corresponding standard deviations, and each analysis was carried out in triplicate. All statistical analyses were performed using Microsoft Office Excel 2016, Minitab statistical software version 14.12.0 (Minitab

Inc., US), and Statistix 8 software (Analytical Software, Florida, USA). To access the significant differences level ($P \leq 0.05$) between each treatment, LSD comparison test was used to produce a 95% confidence interval of the data.

3. RESULTS AND DISCUSSION

3.1 Chemical composition of raw materials

The results of chemical composition of pumpkin seed protein concentrate (PPC) and rice protein isolate (RPI) are provided in Table 1 which were the main source of protein used in the formulation of HMMA. The protein content of PPC and RPI were 65.10% and 84.32%, respectively. The difference in protein content between PPC and RPI can be attributed to the variations in the composition of these two plant-based protein sources, such as fat content [21]. Another reason is that the method of protein separation was different as the pumpkin seed protein is a concentration form and rice protein is the isolation form [37]. Protein concentrate (PC) typically has 30–80% of protein content, while protein isolate (PI) contains protein content up to 80% [38]. PC and PI offered unique functional properties, such as solubility, gelling properties, foam/emulsion ability, water and oil holding capacity, depend on the difference of their source and technique of extraction [39]. Moreover, the different plant proteins sources preparing as an isolate or concentrate form would provide different benefits. From a comparative research work of PC and PI extracted from rapeseed, it reported that PC had a better water holding capacity (WHC) compared to PI because the presence of water-insoluble dietary fibre [40]. In some cases, PI exhibit poor water holding capacity compared to PC because of the purest fraction of protein [38].

Our results showed that RPI had lower content in fat, leading to higher in protein content. Moreover, RPI was found lower in ash content compared to PPC. The unbalanced composition (i.e., fat, ash, fiber, carbohydrate) of individual protein raw materials was attributed to several factors, such as difference techniques of protein preparation, plant species, genetics, cultivation conditions, and analytical techniques [2,19,23,41,42]. However, the higher content of fat in PPC could increase the flexibility and mobility of protein lead to enhance the proteins interaction and rearrangement, thus facilitating of disulfide bonds formation under extrusion [13,43]. In addition, the higher content of ash in PPC, particularly the trace elements (e.g., iron & copper) improved the formation of disulfide bonds since it may catalyze the oxidation of sulfur groups into disulfide bonds [44]. It was reported that the elements of ash, such as iron (Fe), copper (Cu), phosphorous (P), and calcium (Ca) were significantly increased by the extrusion process and it effected to the final extrudate [45]. For instance, the calcium chloride ($CaCl_2$) was reported as an aid for firmness development of protein texturization [46].

Table 6. Chemical composition of pumpkin seed protein concentrate (PPC) and rice protein isolate (RPI)

Chemical composition	PPC	RPI
Moisture content (%, wb)	5.11 ± 0.09[a]	5.61 ± 0.12[a]
Crude protein	65.10 ± 0.24[b]	84.32 ± 0.25[a]
Crude fat	9.70 ± 0.21[a]	1.45 ± 0.27[b]
Crude fiber	1.02 ± 0.01[a]	0.83 ± 0.01[a]
Ash content	10.50 ± 0.05[a]	1.70 ± 0.13[b]
Carbohydrate	8.57 ± 0.06[a]	6.09 ± 0.03[a]

*All values are mean ± standard deviation of three replicates (n = 3).
Different lowercase letters within a row indicate a significant difference ($p \leq 0.05$).

3.2 Moisture content of HMMA

The moisture content of HMMA produced from different mixing ratios of pumpkin seed protein concentrate and rice protein isolate (PPC:RPI) are shown in Figure 2. The moisture content of HMMA was varied from 56.40–58.62%. There was considerably no significant difference ($p>0.05$) in moisture content of all the ratios of PPC and RPI. This could notably that all PPC:RPI mixing ratios were well prepared to obtain the same moisture content as well as the water feed rate during the extrusion process was controlled to ensure uniform water distribution and hydration [47]. These results were similar to the study of Zahari et al. (2020), it proved that different mixing ratios of soy protein isolate (SPI) and hemp protein concentrate (HPC) was no significant variation in moisture content between SPI:HPC mixing ratios [5].

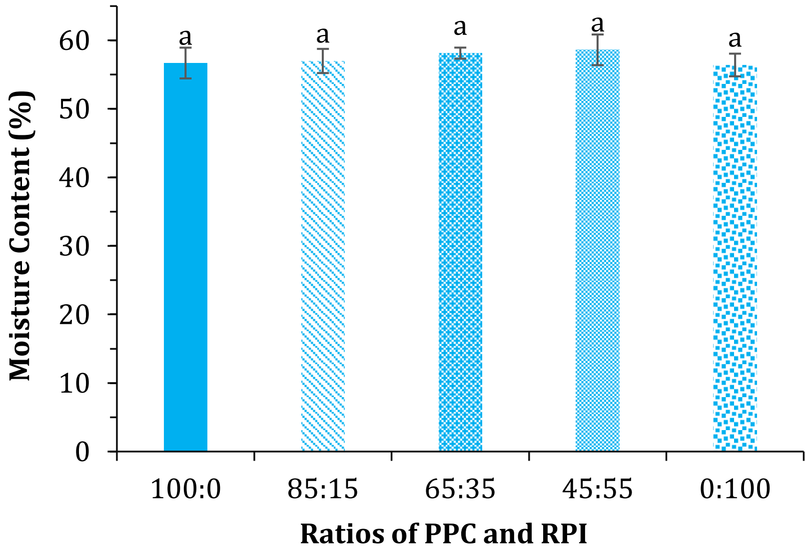

Figure 2. Moisture content of HMMA produced from different PPC:RPI mixing ratios

3.3 Free-SH content and S–S bonds of PPC, RPI, and HMMA

The presence of both free sulfhydryl group (Free-SH) and disulfide bond (S–S bond) are important for the formation of fibrous structure in HMMA, as they determine the degree of protein denaturation and cross-linking during high moisture extrusion process [48,49]. The content of Free-SH and S–S bond of PPC, RPI, and HMMA showed in Figure 3. The content of Free-SH in pre-mixed protein ratios (PPC:RPI) varied from 5.46–6.63 μmol/g. There was no significant change ($p>0.05$) in Free-SH content of all the PPC:RPI ratios before extrusion. However, when the proteins mixture of raw materials passed through the extruder, the Free-SH content of HMMA increased significantly ($p\leq0.05$) compared to all pre-mixed PPC:RPI ratios (Figure 3A). This could be due to the various conditions of the extruder, such as high temperature, pressure, shear force led the native globular structure of plant protein which is stabilized by disulfide bond, hydrophobic interaction, ionic bond, and hydrogen bond unfolded and exposed more reactive Free-SH hidden inside the protein molecules raising to the surface [50,51]. A similar result study on varying the extrusion temperature and screw speed increased the Free-SH content of the textured pea proteins [7]. In our study, the content of Free-SH of HMMA was in range from 9.70–11.44 μmol/g for all the PPC:RPI mixing ratios (Figure 3A). However, the change of Free-SH content of HMMA was not significant difference ($p>0.05$). It could perhaps be due to the relative content of sulfur-containing amino acids presented in PPC and RPI were similarity [21,25].

On the other hand, the result of S–S bond content of pumpkin seed protein concentrate (PPC), rice protein isolate (RPI), and HMMA of PPC:RPI at different ratios showed in Figure 3B. Before extrusion, when increasing the rice protein content in the blended PPC:RPI, the 65:35, 45:55, and 0:100 of PPC:RPI ratio exhibited a significantly increase in S–S bond content compared to pumpkin seed protein at 100:0 of PPC:RPI ratio ($p\leq0.05$). The increasing of S–S bond content could be due to RPI mainly composed of glutelin fraction approximately 80% which the subunits of glutelin are linked together by disulfide bonds [24,52,53]. However, the extrusion process led to a significant increase ($p\leq0.05$) in the amount of S–S bonds for all the PPC:RPI ratios, when compared to the raw materials (Figure 3B). After extrusion, the S–S bond content of 85:15 and 65:35 of PPC:RPI ratio of HMMA increased from 37.37 to 39.07 μmol/g, respectively, which was not significant difference ($p>0.05$) compared to 100:0 of PKS:RPI ratio (33.09 μmol/g). Moreover, the content of S–S bond had raised significantly ($p\leq0.05$) from 43.48–50.79 μmol/g for 45:55 and 0:100 of PPC:RPI ratio, respectively. These results were similar to Xia et al. (2023), the HMMA produced from different ratios between yeast protein and soy protein increased the content of S–S bond significantly ($p\leq0.05$) after high moisture extrusion [17]. The change of the S–S bond content is likely due to the intrachain S–S bonds disruption during extrusion process and then converted into Free-SH groups (as shown in Figure 3A), the oxidation of Free-SH, and the SH/S–S bond interchange reaction [54,51]. However, the content of S–S bond was increased when increasing the rice protein isolate (RPI) content in the ratio, but with non-significant difference ($p>0.05$) in S–S bond content for HMMA using difference of PPC:RPI ratios (85:15, 65:35, and 45:55) after the extrusion. This could also be attributed to the balance between the breaking of S–S bonds in rice protein isolate (RPI) and the formation of new S–S bonds in pumpkin seed protein concentrate (PPC) during extrusion process as well as the interactions between PPC and RPI in the mixing ratios was limited leading more S–S bond would be not obtained [55,29]. On the other hand, using only the plant protein from RPI, it was clearly observed that the S–S bond content

was higher than the others ($P \leq 0.05$) for both the pre-mixed powder and the HMMA one. It is noteworthy that rice protein known for its high content of glutelin fraction that composed of S–S bond cross-linking in glutelin structure, thus the S–S bond content of pre-mixed powder and HMMA with single RPI were found higher compared to others [56].

For HMMA of all different mixing ratios of PKSP:RP after extrusion process, the content of both Free-SH and S–S bond was significantly increased ($p \leq 0.05$) compared to the pre-mixed proteins powder before the extrusion process (Figure 3A and 3B). In this study, it was observed that the content of S–S bonds increased with a rising presence of Free-SH groups of HMMA, especially using 100% of RPI as a pre-mixed powder. The occurrence of this phenomenon implies that the S–S bonds formation was not only reliant on the oxidation of Free-SH groups and SH/S–S bond interchange reactions [17,1], but also the reversible reduction of S–S bond to Free-SH, in particular of the plant protein containing the two residues of sulfur-containing amino acids (e.g., cysteine and methionine) [57].

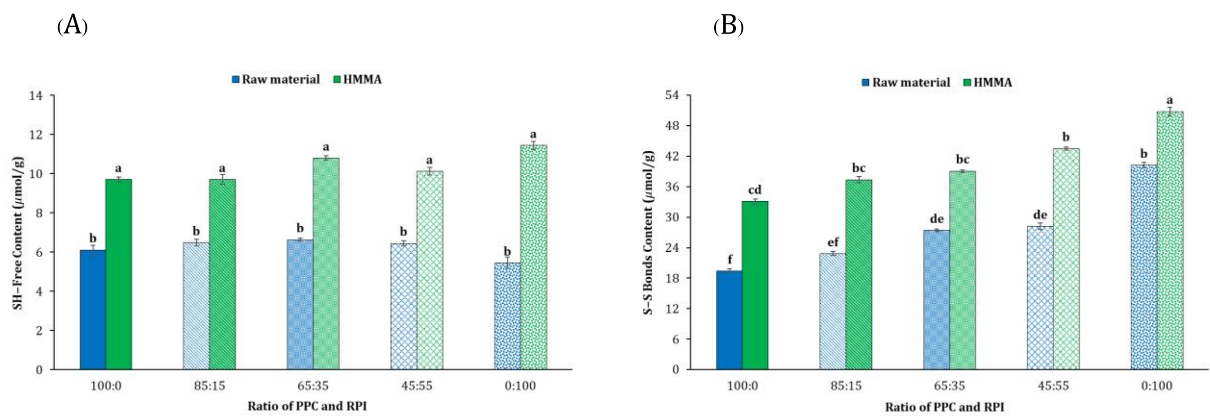

Figure 3. Free-SH and S–S bond content of raw materials and HMMA (PPC:RPI) at different ratios before and after the extrusion process. (A) the Free-SH content and (B) the S–S bonds content.

3.4 Water and oil holding capacity of PPC, RPI, and HMMA

According to a non-significantly difference of the S–S bonding formation among the ratio of PPC:RPI of HMMA as presented in section in 3.3, the functional properties of HMMA were further analyzed. The water holding capacity (WHC) of PPC:RPI at different ratios of proteins before the extrusion were shown in Figure 4A. It was observed that increasing the content of RPI from 0 to 100, the WHC of all PPC:RPI ratios of pre-mixed protein powder increased significantly ($p \leq 0.05$). The increasing of WHC in PPC:RPI ratios of the pre-mixed powder can be explained that the different in polarity on the protein surface and the sulfur-containing amino acids presence in pumpkin seed and rice protein [58]. From previous study reported that the rice protein isolate (RPI) contained more cysteine and methionine than pumpkin seed protein concentrate (PPC), therefore the water holding capacity (WHC) of rice protein was higher than pumpkin seed protein, leading the WHC of PPC:RPI ratios of pre-mixed powder increased in the same trend as the higher content of rice protein isolate in the PPC:RPI ratio was used [54]. Furthermore, the results of WHC for the HMMA of all PPC:RPI mixing ratios increased significantly ($p \leq 0.05$) compared to the PPC:RPI ratios of pre-mixed powder, except 0:100 of PPC:RPI ratio (Figure 4A). The increasing of WHC content of the HMMA caused by

native structure of the protein molecules could undergo significant change and unfold under the application of high temperature, shear, and pressure of the extrusion and the hydrophilic regions known as strong affinity for water molecule become more expose and increasing the surface area, providing more sites available for water molecule to bind and the binding water was trapped inside new alignment of protein structure, leading the increment of water holding capacity [59]. However, the gelation may would not be happened because the method of raw proteins preparation was not involving of protein solubilization, as it was prepared in a wet powder. Thus, there is not available solubilized protein leading to form the gel. After extrusion, the WHC of 85:15 of PPC:RPI ratio of the HMMA first increased significantly ($p \leq 0.05$) compared the control of 100:0 of PPC:RPI ratio (Figure 4A). The increasing of WHC of 85:15 of PPC:RPI ratio of the HMMA considers to a looser structure after the proteins cross-linked during the extrusion process, leading to increase the absorption ability of water molecule [7,55]. However, the WHC of 65:35, 45:55, and 0:100 of PPC:RPI ratio of HMMA decreased significantly ($p \leq 0.05$) in comparison with the 85:15 of PPC:RPI ratio (Figure 4A). The reducing in WHC of 65:35, 45:55, and 0:100 of PPC:RPI ratio due to the proteins were not fully restructured and limited the interaction between protein molecules, resulting in a dense and compact structure decreased the WHC [55,60,7]. The findings result in this study offer several potential benefits for HMMA characteristics in future work include enhanced moisture retention, improved texture, formulation optimization, ingredient selection, and process optimization.

On the other hand, oil holding capacity (OHC) of PPC:RPI at different ratio of pre-mixed protein powder and HMMA were expressed in Figure 4B. It was found that the OHC of all PPC:RPI mixing ratios of the pre-mixed protein powder increased significantly ($p \leq 0.05$). The increasing of OHC could probably be caused by the extraction technique used for rice protein extraction, unfolding the native protein structure and more exposes of hydrophobic groups which buried inside the inner part of rice protein structure by disrupting peptide chain, resulting in high surface hydrophobicity led to increase the OHC when increasing the content of rice protein isolate in the ratio of PPC:RPI of pre-mixed powder [23]. Moreover, OHC of all PPC:RPI ratios of the HMMA increased significantly ($p \leq 0.05$) compared to PPC:RPI ratio of the pre-mixed powder (Figure 4B). The OHC of 85:15 of PPC:RPI ratio of HMMA decreased significantly ($p \leq 0.05$) compared to 100:0 of PPC:RPI ratio because of the reducing of hydrophobicity caused by the hydrophobic interaction after protein denaturation during the extrusion process [61]. However, the 65:35 of PPC:RPI ratio of the HMMA increased significantly ($p \leq 0.05$) in comparison with 85:15 of PPC:RPI ratio. The increasing of OHC of 65:35 of PPC:RPI ratio of HMMA can be attributed to the breakage of chemical bonds during the extrusion process, resulting in a shift in molecular polarity led to increase in surface hydrophobicity [55]. In general, the OHC can be considered as a reliable indicator reflecting the hydrophobic nature of a particular food system [27]. From this finding, it can work towards developing HMMA products with enhanced oil holding capacity and improved overall quality.

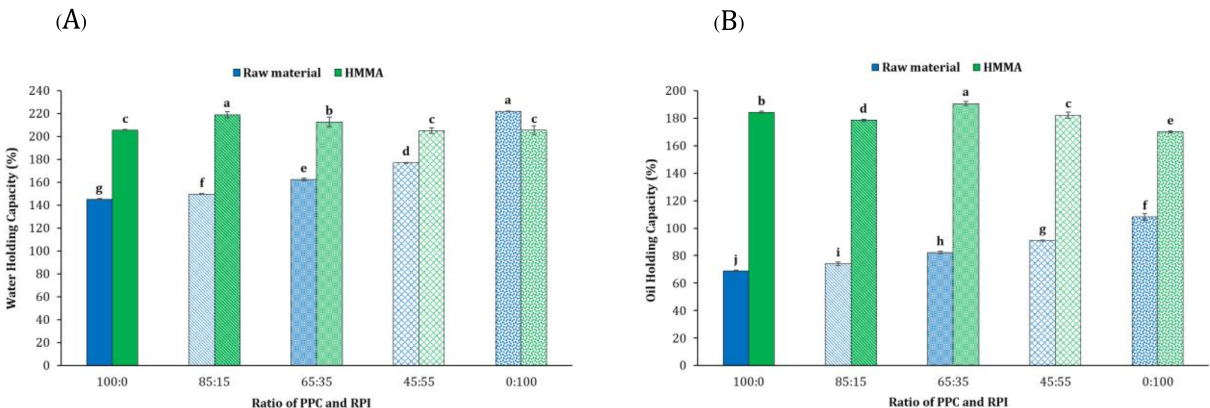

Figure 4. Functional properties of pumpkin seed protein concentrate (PPC), rice protein isolate (RPI), and HMMA of PPC:RPI at different ratios. (A) Water holding capacity (WHC) and (B) Oil holding capacity (OHC).

4. CONCLUSIONS

The different of plant proteins mixing ratios indicated the non-significant impact on the free sulfhydryl (Free-SH) content but it was significant effect to the formation of disulfide (S–S) bonds of HMMA. After the extrusion process, the content of both Free-SH and S–S bond increased in HMMA. However, the content of S–S bond formed in HMMA by using different plant proteins mixing ratios still could not identify an optimal ratio. The different mixing ratios of plant proteins were affected more to the water and oil holding capacity of HMMA. As a result, the 85:15 ratio of HMMA were found in higher water holding capacity and lower oil holding capacity compared to others plant proteins mixing ratios, thus it would provide the juiciness and tenderness to HMMA with low calories in the future work.

5. NOMENCLATURE

Free-SH	free sulfhydryl
HMMA	high moisture meat analogue
PPC	pumpkin seed protein concentrate
RPI	rice protein isolate
S–S bond	disulfide bond
wb	wet basis

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Effect of mung bean protein levels on the physical and textural properties of plant-based ice cream

BP-P209

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ABSTRACT

Ice cream, a delicious frozen dessert and widely consumed globally, is typically made of a mixture of dairy products. However, in order to ensuring food security for the world's growing population, plant-based protein diet has gained increasing attention nowadays. In addition, legume proteins are increasingly being recognized for their potential as an alternative protein source. Therefore, the aim of this study was to investigate the feasibility of using mung bean protein (MBP) for the production of plant-based ice cream and the effect of MBP levels (2, 4 and 6%) on the physical and textural properties of the ice cream. Viscosity of the ice cream mix, %overrun, destabilised fat index (DFI), melting behavior, melting rate and hardness of ice cream were determined in each sample (2, 4 and 6% MBP in ice cream formula). The result showed that increasing the MBP levels from 2 to 6 % led to increase the viscosity of ice cream mix, resulting in a reduction of overrun and DFI of the ice cream. High MBP levels tended to reduce the melting down, however, it affected the hardness of the ice cream. Ice cream with 6% MBP reached the highest hardness and lowest overrun. All results demonstrated that the physical and textural properties of MBP-based ice cream are affected by the level of MBP added.

1. INTRODUCTION

The global interest in veganism has seen a notable increase in recent years, with approximately 1 to 5% of the population now opting for plant-based meals, as reported by Radnitz et al. [1]. This growing trend reflects a significant shift towards more sustainable, ethical, and health-conscious dietary choices. For this reason, plant-based proteins are becoming more popular among consumers. In this scenario, dairy alternative products composed of plant-based proteins serve as suitable options for addressing allergies, lactose intolerance, and adhering to vegan diets [2-4].

Mung bean (*Vigna radiata*) is primarily cultivated in regions across Asia, Africa, South and North America, and Australia. Renowned for its nutritional richness, it serves as an excellent source of vitamins, minerals, and protein. Mung bean have about 20-27% protein [5]. Moreover, its essential amino acid profile rivals that of soybeans and kidney beans [6]. Mung bean protein isolate is notable for its amino acid composition, containing sufficient quantities of all essential amino acids, including lysine. Siemensma et al. [7] reported that its lysine levels closely approach those found in eggs. The amino acid profile of albumin closely resembles that of the human body, earning it recognition as a high-quality natural plant protein [8]. Mung bean protein isolate has been demonstrated to exhibit desirable functions in processed foods, including foaming, emulsification, and water absorption [9].

Ice cream, a multiphasic frozen system, is the favored frozen desserts product. Within its frozen matrix structure contains air bubbles, ice crystals, fat globules, and an unfrozen serum phase [10,11]. The substitution of cow milk with a variety of non-dairy milks, including soy milk, coconut milk, watermelon seed milk, soy protein and pea protein has been explored in the production of functional ice cream. This effort stems from the nutritional and health properties offered by these alternative raw material sources [12-15]. Although the effect of non-dairy milk and also some of plant proteins on the properties of plant-based ice cream have been identified, no study has been performed on potential used of MBP. This study aimed to investigate the effect of MBP levels on the physical and textural properties of the ice cream and studied the feasibility of using MBP for the production of plant-based dairy free ice cream.

2. MATERIAL AND METHODS

2.1 Materials

MBP containing 80% of protein content (Chemecosmetics, China), sugar (Mitr Phol, Thailand), coconut oil (Naturel, Thailand), tween 80 (Krungthepchemi CO.,LTD, Thailand), xanthan gum (Krungthepchemi CO.,LTD, Thailand), carrageenan (Krungthepchemi CO.,LTD, Thailand) and water were used for ice cream production.

Table 1. Formulations of MBP ice cream (%w/w)

Sample	2% MBP	4% MBP	6% MBP
MBP	2	4	6
Water	72.5	70.5	68.5
Sugar	15	15	15
Coconut oil	10	10	10
Tween80	0.2	0.2	0.2
Xanthan gum	0.275	0.275	0.275
Carrageenan	0.025	0.025	0.025

2.2 Ice cream production

Three ice cream formulations were prepared by using different levels of MBP (2%, 4% and 6%). All ingredients were mixed and heated to 60°C, then cooldown to room temperature for homogenization by blender (Toshiba, Japan) at speed no. 2 for 2 minutes and followed by pasteurization at 80°C for 2 min. The mixes were cooled to room temperature and aged at 4°C for 18 hr. Then the ice cream mixes were frozen in a batch-type ice cream machine (Frigomat machine per gelato, Model TS2, Italy) and packed in 2 oz. plastic containers and hardened at -18°C for 18 hr.

2.3 Measurement of viscosity of the ice cream mix

The viscosity was measured according to the modified method of Pisalwadcharin et al. [16]. by Brookfiled ametek Model DV2T RV (Ametek, USA) with small sample adapter Model SC4-27. Speed of viscosity analysis was 50 rpm, single point mode and end condition at 30 s.

2.4 Overrun determinations

Overrun was determined according to the method described by Muse & Hartel [17]. Three overrun measurements in percentage were taken per sample using the following equation:

$$Overrun\ (\%) = \frac{\text{weight of ice cream mix} - \text{weight of ice cream}}{\text{weight of ice cream}}\ x\ 100$$

2.5 Determination of destabilised fat index (DFI)

DFI was determined according to the method described by Segall and Goff [18]. 3g of ice cream mix and melted ice cream were diluted with 30 g of water. 1 ml of solution was diluted to 50 ml in a volumetric flask giving a total dilution of 1:500. Then the solution were measured turbidity by UV-Visible spectrophotometer (Thermo scientific Model genesys 10S UV-Vis, UK) at 540 nm. Turbidity of solution was used to determine the amount of destabilised fat in the sample using equation:

$$DFI\ (\%) = \frac{absorbance\ of\ ice\ cream\ mix - absorbance\ of\ melted\ ice\ cream}{absorbance\ of\ ice\ cream\ mix}\ x\ 100$$

2.6 *Meltdown test*

The melting rate and melting behaviour of ice cream were determined according to Narala et al. [19]. with slight modifications. Ice creams were removed from containers and measure the initial weight of ice cream. Then placing the ice cream on a stainless steel mesh at room temperature (25±1 °C), and weight of the melted ice cream was noted every 15 min to calculate the percentage of melted ice cream. Plotting melting behaviour graphs obtained by the percentage of ice cream melted and time (min). Melting rates were calculated from the slope of each meltdown curve.

2.7 Hardness measurement

The hardness of ice cream was evaluated according to the method of Narala et al. [19]. by Texture analyzer (TA-XT. Plus, UK) load cell 50 Kg with 2.5 cm diameter stainless steel cylindrical probe (P/25). The ice cream stored at -18 °C was removed from freezer and waited until the temperature of the ice cream at 1.5±0.3 °C before the analysis. The texture analysis conditions were as follows: compression mode, strain 50%, trigger force 5.0 g, pre-test speed 3.00 mm/sec, test speed 3.30 mm/sec, post-test speed 3.00 mm/sec. Hardness were calculated as peak force (N) during penetration

2.8 Statistical analysis

The data were subjected to analysis of variance and mean comparisons were carried out using Duncan's new multiple range test. Statistical analyses were performed using IBM SPSS Statistics Version 28.0 (Thaisoftup Co., Ltd., Thailand).

3. RESULTS AND DISCUSSION

3.1 *Appearance of ice cream*

The overall appearance of the three ice cream recipes was similar, which was light yellow to almost white. As shown in Figure 1, it can be seen that 6% MBP has the darkest colour, followed by 4% MBP and 2% MBP, respectively. This result might be due to the effect of MBP colour which was yellow. Therefore, the more level of protein could induce the more yellow ice cream. Sivasankari et al. [20] reported that as the concentration of pulses protein increased, the b* values of ice cream also increased.

Figure 1. Appearance of ice cream with 2, 4 and 6% of MBP

3.2 *Viscosity*

The result in Table 2 showed the viscosity of the ice cream mix at different MPB levels. Ice cream mix with 6% MBP had the highest viscosity, followed by 4% MBP and 2% MBP, respectively. These results indicated that the viscosity of ice cream mix increased with an increase of MBP level. This outcome was attributed to the ability of proteins to form a more stable gel matrix due to their high-water retention and water binding properties [21]. Similar results have been observed in various studies on ice cream production when plant protein or milk with high protein content are added, indicating a consistent effect on ice cream viscosity [12,22]. Daw and Hartel [23] explained that an increase in protein content tended to increase the consistency coefficient, and consequently viscosity which could be attributed to the increasingly large dispersion of colloidal particles.

Table 2. Changes in viscosity, overrun of ice cream with 2,4 and 6% MBP.

Sample	Viscosity (cP)	Overrun (%)
2%MBP	410.50±3.06^{c}	20.06±1.92^{a}
4%MBP	758.33±23.57^{b}	20.31±2.47^{a}
6%MBP	1283.33±21.21^{a}	17.05±1.44^{a}

* Values are mean ± SD from three determinations; different superscript letters in the same column show significant differences ($p \leq 0.05$).

3.3 *Overrun*

Overrun of the ice cream with various MBP levels are present in Table 2. Overrun of the ice cream with 2% MBP and 4% MBP were 20.06±1.92% and 20.31±2.47%, respectively, while that of 6%

MBP was 17.05±1.44%. Although all of the samples had no significant difference ($p > 0.05$) in overrun values, it trended to decrease with increasing MBP level to more than 4%. This result was probably due to the formation of a highly viscous gel matrix by MBP in sample 6% MBP, which can obstruct the incorporation of air during the freezing process, thus affecting the overrun values. Gracas-Pereira et al. [12] and Badıllı [24] reported that the increment in soy protein extract and chickpea flour led to increased viscosity, consequently resulting in decreased overrun values of ice creams.

3.4 *DFI*

Changes in the turbidity of diluted ice cream and the turbidity of diluted ice cream mix used to determine the extent of fat destabilization [17]. Figure 2. showed that ice cream with 6% MBP has the lowest DFI that implying stable emulsion. The DFI of ice cream gradually decreased as the level of protein increases. When protein content of the ice cream increased, the adsorbed protein layer would become denser and thicker, impeding partial coalescence of fat to a greater extent, therefore, the degree of partial coalescence of fat was decreased [23]. The formation of an aggregated network of partially destabilized fat could reduce melting rate of ice creams [17].

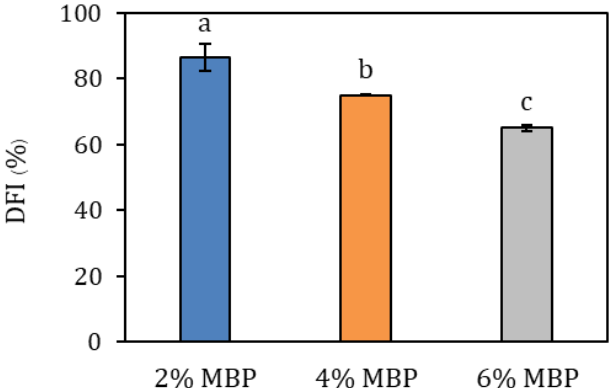

Figure 2. Destabilised fat index (%) of ice cream with 2, 4 and 6% of MBP. All values were based on three different samples and data are presented as means ± SD; different letters indicate significant differences ($p \leq 0.05$)

3.5 Melting rate and melting behaviour

The result in Table 2. showed that melting rate of the ice creams tended to decrease with increasing MBP level. However, there were no significant difference ($p > 0.05$). When considering melting behaviour graph (Figure 3), it was found that ice cream with 2% MBP melted fastest followed by ice cream with 4% MBP and 6 % MBP, respectively. However, none of the ice creams showed signs of melting until 30 min from the initial time. These durations surpass the conventional melting times observed in dairy ice creams. According to da Silva & Lannes [25], Guler-Akin et al. [15], and Loffredi et al. [26], the time taken for the first dripping was typically below 20 minutes, with complete meltdown occurring within 60 minutes in ice creams made with milk-based ingredients. The water binding and retention capacities of MBP used in the formulation could forming a stable gel matrix with water molecules, thus resulting in the slower melting of the ice cream. Sivasankari et al. [20] noted that the addition of pulse protein leads to the immobilization of water molecules, restricting their free movement among other molecules in the mix due to the formation of a stable gel network.

This phenomenon ultimately contributes to a lower melting rate of the ice cream. Moreover, the results of melting down were related to the viscosity of the ice cream mix (section 3.2). Previous studies have demonstrated that an increase in apparent viscosity enhanced the resistance to meltdown of ice cream [27,28]. The increase of viscosity in serum phase (unfrozen phase) could prolong the time required for the melted water phase to diffuse into the concentrated serum phase before it starts flowing from the interior to the exterior of the ice cream [29].

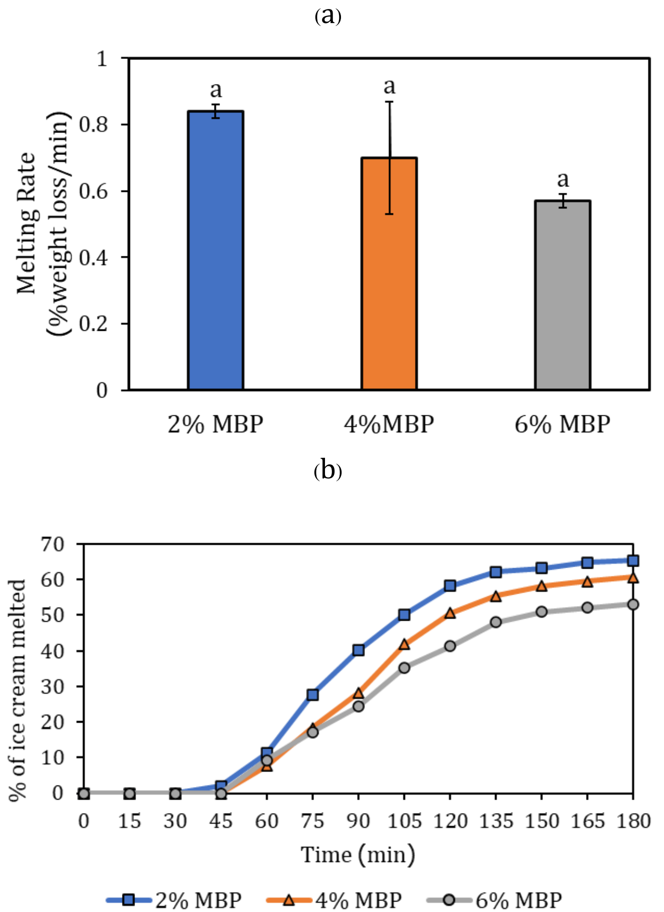

Figure 3. Melting rate (a) melting behaviour (b) of ice cream with 2, 4 and 6% of MBP

3.6 Hardness

There was a significant impact ($p \leq 0.05$) on the hardness values of the ice cream when increasing MBP level. The result in Figure 4. showed that 6% MBP had the highest hardness value, followed by 4% MBP and 2% MBP, respectively. This result was in line with the study of Atalar et al. [30], who explained that an increase in viscosity contributed to the hardness of the ice cream by increasing the resistance to probe penetration. It could be attributed to the water-binding properties of proteins, which result in a reduction of molecular mobility during storage, thereby establishing a stable and uniform emulsion structure.

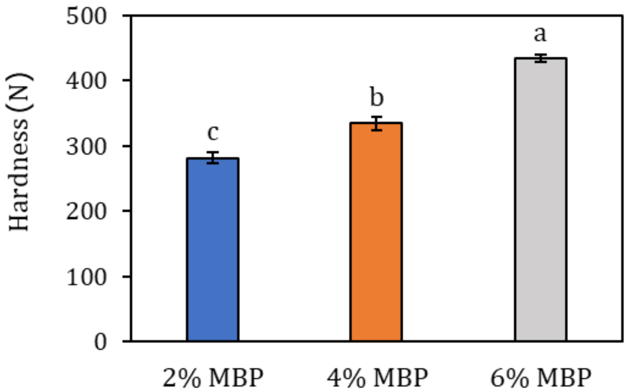

Figure 4. Hardness of ice cream with 2, 4 and 6% of mung bean protein. All values were based on three different samples and data are presented as means ± SD; different letters indicate significant differences ($p \leq 0.05$)

4. CONCLUSIONS

The present study revealed that the level of the MBP used in the ice cream affected the physical and textural properties of ice cream. By increasing MBP in the range of 2 to 6%, viscosity of the ice cream mix increased while DFI of the ice cream decreased. When MBP levels exceed 4%, overrun of the ice cream trended to decrease. Ice cream with high MBP content resulted in a reduction of melting down of ice cream. However, hardness of the ice cream greatly increased with increasing MBP levels, especially at 6% MBP. These results suggest that MBP has the potential to serve as a main ingredient in plant-based ice cream.

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Chemical composition and functional properties of raw, roasted and autoclaved tiger peanut (*Arachis hypogaea L.*) powder from Mae Hong Son, Thailand

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ABSTRACT

Currently, consumers are increasingly interested in plant-based protein products. However, most plant-based proteins are imported, and research on Thai plant proteins is still limited. Tiger peanut, which has a Geographical Indication (GI) from Mae Hong Son province, is a significant plant protein. This study aims to examine the effects of processing tiger peanut residue powders, including raw (RAW_PEA), roasted (ROS_PEA), and autoclaved (121 degrees Celsius for 15 minutes; CLAV_PEA), on their chemical composition and functional properties. The processed tiger peanut residue powders were found to have high protein content (42.84-44.79 grams/100 grams) and low fat content (6.74-9.07 grams/100 grams). Roasting and autoclaving slightly reduced the amino acid content. For example, the valine content of ROS_PEA and CLAV_PEA decreased by 2.44% and 12.20%, respectively, compared to RAW_PEA. The leucine content of ROS_PEA and CLAV_PEA decreased by 1.56% and 10.94%, respectively, compared to RAW_PEA. After roasting, water absorption capacity and fat content decreased by 9.03 and 25.69% respectively, whereas foam expansion and solubility increased by 91.42 and 42.03% respectively. These increases were statistically significant. As a result, roasted tiger peanut residue powders have a chemical composition and functional properties suitable for use as a food ingredient, making them a beneficial addition for consumers.

1. INTRODUCTION

Plant-based protein is classified as an alternative protein, which uses plant-based ingredients that are high in protein, such as beans, mushrooms, algae, oats, and almonds. The taste, smell, and color are developed to resemble animal products. Most of the main ingredients, such as soybeans, peas, and others, have to be imported from abroad. However, currently, there is limited information available about the nutritional value, quality, processing, and utilization of plant-based proteins in Thailand. If such information were available, it would promote the use of domestic raw materials in the processing of health-promoting products and reduce the import of raw materials from other countries.

Mae Hong Son Tiger Stripe Peanut, also known as Tua Lai Suer Mae Hong Son or Kalasin 2 peanut, has the scientific name Arachis hypogaea L. It is an annual plant in the Fabaceae or Leguminosae family. This peanut variety features long pods with thin shells and deep, distinct stripes on the shells. The seeds are large, with a white seed coat and purple stripes resembling tiger stripes. Each pod contains 2-4 large seeds. The Mae Hong Son Tiger Stripe Peanut is a protein-rich plant that is geographically specific to Mae Hong Son province [18].

Legumes are an important source of protein in human diets [8]. The nutritional composition of legumes can provide protein, fats, carbohydrates, dietary fiber, B vitamins (thiamine, riboflavin, niacin), and minerals in high proportions [15]. This composition can vary depending on the variety, growing location, climate, environmental factors, and type of soil in which the legumes are grown [8]. Generally, peanuts are seeds with a relatively high oil content (about 50% oil). After oil extraction, the peanut meal or cake contains approximately 6-7% oil, making it a good source of protein and amino acids [14]. Particularly when this peanut meal is processed into food, it has significant nutritional benefits. However, cooking or heating, for food safety purposes to inhibit microbial growth, can reduce the essential compounds in the seeds. Studies on the effects of cooking on the nutritional composition of vegetables have shown that different cooking methods can alter the amino acid content [13]. Therefore, this research aims to study the influence of processing methods on Mae Hong Son Tiger Stripe Peanut, including raw, roasted, and autoclaved peanuts.

2. MATERIAL AND METHODS

2.1 Raw materials and sample preparation

2.1.1 Prepare ingredients for tiger peanuts.

Tiger Peanuts, also known as Kalasin 2 Peanut, from Pai District in Mae Hong Son Province, is harvested from November to December. The harvest period is 100-120 days from the planting date. The preparation of the Tiger Peanut involves removing the seeds from the pods and discarding any damaged seeds.

2.1.2 Raw tiger peanut powder (RAW_PEA)

The raw tiger peanut seeds are processed using an oil extraction machine to obtain Tiger Peanut oil. The remaining Tiger Peanut meal is then ground into a powder and sieved using a 60-mesh screen to obtain tiger peanut residue powders (RAW_PEA).

2.1.3 Roasted tiger peanut powder (ROS_PEA)

The raw tiger peanut seeds are roasted using a 1.5L Simplus electric frying pan at 600W for 10 minutes. The roasted peanuts are then extracted for oil using an oil extractor, resulting in both oil and tiger peanut residue. The residue is ground into powder and sieved through a 60-mesh sieve to obtain roasted tiger peanut residue powder (ROS_PEA).

2.1.4 Tiger peanut powder autoclave (CLAV_PEA)

The raw tiger peanut seeds are heat-treated in an autoclave at 121 degrees Celsius for 15 minutes. The treated seeds are then used to extract oil, resulting in both oil and tiger peanut kernel residue. The residue is ground into powder and sieved through a 60-mesh sieve to obtain autoclave-treated tiger peanut residue powder (CLAV_PEA).

2.2 Chemical and physicochemical properties

2.2.1 Protein content analysis

Protein content was determined using the Kjeldahl method [3]. The crude protein content was calculated using a conversion factor of 6.25, as shown in the formula below:

$$Crude\ protein\ (\%) = \frac{(titration\ Sample - titration\ blank) \times 1.4 \times 6.25}{Sample\ Weight\ (grams)}$$

2.2.2 Fat content analysis

Fat extraction was performed using the Soxhlet extraction method [3], with hexane as the solvent. After solvent evaporation, the residue was dried at 105°C until a constant weight was obtained. The fat content was determined by the difference in weight using the following formula:

$$Crude\ fat\ (\%) = \frac{Fat\ weight\ after\ extraction\ (grams)}{Sample\ Weight\ (grams)} \times 100$$

2.2.3 Analysis of amino acid composition

The analysis of amino acid composition was carried out using the Amino acid Analyzer [4] model Hitachi - LA8080 AminoSAAYA. The reagents used were Ninhydrin Solution and Buffer for Amino acid Analyzer.

2.3 Functional properties

2.3.1 Analysis of foam properties

Foam property analysis [19] was performed by preparing a 30 ml sample solution (V0) with a sample-to-water ratio of 3:30 (w/v). The sample solution was transferred into a 50 ml volumetric flask. The solution was then stirred at 20,000 rpm for 1 minute using a homogenizer. The total volume was immediately measured after stirring (A1) and again after 30 minutes (A0). The values obtained were used to calculate Foam Expansion (FE) and Foam Stability (FS) using the following equations:

$$Foam\ Expansion\ (\%) = \frac{A1 - V0}{V0} \times 100$$

$$\textit{Foam Stability (\%)} = \frac{A0}{A1} \times 100$$

2.3.2 Analysis of emulsion properties

The analysis of emulsion properties [9] was conducted by dissolving a 4 g sample in 100 ml of distilled water. The sample solution was mixed with 10 ml of sample solvent and 2.5 ml of soybean oil. The emulsion was then prepared using a high-speed homogenizer at 10,000 rpm for 2 minutes. Fifty microliters of the emulsion were withdrawn from the bottom and diluted 100 times with a 0.5% (w/v) sodium dodecyl sulfate solution (SDS). The light absorbance at 500 nm was then measured using a spectrophotometer. The obtained values were used to calculate the Emulsion Activity Index (EAI) and Emulsion Stability Index (ESI) using the following equations:

$$EAI\ (\%) = \frac{2 \times 2.303 \times abs\ 0\ min \times 100}{protein\ concentration \times 0.25 \times 10{,}000} \times 100$$

$$ESI\ (min) = \frac{abs\ 0\ min}{abs\ 0\ min - abs\ 10\ min} \times 100$$

2.3.3 Analysis of water absorption capacity

The analysis of water absorption capacity (WAC) [2] involved preparing a sample solution by dissolving 1 grams of sample in 10 ml of distilled water. The mixture was stirred for 5 minutes and then homogenized at 5000 rpm for 30 minutes. The liquid portion was then transferred into a volumetric flask, and the solubility was calculated using the equation derived from this method:

$$WAC\ (\%) = \frac{Mass\ of\ absorbed\ water\ (grams)}{Initial\ water\ volume\ (ml)} \times 100$$

2.3.4 Analysis of solubility.

For the solubility analysis [22], a sample of 0.5 grams was dissolved in 25 ml of distilled water. The solution was stirred for 30 minutes and then homogenized at 3000 rpm for 10 minutes. The supernatant was then separated from the sediment and dried. The solubility was expressed as a percentage calculated using the equation derived from this method:

$$Solubility\ (\%) = \frac{weight\ of\ sample\ after\ baking\ (grams)}{Sample\ Weight\ (grams)} \times 100$$

2.4 Statistical analysis

Design of experiments using Completely Randomized Design (CRD). The analysis of variance (ANOVA) was calculated using the SPSS package and the mean differences between samples were compared using Duncan's New Multiple Range Test (DMRT) at a confidence level of 95%. The experiment was repeated 3 times. and specify the standard deviation.

3 RESULTS AND DISCUSSION

3.1 Chemical and physicochemical properties

Protein and Fat Content (Table 1) shows the differences between the processing methods. Analysis of the protein content in the three types of processed peanut residue powders—RAW_PEA, ROS_PEA, and CLAV_PEA—revealed that the protein content did not differ significantly among the three types ($p<0.05$). The protein content was 43.05±0.17%, 42.84±0.26%, and 44.79±1.46%, respectively. The study found that autoclaving was the most suitable processing method for preserving the nutritional quality of the samples.

Analysis of fat content revealed that ROS_PEA had the lowest residual fat content after oil extraction, with a fat content of 6.74±0.25%. CLAV_PEA followed with a fat content of 7.93±0.13%, and RAW_PEA had the highest residual fat content at 9.07±0.10%. These differences were statistically significant. During roasting, the temperature of the sample rises nearly 100°C at dry condition, approaching hot air temperature, depending on the process conditions. Dehydration during roasting occurs as soon as the evaporation temperature of water is reached. Due to the low moisture content of the peanuts used in the roasting process, typically between 4 to 6 grams per 100 grams of weight, the evaporation temperature increases rapidly, resulting in a decrease in the weight and moisture content of the sample over time at varying roasting temperatures [1]. The oil trapped within the cells of the peanuts may flow from one cell to another. However, during roasting, the flow of oil between cells increases the amount of substrate available for fat oxidation [21]. During roasting, the structure of the peanuts undergoes significant changes, with the extent of these changes varying depending on the hot air temperature, roasting time, and hot air flow [24]. Higher roasting temperatures significantly impair the stability of the fats. Generally, peanuts should be roasted at high temperatures but within a limited temperature range to enhance the formation of antioxidants and improve storage stability [21].

The analysis of amino acid composition in the tiger peanut residue powder, as shown in Table 2, revealed that RAW_PEA and ROS_PEA contain higher amounts of essential amino acids like Leucine, Isoleucine, and Valine compared to CLAV_PEA, although the differences were statistically significant ($p<0.05$). These amino acids are classified as BCAAs (Branched Chain Amino Acids), which aid in muscle building, especially Leucine, which helps increase protein synthesis and decrease protein degradation during muscle rest in humans. Additionally, during recovery from endurance exercise, BCAAs have been shown to affect muscle building in human muscles [5]. Therefore, these tiger peanut residue powder can be used as ingredients in food products..

Table 1. Protein and Fat content of tiger peanut residue powders.

Parameter	RAW_PEA	ROS_PEA	CLAV_PEA	Unit	*p* value
	Mean±SD	Mean±SD	Mean±SD		
Protein	43.05±0.17	42.84±0.26	44.79±1.46	g/100g	.061
Fat	9.07±0.10	6.74±0.25	7.93±0.13		<.001*

*$p<0.05$ (statistical significance).

Table 2. Composition of essential amino acids of tiger peanut residue powders.

Essential amino acids	RAW_PEA	ROS_PEA	CLAV_PEA	Unit	*p* value
Threonine	0.26	0.27	0.25	g/100g	<.001*
Valine	0.41	0.40	0.37		<.001*
Methionine	0.09	0.09	0.09		<.001*
Isoleucine	0.33	0.32	0.29		<.001*
Leucine	0.64	0.63	0.57		<.001*
Phenylalanine	0.51	0.50	0.45		<.001*
Tryptophan	ND	ND	ND		ND
Lysine	0.34	0.33	0.29		<.001*
Histidine	0.22	0.22	0.19		<.001*

*$p<0.05$ (statistical significance).

3.2 Functional properties

Functional properties such as solubility, water-holding capacity, emulsion activity, emulsion stability, and foaming capacity are very important in food processing and formulation. They significantly impact product quality and production efficiency.

The analysis of the water absorption capacity of the tiger peanut residue powder revealed no significant differences among the three types. ROS_PEA had the lowest value, followed by RAW_PEA and CLAV_PEA, with values of 32.73±1.27, 36.00±1.27, and 39.43±1.97, respectively, showing not significant difference.

Foaming capacity (FC) and foam stability (FS) primarily depend on the film-forming properties of proteins and their gas permeability. The analysis of the foaming properties and foam stability of the tiger peanut residue powder showed that CLAV_PEA had the lowest foaming capacity at 5.36±1.41%, while ROS_PEA had the highest at 14.72±1.01%. Generally, proteins that are absorbed quickly can foam better than those that are absorbed slowly [23]. Regarding foam stability, as shown in Table 1, the foam produced from CLAV_PEA had the highest foam stability at 97.81±1.41%. FC and FS are crucial variables in determining the functional properties of proteins. Fat is the main cause of foam instability in protein concentrates and isolates. It is evident that ROS_PEA showed a significantly increased foaming capacity compared to RAW_PEA. Additionally, the foaming properties of these proteins improve when the product is heated to 75-80°C. Although peanut proteins exhibit good foaming capacity after heating and fat extraction, their practical use remains limited due to structural instability. [11],[12]

From the analysis of the emulsifying properties of the peanut residue powders, it was found that RAW_PEA had the best emulsifying stability, followed by ROS_PEA and CLAV_PEA, respectively (Table 3). Changes in emulsifying activity may be due to the number of hydrophobic groups on the protein surface. High surface hydrophobicity enables proteins to interact with oil or fat, leading to

the formation of a strong protein film, which tends to become superior emulsifiers [23]. Emulsifying activity index (EAI) and emulsifying stability index (ESI) are important parameters influencing the selection of proteins for industrial processes. Proteins can reduce interfacial tension between water and oil and help prevent coalescence [16].

Two key functional properties of proteins in food are their ability to form and stabilize foams and emulsions by adsorbing at the air-water or oil-water interfaces. This reduces surface tension and stabilizes the interfacial film [20]. The emulsifying and foaming properties of proteins can be improved through appropriate chemical modification strategies [7]. A major limitation of chemical modification to enhance protein functionality is the nutritional safety of the protein derivatives. Enzyme modification is an attractive approach to improving the foaming and emulsifying properties of proteins. The most practical modifications are hydrolysis and polymerization reactions. Limited enzymatic hydrolysis of proteins using proteases can enhance these properties [10]. However, extensively hydrolyzed proteins produce highly unstable emulsions and foams [17].

Commercial peanut proteins exhibit reduced emulsifying properties compared to laboratory-produced proteins. Most commercial proteins have low solubility and relatively high surface hydrophobicity. High-pressure homogenization can break down large, insoluble aggregates in commercial peanut proteins, leading to a significant increase in protein solubility [6].

From the analysis of solubility of the three types of peanut residue powders, it was found that ROS_PEA had the highest solubility at 26.19%. This is because ROS_PEA contains the least amount of hydrophobic fat, resulting in the highest solubility.

Table 3. Functional properties of tiger peanut residue powders.

Parameter	RAW_PEA	ROS_PEA	CLAV_PEA	Unit	*p* value
	Mean±SD	Mean±SD	Mean±SD		
Water absorption capacity	36.00±1.27	32.73±1.27	39.43±1.97	g water g^{-1}	.110
Foam Expansion	7.69±1.03	14.72±1.01	5.36±1.41	g/100g	<.001*
Foam Stability	93.66±0.68	92.78±4.27	97.81±1.41	g/100g	.206
Emulsifying Activity Index	$3*10^{-4}$±0.00	$3*10^{-4}$±0.00	$4*10^{-4}$±0.00	m^2g^{-1}	.141
Emulsion Stability Index	5.52±0.58	3.22±0.47	1.57±0.80	g/100g	.002*
Solubility	18.44±0.64	26.19±0.72	18.59±1.22	g/100g	<.001*
Bulk density	0.49±0.15	0.49±0.23	0.56±0.17	g mL^{-1}	<.001*

*$p<0.05$ (statistical significance).

4 CONCLUSIONS

Processing affects the chemical composition and functional properties of tiger peanut residue powders. Roasting results in the lowest fat content as it alters the tiger peanut structure. Higher roasting temperatures reduce the weight and moisture of the samples. It is observed that processed tiger peanut residue powders have high protein content, which is not significantly different across samples, and low fat content (6.74-9.07 grams/100 grams). Roasting and autoclaving slightly decrease amino acid content. Comparing the processing methods, roasting yields a higher content of BCAAs compared to autoclaving, with significant differences. The functional properties of protein from tiger peanut residue powders are crucial in processing. After roasting, water absorption capacity and fat content decrease by 9.03% and 25.69%, respectively, while foam expansion and solubility increase by 91.42% and 42.03%, respectively, with statistically significant differences. From the analysis of the chemical composition and functional properties of tiger peanut residue powders, it is concluded that roasted tiger peanut residue powders have suitable chemical composition and functional properties for use as a food ingredient. The emulsion properties of the protein can be improved through appropriate chemical modification strategies. However, this study may be beneficial in improving the functional properties of peanut protein, potentially promoting its use in the food industry more widely.

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Effects of Ultrasound and High-Pressure Pretreatments Combined with Supercritical CO_2 Extraction on Oil Yield and Properties of House Cricket (*Acheta domesticus*)

BP-0229

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Keywords

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ABSTRACT

House crickets (*Acheta domesticus*) have emerged as a promising alternative source of high-quality proteins and oils with potential health benefits. This study investigated the effects of pretreatments (high-pressure processing and ultrasound) combined with supercritical CO_2 extraction (25 – 35 MPa, 30 min, 37 °C) on the yield and properties of house cricket oils. The extracted oils were analyzed for yield, peroxide value, acid value, thermal properties, and fatty acid profile. The results showed that the oil yield ranged from 36.23 to 102.82% compared to the initial oil content obtained using the Soxhlet method. The peroxide value and acid value of the extracted oils ranged from 1.11 to 21.18 mEqvO_2/kg and 3.80 to 78.98 mgKOH/g, respectively. Ultrasonic pretreatment at 37 kHz for 15 min altered the thermal properties of the extracted oils compared to the control. The extracted house cricket oil presented a wide range of melting peaks and crystallization peak, from -35.26 to 22.56 °C and -53.71 to 12.90 °C, respectively, which makes them liquid-like at room temperature. Ultrasound had effected on thermal properties that changed melting and crystallization of house cricket oils. The fatty acid profile of the extracted oils revealed a significant content of health-beneficial unsaturated fatty acids, such as oleic acid (ω-9) at 31.60 g/100 g and linoleic acid (ω-6) at 31.01 g/100 g.

1. INTRODUCTION

In recent years, the growing demand for alternative sources of protein and oils has been driven by concerns over the sustainability and safety of traditional meat proteins and plant oils [1,2].

Edible insects have emerged as a promising alternative, with over 2,000 species identified worldwide [3]. Among these, crickets have gained popularity due to their high protein (42.0-70.0% dry basis) and fat (9.71-29.1% dry basis) content [4,5]. Consequently, cricket proteins and oils have the potential to be incorporated into human diets and animal feeds. Ugar, A.E. et al. [6] reported that the major of fatty acids of cricket oil includes are omega-6 (30-40%), omega-9 (23-27%), palmitic (24-30%) and stearic (7-11%). Their content of potential oil as omega-3, omega-6, which can inhibit the production of inflammation-inducing prostaglandin hormones and shown potential of reducing the risk of coronary artery disease [7]. It has been found to benefit the health of mice and pigs [8]. However, to fully exploit this potential, it is crucial to understand the qualities, fatty acid compositions, and biological properties of cricket oils.

Pretreatment processes prior to oil extraction have gained increasing attention as a means to enhance extraction efficiency. Novel extraction techniques, such as high-pressure processing (HPP) and ultrasound (UL), have become popular due to their reported ability to improve oil extraction yield and reduce extraction time compared to conventional methods [9,10]. The combination of these high-potential technologies with supercritical CO_2 (SC-CO_2) extraction is of particular interest. HPP can disrupt lipid bilayers [11], while ultrasound generates cavitation, resulting in the breakdown of cell walls [12]. These mechanisms may enhance the efficiency of SC-CO_2 extraction.

The objective of this study was to extract oils from house crickets using SC-CO_2 extraction by comparing HPP and UL pretreatment processes. Oil yield, oil qualities, oil characteristics, and fatty acid composition of the extracted cricket oils were evaluated.

2. MATERIAL AND METHODS

2.1 Materials

House crickets (*Acheta domesticus*) were obtained from Thai Ento Food Company Limited, Thailand. The raw materials were cleaned using distilled water to remove extraneous materials and boiled at 100 °C for 5 min to reduce biocontamination. One kilogram of cleaned fresh cricket were packed in polyethylene 8 × 12 inch and stored at -18 °C until use. All reagents were of analytical grade and purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2 Pretreatment processes

UL pretreatment was performed using an ultrasonic bath (E100H, Elma, Germany). Cleaned fresh house cricket was thawed before UL pretreatment. Then, the samples were separated to three portions. Each 100 grams of house crickets were weighed, placed in a polyethylene bag (4x6 inches) and 50 ml of distilled water were added. The sample was then placed in the ultrasonic bath containing distilled water as the liquid medium. UL pretreatment was conducted at 37 kHz for 5, 10, and 15 min. The pretreatment temperature was maintained at 37 ± 1°C using an ice bath.

HPP pretreatment was carried out using a high-pressure machine (HPP600MPa/ 3-5L Pilot Scale; Baotou Kefa Inc., Baotou City, China) with a maximum operating pressure capacity of 600 MPa. Fifty grams of house crickets were placed in a polyethylene bag (4x6 inches) and then loaded into the 5 L high-pressure vessel containing distilled water as the hydrostatic fluid. HPP pretreatment was performed at pressures of 200, 400, and 600 MPa, at 37 ± 1°C for 5 min.

Non-pretreated house crickets were used as a control sample for comparing the oil extraction yield and oil quality with the pretreated samples. All samples were measured in trip After finished pretreatment processes, all samples were dried using hot air oven at 70 °C for 8-10 h.

2.3 Supercritical CO_2 extraction

SC-CO_2 extraction was performed using a pilot-scale SC-CO_2 extraction machine (SEF-01L, CAREDDI SCF, China). The extraction process was carried out in a stainless-steel container (1 L) at various pressures (25, 30, and 35 MPa) and a constant temperature of 37 ± 1°C for 30 min. The SC-CO_2 flow rate was maintained at 24-26 L/h. For each extraction, 100 g of pretreated or non-pretreated dried house cricket, milled and sieved through 18 mesh. House cricket powders was filled in the SC-CO_2 extractor chamber and sealed. The extraction time was initiated once the SC-CO_2 system reached the desired pressure. After the extraction process was completed, the extracted house cricket oils and defatted house cricket powder were collected. The oils were then centrifuged at 6000 rpm for 20 min to remove any residual liquid contaminants.

2.4 Oil extraction yield

The yield of house cricket oils was calculated using the following equation (1).

$$\text{Yield (\%)} = \frac{W_1}{W_2} \times 100 \qquad (1)$$

where W_1 is the total oil yield (g) of house cricket successive fraction by SC-CO_2 and W_2 is the weight of ground house cricket (g).

2.5 Oil quality

Peroxide value (PV) and acid value (AV) were used to indicate the quality of oils. The PV and AV of house cricket oils was determined using the acid-base titration method according to the AOAC (1990) [13]. All analysis was done in triplicates.

2.6 Oil characterization

Differential scanning calorimeters (DSC) (TA Instruments DSC 25; New Castle, DE, USA.) was used to analyze the crystallization and melting properties of house cricket oils, according to the modified procedure by Chou, T.H. et al. [14]. Ten milligrams of selected house cricket oils were weighted into an aluminium pan and sealed with aluminium lid. An empty aluminium pan was used as a reference. The DSC condition was set at a temperature range of -60 to 60 °C with a heating of 5 °C per min.

2.7 Fatty acid profile

Fatty acid profiles of selected house cricket oils were performed according to the procedure of AOAC (2012) 996.06 [15] using the GC-MS technique. Identification of peaks was done by comparison with relative retention times for the standard fatty acid methyl esters (FAMEs). Concentrations of each fatty acid were reported as g/100 g oil.

2.8 Statistical analysis

For statistical analysis, all samples were performed in triplicate. Three time of SC-CO_2 were performed for each pretreated sample. All data were analysed using the general linear model procedure in SPSS (IBM SPSS Statistics Ver. 26), are expressed as the mean ± SD. The $P<0.05$ was considered to indicate a statistically significant difference with Duncan's multiple range test.

3. RESULTS AND DISCUSSION

3.1 Oil extraction yield

Oil extraction yields of the control and pretreated (HPP or UL) house cricket obtained by SC-CO_2 extraction are presented in Figure 1. Among of all, UL15 combined SC-CO_2 300 MPa gave the highest of the yield that increasing oil extraction yield up to 102.82% Roselló-Soto E. et al. [16] reported the effect of UL for extracted oil that UL induces cavitation and shear forces. It can create pores on cell membrane of sample and oil can be easier released. The results of HPP combined SC-CO_2 extraction, it was expected that HPP would increase the extracted oil yield because of its ability on the cell wall disruption of samples [6]. In contrast, HPP had slightly effect on oil extraction yield. Some of them were a bit decreased from control. It was hypothesized that pressure might have disrupted the structures of triglycerides [6]. Kim, S.W. et al. [17] showed the oil yield of black soldier fly increased with pressure, from 16.0% at 15 MPa to 24.5% at 35 MPa, which might be due to the increasing oil solubility. Thus, more SC-CO_2 could penetrate into cells and leach more oil [6]. The pores and damage allow increased diffusion and mass transfer of intracellular oil components [18].

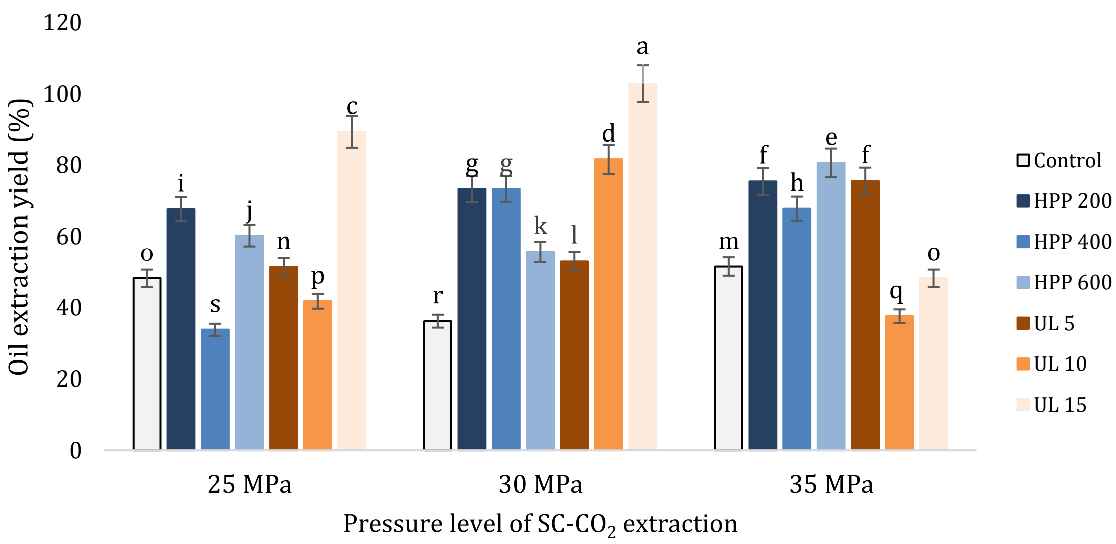

Figure 1. Oil extraction yield of pretreated house cricket using SC- CO_2 extraction. Different superscript letters of all samples were indicating statistical significance.

3.2 Peroxide and acid value of house cricket oils

Peroxide value of house cricket oil was evaluated and shown in Figure 2. A general rule is that the peroxide value of oils should not be above 10 mEqv.O_2/kg fat to avoid rancidity [19]. Only SC-

CO_2 oils extracted from all UL pretreated samples had the peroxide value below the standard, while most of the oils obtained from HPP pretreated samples possessed peroxide values above 10 mEqv.O_2/kg fat.

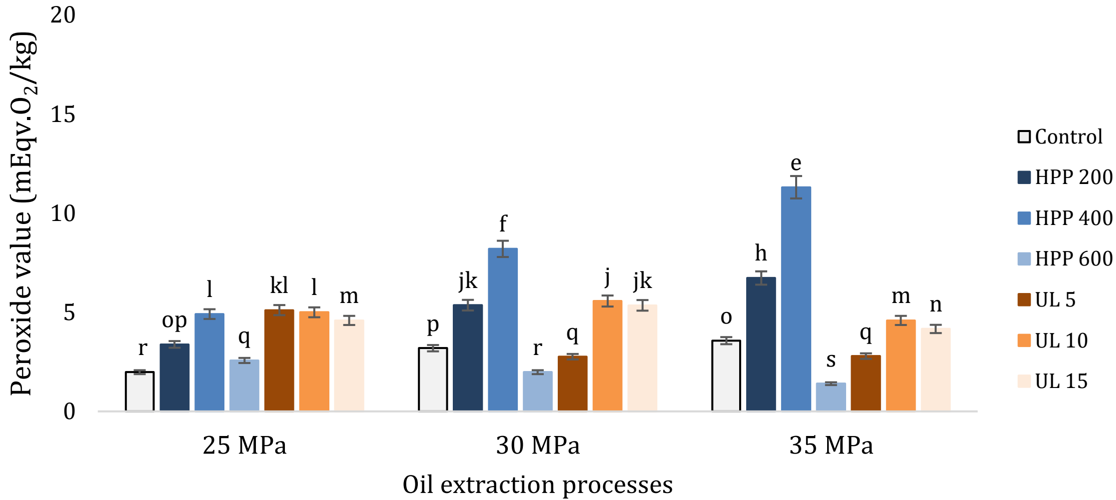

Figure 2. Peroxide value of pretreated house cricket oils obtained by SC-CO_2 extraction. Different superscript letters of all samples were indicating statistical significance.

HHP had a negative effect on the oxidation status of cricket oils. The pressure <300 MPa had a slight effect on lipid oxidation, but the oxidation increased above 300 MPa [6]. A combination of CO_2 and HPP can reduce lipid oxidation [20]. Increasing the pressure level can increase solvent solubility [21], which may explain why the 600 MPa HPP pretreatment resulted in lower lipid oxidation compared to the 400 MPa pretreatment. The higher pressure likely allowed for better CO_2 penetration into the samples, leading to more efficient extraction and reduced exposure of the lipids to oxidative conditions. On the other hand, Chemat et al. [22] reported that ultrasonic pretreatment might cause the release of free radicals in the sample, potentially contributing to an increase in peroxide value.

The acid value of house cricket pretreated using UL + SC-CO_2 decreased when increasing the pressure and extraction time (Figure 3). This result is similar to Kerras, H. et al. [23]. HPP 200 and 400 MPa, acid value was increased when increasing pressure of SC-CO_2. Wang, H. et al. [24] found that the pressure of 200 MPa enhanced lipase activity by 18%. After the researcher received the house cricket samples. House crickets were kept at -18 °C for a long time before being boiled, which may have promoted the lipid oxidation reaction. The effects of pressure on lipid oxidation depend on the food matrix and treating conditions including pressure level, holding time, etc. In addition, the unsaturated fatty acid in oil at a pressure of 400 MPa and above became more sensitive to lipid oxidation, and lipid membranes were changed [25].

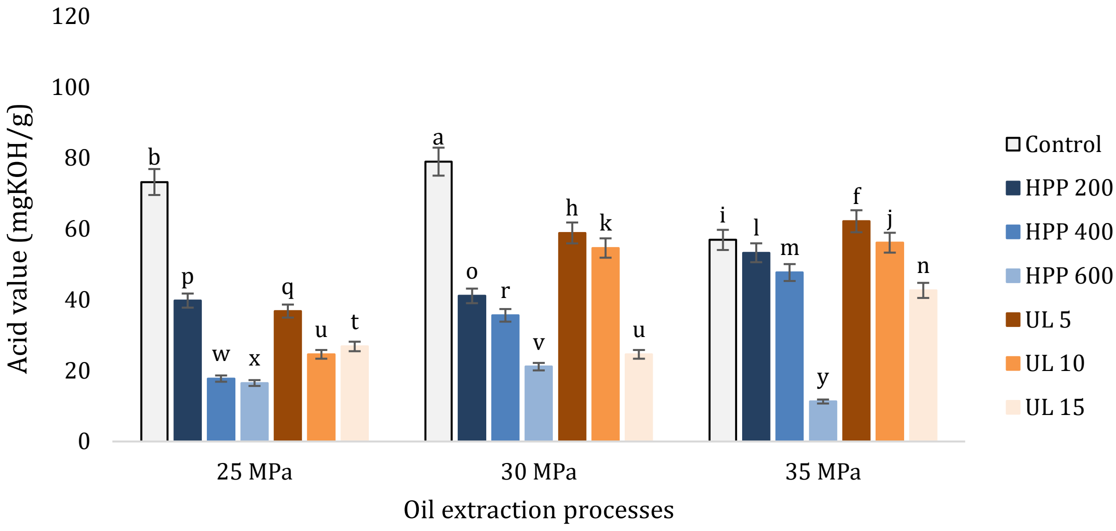

Figure 3. Acid value of pretreated house cricket oils obtained by SC-CO_2 extraction. Different superscript letters of all samples were indicating statistical significance.

3.3 Thermal properties of house cricket oils

After SC-CO_2 extraction, oil yield and oil quality were evaluated. UL15 combined SC-CO_2 at 30 MPa gave the highest oil yield and acceptable oil quality. House cricket oil pretreated by UL 37 kHz, for 15 min was determined for the melting and crystallization temperatures using DSC (Table 1 & Figure 4).

Table 1. Thermal properties of house cricket oils

Treatments	Peak	Crystallization (°C)	Melting point (°C)
Control	1	-53.71	-21.18
	2	-44.32	-14.56
	3	-18.37	-4.70
	4	3.38	2.71
	5	-	14.67
UL15	1	-22.97	-35.26
	2	-14.53	-15.10
	3	0.34	-0.93
	4	12.90	10.74
	5	19.81	22.52

* Control = house cricket oil control extracted using SC-CO_2 30 MPa, UL15 = house cricket oil pretreated by ultrasonic 37 kHz for 5 min and extracted oil using SC-CO_2 30 MPa.

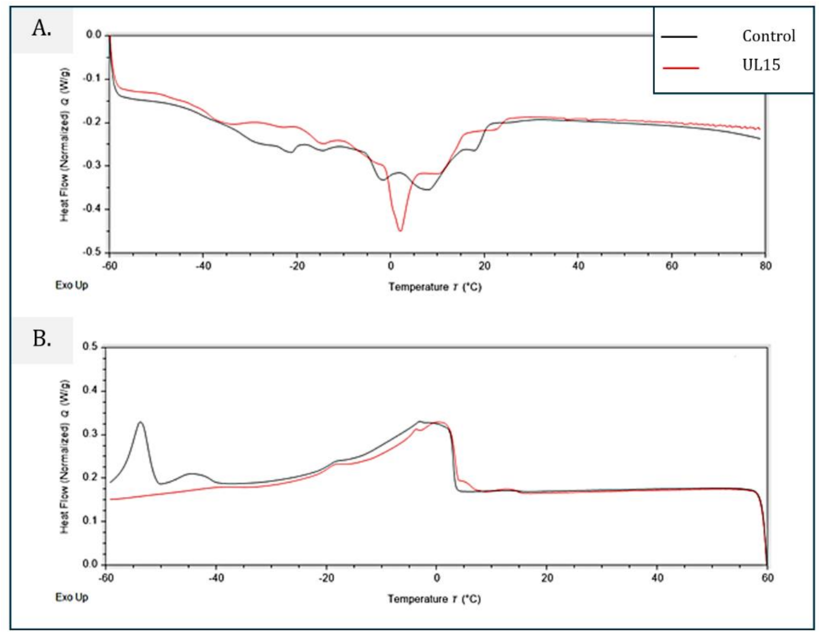

Figure 4. DSC thermogram of house cricket oil. (A.) crystal point and (B.) melting point.

House cricket oil pretreated by UL15 combined SC-CO_2 30 MPa was evaluated thermal properties and compared with control combined SC-CO_2 30 MPa. UL15 showed at least five crystal point that ranged from -22.97°C to 19.81 °C, for control was from -53.71 °C to 3.38 °C. Changing of crystallization points indicates that the effect by different of triacylglycerol in oil composition [26]. For melting point of UL15 was from -35.26 °C to 22.52 °C, control was from -21.18 °C to 14.67 °C. The fatty acid composition affected the thermal properties of oils, which has a high concentration of unsaturated fatty acids, has a low melting point [27]. Ugar, A.E. et al. [6] reported that house cricket oil extracted by hexane 1:15 w/v had crystallization and melting point at -2.26 and 1.11°C, respectively. It is different from this study because used difference oil extraction method.

3.4 Fatty acid profiles

The fatty acid of house cricket oil, subjected to UL 37 kHz, for 15 min combined SC-CO_2 at 35 MPa pressure, were analyzed and presented in **Table 2**.

Table 2. Fatty acid composition of UL15 combined SC-CO_2

Fatty acid compositions	Fatty acid content (g/ 100 g)
Oleic acid (C18:1c9)	31.44
Linoleic acid (C18:2)	30.83
Linolenic acid (C18:3)	0.84
Saturated fatty acid	35.83
Unsaturated fatty acid	64.18
Monounsaturated fatty acid	32.39
Polyunsaturated fatty acid	31.79
Tran fat	0.10
Omega 3	0.78
Omega 6	31.01
Omega 9	31.60

House cricket oil has high levels of omega-6 and omega-9 fatty acids. The fatty acid profiles of the house cricket oils also revealed significant amounts of C18: 1c9, and C18: 2, which are known for their health benefits. Additionally, all insect oils were found to contain high levels of omega-6 and omega-9 fatty acids, which play crucial roles in blood clotting, wound healing, immune system support, and cholesterol regulation [28,29]. This highlights the potential of insect oils as nutraceuticals.

4. CONCLUSIONS

This study demonstrated that ultrasound (UL) and high-pressure processing (HPP), can enhance oil extraction from house crickets using supercritical CO_2 (SC-CO_2) extraction. The combination of UL pretreatment for 15 min with SC-CO_2 extraction at 30 MPa proved to be the most suitable condition, yielding the highest oil extraction efficiency (102.82 %) while maintaining lower peroxide and acid values, indicating reduced lipid oxidation. The application of UL and HPP pretreatments in combination with SC-CO_2 extraction offers a promising approach for the efficient extraction and utilization of these valuable components from house crickets.

5. ACKNOWLEDGEMENTS

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Improvement of Tamarind Seed Flour by Pre-gelatinization Treatment

BP-P234

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Keywords

Tamarind seed flour
Pre-gelatinization
Boiling
Steaming
Superheated steam.

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ABSTRACT

This research aims to improve the properties of tamarind seed flour using pre-gelatinization treatment: boiling at 100 °C 20 min, steaming at 75 °C 10 min, and superheated steam treatment at 105 °C 30 min. The color value, total phenolic content, functional properties, and particle size distribution of pre-gelatinized flour were analyzed. The color difference (ΔE) values of all samples were greater than 5, suggesting that pre-gelatinization flour created a noticeable color difference compared to wheat flour. The total phenolic contents of superheated steam and steam modified flour gave the highest phenolic contents at 10.88 ± 0.92 mg GEA/g DW and 10.67 ± 0.03 mg GEA/g DW, respectively. The water absorption indices of all modified flours (3.22 - 3.37 g/ml) were significantly higher than of control (0.30 g/ml). The flour treated with boiling water gave the highest gelatinization degree at 80.37 ± 0.64 %, while the flour treated with superheated steam gave the highest viscosity at 1357.00 ± 1.73 cP. The superheated steam flour gave the smallest particle size of 190.08 ± 0.60 µm.

1. INTRODUCTION

Starch is an important major component in food. It plays a crucial role in providing energy to humans and animals [1] and is widely used as a gelling agent, thickening agent, and stabilizer in the food industry [2, 3]. However, the general structure of native starch limits its properties. This makes it difficult to use in practice. For example, native starch without gelatinization has a relatively low water absorption capacity and swelling power. Therefore, it is difficult to produce heat-resistant foods (such as foods containing colors, seasonings, vitamins, and bioactive substances) or low-temperature processed foods (such as instant puddings, chilled desserts, and baby food) due to its low water absorption capacity and low swelling power. Therefore, starch modification is required to improve its functional properties and make it suitable for specific applications [5].

Starch modification is usually achieved by chemical, physical, enzymatic, and genetic methods, or their combinations [6].

The physical method has attracted much attention because of its advantages, such as no chemical usage, environmental protection, safety, and simplicity [7, 8]. As a common physical modification method, pre-gelatinization can enhance the absorption and swelling power of starch in cold water. Therefore, pre-gelatinized starch can improve the system's viscosity and have a suitable thickening property [9, 10]. In addition, pre-gelatinized starch has better water retention capacity, making it very suitable to produce high moisture flour products for the elderly and children. Because it is a physically modified starch, pre-gelatinized starch is green, safe, and easier to accept. Therefore, the application of pre-gelatinized starch in wheat flour products has a good research prospect. The physicochemical properties of pre-gelatinized starch obtained by different drying methods are different, so do their influences on the flour products. In order to provide a theoretical basis for the application of pre-gelatinized starch in wheat flour products, the physical and chemical properties of pre-gelatinized starch and its application in wheat flour products were reviewed in Ma et al. [5]. Physical treatments such as moist heat, conventional air ovens or other treatments are applied to change the physicochemical, thermal, structural, and functional characteristics of starch [11]. Generally, thermal processing leads to the loss of bioactive phytochemicals, but this is not always the case. The effect of thermal processing on the quality of foods depends on processing methods, moisture content, oxygen, source of the food, and thermal history [12].

Soaking and boiling can improve the digestibility of plant seeds by reducing anti-nutritional factors and the hard-to-digest phenomena. Pre-treatments affect the particle size distribution in flours, as boiling causes the flour particles to aggregate (> 1000 μm) due to the gelatinization of starch [13]. The superheated steam (SHS) treatment is an effective and novel thermal processing technology widely employed in food processing [14]. For instance, it has numerous noticeable advantages over traditional methods, such as reduced processing time, undesirable odour, higher enthalpy, energy efficiency, heating rate, palatability, acceptability, and retain nutritional and techno-functional properties [15, 16, 17]. Moreover, SHS is produced by secondary heating, and the heat is transferred through condensation of steam on the food surface, thereby increasing food temperature in less time, and positively correlated with the contact surface area, pressure difference, and flow pattern of steam [18, 19]. SHS retains the nutrients and improves the functional, pasting, and thermal properties of several food materials (wheat, kejirak, rice, and buckwheat. Similarly, Hu et al. [20] found that the SHS treatment (110 – 170 °C, 1 – 3 min) reduced the gelatinization temperatures and increased the viscosities of wheat flour. Kim et al. [21] revealed that the SHS treatment (250 °C, 10 min) increased carbohydrate content, water absorption capacity, and reduced gelatinization temperatures of rice flour. Likewise, Shaharuddin et al. [22] reported an increase in physicochemical properties (total phenolic content, total flavonoid content, antioxidant activity) in kejirak after SHS treatment (170 °C, 15 min). As observed from the literature, the primary focus of previous studies was improving cereal's functional, thermal, and pasting properties.

Tamarind (*Tamarindus indica* L.) is a tall tree that can grow up to 30 meters height. This tree is known for its fruit pulp, which is rich in vitamin C, tartaric acid, malic acid, and sugar [23]. Tamarind has been planted and harvested extensively in Bangladesh, India, Myanmar, Malaysia, Sri Lanka, Thailand, Australia, and several African, Central American, and South American countries [23]. Tamarind seed is an available by-product of tamarind pulp industry, containing as high as 72 % w/w of polysaccharides [24], which is composed of β-(1,4)-D-glucan backbone substituted with side chains of α-(1,4)-D-xylopyranose and (1,6) linked (β-D-galactopyranosyl-(1,2)-α-D-xylopyranosyl) to glucose residues, where glucose, xylose and galactose units are present in the ratio of 2.8 : 2.25 : 1.0 as the

monomer units [25] and with a molecular weight of 720-880 kDa [24]. Moreover, the nutrient value of food can be changed by the way it is processed, cooked, and stored. Some food processing methods (soaking, boiling, roasting, blanching, autoclaving and fermentation) can enhance quality of processed foods through detoxification of anti-nutrients, flavor and color development, among others [26]. Previous studies had reported the use of soaking, boiling, roasting, germination and fermentation to produce detoxified flours from tamarind nuts [27, 26]. However, information on the use of autoclaving in processing of tamarind seeds and pasting properties of processed flours from the seeds based on previous methods are limited [28].

The objective of this research is to study the improvement of the quality of tamarind seed flour by pre-gelatinization method, in terms of color changes, total phenolic content, functional properties and particle size distribution of tamarind seed flour. This information will serve as a basis for further development of food products.

2. MATERIAL AND METHODS

2.1 *Raw flour preparation*

Local sour tamarind seeds from Grandpa and Grandma's Sufficiency Farming Shop, Thailand are used as raw materials for producing raw flour. The preparation method was adapted from the method of Sun et al. [29]. The tamarind seed batter was prepared by roasting the tamarind seeds in a metal pan with direct flame at 140 °C for 15 min and allowing the roasted tamarind seeds to cool down. The roasted seeds were shell cracked with a pestle, washed with water thoroughly, and dried at 60 °C for 6 hours in a hot air oven. The dried seeds were then finely ground, sieved, packed into plastic bags, and kept in a desiccator for further modification. The received tamarind seed flour was used as a control sample and as a material for steaming, and superheated steam treatments.

2.2 *Pre-gelatinization treatment*

2.2.1 *Boiling treatment*

The unshelled tamarind seeds were prepared by roasting the tamarind seeds in a metal pan with direct flame at 140 °C for 15 min and allowing the roasted tamarind seeds to cool down. The roasted seeds were shell cracked with a pestle. The unshelled tamarind seeds and water at a ratio of 1 : 5 (w/v) were boiled at 100 °C for 20 min, filtered, air-dried at room temperature for 1 hr, dried at 60 °C for 6 hours in a hot air oven, finely ground, sieved, packed into plastic bags, and kept in a desiccator until used (adapted from the method of Sun et al. [29]).

2.2.2 *Steaming treatment*

Raw flour from 2.1 wrapped in cheesecloth was placed in a steamer which gave the inner temperature of the sample at 75 °C for 10 min, let it stand at room temperature for 1 hr, dried at 60 °C for 6 hr in a hot air oven, finely ground, sieved, packed into plastic bags, and kept in a desiccator until used (adapted from the method of Sun et al. [29].

2.2.3 Superheated steam treatment

Raw flour from 2.1 was mixed with adequate amount of distilled water, kept overnight at 4 °C, placed in a hot air oven at 60 °C, then measured the moisture content of flour to achieve a final moisture content of 20 %. The obtained flour was filled in a Duran bottle, autoclaved at 105 °C (0.2 kg/cm^2 pressure) 30 min, dried at 60 °C for 6 hr in a hot air oven, finely ground, sieved, packed into plastic bags, and kept in a desiccator until used (adapted from the method of Lim et al. [30]).

2.3 Analysis

A Hunter Lab Spectrophotometer (Hunter Lab, Mini Scan XE Plus, and Reston, Virginia, USA) was used to measure the CIE color values of the flour samples. The instrument was calibrated with a standard white plate (L* = 97.21; b* = 0.14; a* = 1.99). The L*, a* and b* were obtained directly from the spectrophotometer. Where lightness (L*) quantifies the sample on a black (0) to white (100) spectrum. The a* denotes redness (+) to greenness (−), while the b* gauges the yellowness (+) to blueness (−) colors. ΔE was calculated based on the color of wheat flour as a reference (L* = 93.00; a* = -0.70; b* = 12.20) using Equation (1) according to the modified method of Carvalho et al. [31].

$$\Delta E = \sqrt{\Delta L *^2 + \Delta a *^2 + \Delta b *^2} \quad (1)$$

The total phenolic content was evaluated using the Folin-Ciocalteu colorimetric method. The 0.3 ml of the extract (1 g of sample mixed with 10 ml 80 % methanol, placed in the dark for 15 min, centrifuged) from tamarind seeds powder was mixed with 1.5 ml of freshly diluted Folin Ciocalteu phenol reagent (10 % v/v) and 1.2 ml of 7.5 % sodium carbonate. The mixture was then kept in the dark for 2 hours to complete the reaction (the solution will turn blue green). The absorbance was measured at 765 nm using a spectrophotometer (UV-VIS Spectrophotometer, UV-1900i, SHIMADZU). Gallic acid was used as the standard for calibration, and TPC was reported in milligrams of gallic acid equivalent (GAE) per gram of dry weight (adapted from the method of Akomeng et al. [32]).

To determine the solubility index, supernatants (weigh 2.5 g sample in 30 ml distilled water, centrifuge at 2,200 rpm 15 min) were transferred into moisture can (pre-weighed) and dried in an air oven at 105 °C for 4 hours and the weight of the residue was measured. The water solubility index was calculated using the number of dried solids obtained by evaporating the supernatant. Water solubility index presented as gram dried solids in 100 g of sample on a dry weight basis according to Equation (2) using the method of Hettiarachchi et al. [33]. For the inclined tube, weigh the remaining part of the tube and calculate the water absorption index according to Equation (3) using the method of Gu et al. [34]:

$$Water\ solubility\ index\ (\%) = \frac{dry\ supernantant\ weight}{dry\ sample\ weight}\ x\ 100 \quad (2)$$

$$Water\ absorption\ index\ (g/g) = \frac{wet\ sediment}{dry\ sample\ weight}\ x\ 100 \quad (3)$$

The level of gelatinization was calculated by utilizing the principle of interaction between amylose and iodine solution which results in a blue-color substance. The standard curve of the relationship between the gelatinization degree (X) and the light absorption value (Y) was created using ungelatinized flour as the 0 % gelatinization degree and completely gelatinized flour as the 100 % gelatinization degree. The 0.2 g flour sample, 98 ml distilled water, and 2 ml 10 M KOH solution were mixed, gently agitated for 5 min, and centrifuged. The 1 ml supernatant were added with 0.4 ml 0.5 M HCl, and the mixture was made up to 10 ml volume with distilled water. The 0.1 ml iodine reagent (1 g iodine and 4 g potassium iodide per 100 ml water) were then added, mixed thoroughly, measured the absorbance at 620 nm (adapted from the method of Birch et al. [35]).

Apparent viscosity of the sample solution was evaluated by dissolving 16 g sample in 200 ml water and boiling until the sample was completely dissolved. The cooled starch slurry was then measured the viscosity using a Brookfield Viscometer (Brookfield AMETEK LV DV2T Viscometer) at 50 rpm.

The particle size distribution of the flour was analysed using a laser diffraction technique with a dry feed unit from SYMPATEC model HELOS (Germany), The values of X 10, X50, and X90 represent the average particle size (µm) calculated from two repeated measurements (adapted from the method of Sammalisto et al. [36]).

2.4 Statistical analysis

An analysis of variance (ANOVA) was performed. The mean comparison was carried out by Duncan's Multiple Range Tests. The significance of difference was defined at $p \leq 0.05$ The analysis was performed using an SPSS package (Version 16).

3 RESULTS AND DISCUSSION

3.1 CIE color

The color values of pre-gelatinized starch are shown in Table 1. The results showed that different modification methods significantly affected the L*, a* and b* values of the starch ($p \leq 0.05$). Starch processed by superheated steam and steam tended to have a decreased brightness value (L*), while the a* and b* values tended to increase compared to the control sample. In general, the heat treatment caused a slight, although significant, decrease of lightness (L* values), indicating that the color of the treated samples became darker [37]. In addition, thermal treatment induced non-enzymatic browning which led to the formation of a brown color [38]. This browning reaction was enhanced by the increased temperature level and heating time [39], thus the color of pre-gelatinized flour tended to become darker with increasing pre-gelatinized temperature. The color of pre-gelatinized tamarind seed flours could be differentiated from wheat flour with visual inspection concluded by ΔE stating that the ΔE values were greater than 5 ($\Delta E > 5$) [40]. The natural color of tamarind seed and heating process for flour preparation caused tamarind seed flours to have light brown color. The change of color in heated samples is attributed to Maillard reaction [41].

Table 1. CIE color

Pre-gelatinization treatment	***L****	***a****	***b****	***ΔE***
Control	41.08 ± 0.01[b]	0.22 ± 0.01[c]	11.00 ± 0.00[c]	51.94 ± 0.01[c]
Boiling water 100 °C, 20 min	42.72 ± 0.00[a]	-0.50 ± 0.01[d]	8.60 ± 0.01[d]	50.41 ± 0.00[d]
Steam 75 °C, 10 min	40.57 ± 0.01[c]	0.81 ± 0.02[b]	11.78 ± 0.02[a]	52.45 ± 0.01[b]
Superheated steam 105 °C, 30 min	39.53 ± 0.01[d]	0.86 ± 0.01[a]	11.63 ± 0.02[b]	53.50 ± 0.01[a]

*Values expressed mean ± standard deviation. Data of different alphabets in the same column were different with statistical significance ($p \leq 0.05$).

3.2 Total phenolic content

From Table 2, the total phenolic content of all modified flours significantly increased compared to control ($p \leq 0.05$) which superheated steam and steam modified flour gave the highest phenolic contents at 10.88 ± 0.92 mg GEA/g DW and 10.67 ± 0.03 mg GEA/g DW, respectively. A similar result was reported by Locali-Pereira et al. [13] that the flour produced with raw seeds sample had the highest total soluble phenolic compound content (7.2 mg GAE/g), which is in the range of values reported for dried beans [42]. Soaking reduced the content of total soluble phenolic compounds by 16 %, whereas boiling treatment led to further reduction (57 %) of total soluble phenolic compound content in flour, this may result from the leaching of phenolic compounds during soaking, as well as from degradation of these compounds by high temperature during the boiling treatment, Leaching of phenolic compounds during soaking depends on the rate of water absorption, coat permeability, hilum size and cotyledon composition [13]. Wang et al. [43] claimed that thermal treatments could weaken the constraints of the cell wall matrix, promoting the release of bound phenolics. The same result was also observed by Drawbridge et al. [44] that boiling treatment significantly increased the extractability of bound phenolics in four types of boiled Canadian barley due to the breakdown of structural components and loosening of grain matrix. Hatamian et al. [45] also found that roasting significantly increased ($p \leq 0.05$) the total phenolic content in samples probably due to the effect of heat on the cell wall and breakdown of bonds between phenolic compounds and proteins, thereby increasing the total phenolic content in samples. Roasting time from 15 to 25 min significantly increased ($p \leq 0.05$) antioxidant activities of samples. The formation of Maillard reaction products in foods facilitates bioactivity in vitro that may augment the phenolics and flavonoids [46]. The total phenolic content of pre-gelatinized tamarind seed flour was comparable to those of wheat flour (6.96 ± 0.1 mg/g), amaranth flour (2.7 ± 0.1 mg/g), buckwheat flour (7.25 ± 0.2 mg/g), and quinoa flour (2.8 ± 0.1 mg/g) reported by Chlopicka et al. [47].

Table 2. Total phenolic content

Pre-gelatinization treatment	Total phenolic content (mg GEA/g DW)
Control	4.31 ± 0.03[c]
Boiling water 100 °C, 20 min	6.50 ± 0.02[b]
Steam 75 °C, 10 min	10.67 ± 0.03[a]
Superheated steam 105 °C, 30 min	10.88 ± 0.92[a]

*Values expressed mean ± standard deviation. Data of different alphabets in the same column were different with statistical significance ($p \leq 0.05$).

3.3 Functional properties

The water solubility index of the modified flour samples increased slightly but did not significantly differ ($p > 0.05$) compared to the control flour, which could be related to the non-starch components as claimed by Yu et al. [48]. The study of Dudu et al. [49] had an adverse effect as the solubility of flour was increased by modification with steam, heat, and moisture. The increased solubility may be a result of shrinkage or breakage of the starch granules, which may weaken the amylose-amylopectin bond, this, in turn, increases the interaction between amylose and water [50, 49].

As shown in Table 3, the water absorption indices of the modified samples were higher than of control ($p \leq 0.05$). This may be caused by the starch granules being destroyed during heating, as a result, the flour sample has a porous structure [7]. Pre-gelatinized starch has better water-retention capacity which is suitable to add a proper amount of pre-gelatinized starch in bread production for improving the water retention of bread, thus reducing the hardness and increasing the cohesion of breadcrumbs as reported by Ma et al. [51]. Similar result was reported by Keppler et al. [52] that heat treatment significantly increased the water retention capacity. These results might be ascribed to the interaction between water and polar groups of carbohydrates, in addition, other polar groups present inside flour could also increase the water absorption indexes. However, the primary reason for the increase of water absorption indexes was regarded as the changes of starch, since flour contained about 85 % component in starch [53, 54]. Anuntagool et al. [55] reported that the solubility of whole jack bean flour and jack bean seed kernel flour were 17.3 g/100 g sample and 16.4 g/100 g sample, respectively, at 75 °C and slightly changed to 15.9 g/100 g sample and 17.2 g/100 g sample, respectively, at 95 °C, while the starch sample had 1.1 g/100 g sample solubility at 75 °C which increased to 24.5 g/100 g sample solubility at 95 °C. Starch granules are insoluble and have limited water absorption ability at a temperature below its gelatinization temperature, As the temperature increases above its gelatinization temperature, starch granules rupture, releasing amylose into the solution and increasing the solubility.

The flours modified by boiling water gave the highest gelatinization degree at 80.37 ± 0.64 %. This was because water made starch granules swell more quickly and gelatinize more easily [55].

Van Steertegem et al. [57] states that the flour subjected to prolonged and intense heat treatment led to the cross-linking of amylose and amylopectin in the gluten protein. Polymer proteins will make the starch particles harder and help them expand longer, resistant to external forces, increasing the elasticity of the starch after processing. Table 3 reveals a significant variation in

viscosity among all types of flour tested ($p \leq 0.05$). The maximum viscosity obtained from superheated steam flour at 1357.00 ± 1.73 cP.

Table 3. Functional properties

Pre-gelatinization treatment	Water Solubility index^{ns} (%)	Water absorption index (g/ml.)	Gelatinization degree (%)	Apparent viscosity (cP)
Control	12.20 ± 0.28	0.30 ± 0.10^{b}	58.89 ± 1.11^{c}	50.89 ± 0.29^{d}
Boiling water 100 °C, 20 min	13.00 ± 2.55	3.22 ± 0.30^{a}	80.37 ± 0.64^{a}	208.20 ± 9.24^{c}
Steam 75 °C, 10 min	13.40 ± 0.28	3.37 ± 0.15^{a}	58.89 ± 1.11^{c}	390.00 ± 3.12^{b}
Superheated steam 105 °C, 30 min	13.40 ± 1.41	3.25 ± 0.09^{a}	69.26 ± 0.64^{b}	1357.00 ± 1.73^{a}

*Values expressed mean ± standard deviation. Data of different alphabets in the same column were different with statistical significance ($p \leq 0.05$).

3.4 Particle size distribution

Figure 1 shows that the particle size distribution of flours presented a clear bimodal distribution. Similar results were reported by Zhang et al. [58], with the lower peak representing free starch granule fraction and the higher peak representing cell clusters fraction which contained packed starch aggregates [59]. Overall, the volume proportion of free starch granules was less than that of starch aggregates.

Table 4 shows the particle size distribution levels of pre-gelatinized flours, including the particle sizes X10, X50, and X90. It is observed that the particle size distribution of the flours changed with modification methods. The modified flours have a higher particle size distribution than the control flour, and at 10 % to 90 % showed a decreasing trend. The superheated steam flour has the smallest particle size of 190.08 ± 0.60 µm. The possible reason was that the superheated steam might soften and destroy the cell wall [60]. More starch granules were liberated from intact cells due to the superheated steam, thus reducing the average particle size of flour [61]. Flours with smaller particles sizes absorb more water, have lower gelatinization temperature and higher viscosity [62]. Lin et al. [63] reported that the decrease in particle size could improve the gluten network of whole wheat flour dough, resulting in an increase in gas retention during proofing. Liu et al. [64] found that decreasing particle size of whole-wheat flour could improve the breaking force and extensibility of tortilla, suggesting an improvement of the tortilla quality, all these studies indicated that particle size of flour plays an important role in the structure and quality of products.

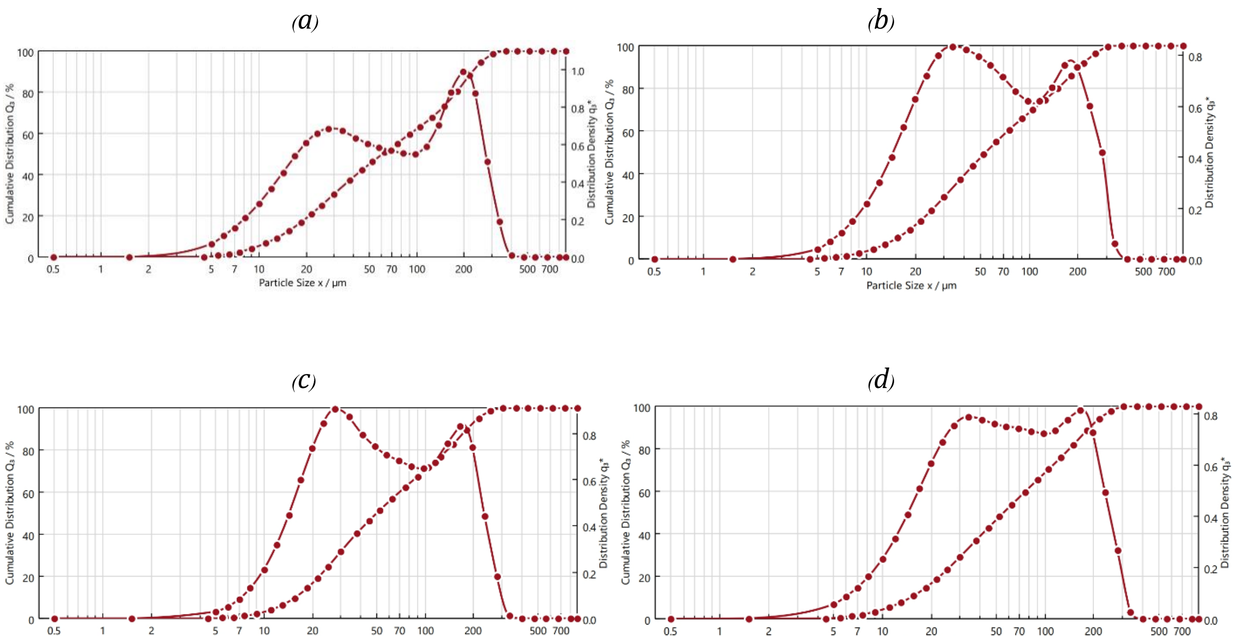

Figure 1. The Particle size distribution of pre-gelatinized tamarind flours. (a)control, (b) boiling water, (c) steam, and (d) superheated steam.

Table 4. Particle size distribution

Pre-gelatinization treatment	Particle size (X10/µm)	Particle size (X50/µm)	Particle size (X90/µm)
Control	13.63 ± 0.15^{c}	61.92 ± 1.39^{a}	228.21 ± 0.93^{a}
Boiling water 100 °C, 20 mins	15.69 ± 0.09^{a}	53.44 ± 1.13^{bc}	201.34 ± 4.26^{b}
Steam 75 °C, 10 mins	15.72 ± 0.08^{a}	50.83 ± 0.90^{c}	190.61 ± 10.77^{b}
Superheated steam 105 °C, 30 mins	14.77 ± 0.18^{b}	55.68 ± 0.40^{b}	190.08 ± 0.60^{b}

*Values expressed mean ± standard deviation. Data of different alphabets in the same column were different with statistical significance ($p \leq 0.05$).

4 CONCLUSIONS

The tamarind flour that has undergone pre-gelatinization improves the properties of the flour in terms of total phenolic content, viscosity, and particle size distribution. it is recommended to use pre-gelatinized tamarind seed flour as a functional food ingredient and as a stabilizer for future food products.

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Effects of maltodextrin and air inlet temperature on the properties of spray-dried Chrysanthemum juice powder

BP-P239

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ABSTRACT

Chrysanthemum juice is considered as a healthy drink that quenches thirst and helps prevent and relieve various diseases. The major problem of chrysanthemum juice is the short shelf-life. This study was therefore aimed to prolong the shelf-life using spray drying process. The process variables were maltodextrin content at 20%, 25% and 30% w/w as well as air inlet temperature at 180, 190, and 200°C. The process condition delivered the Chrysanthemum juice powder at the product yields of 12.70 - 20.18%, the moisture content from 3.47% to 0.56%, the water activity less than 0.6, and wettability from 590 to 92 seconds. An increase in air inlet temperature decreased the bulk density but did not significantly affect the solubility of Chrysanthemum juice powder ($p>0.05$). The spray-dried product was later reconstituted using water at the same amount of evaporated water. Increases of maltodextrin content and air inlet temperature decreased total phenolic content but had no effect on DPPH radical scavenging activity. The lowest DPPH radical scavenging activity of reconstituted Chrysanthemum juice was derived from 200°C air inlet temperature and 30% w/w maltodextrin spray drying condition. The suitable condition to produce spray- dried Chrysanthemum juice powder was at 180°C air inlet temperature and 20% w/w maltodextrin due to the highest wettability of powder, and the highest b* value and total phenolic content of reconstituted juice.

1. INTRODUCTION

Chrysanthemum, also known as the Garden Mum, is a medicinal herb native to China and Japan. Its distinctive characteristics include a pungent smell, bitter and sweet taste, and cooling properties. It is widely used in traditional medicine in China and other East Asian countries for use as an ingredient in food products and consumed in the form of beverages [1].

The Chrysanthemum flower part is more commonly used than the leaves, roots, and young shoots. The dried bloom Chrysanthemum flower is boiled with hot water to give beverage that help quenching thirst. Furthermore, it is beneficial for health as Chrysanthemum tea contain antioxidant compounds such as flavonoids, chrysanthemin, adenine, stachydrine, choline, amino acids, and essential oils which act as antioxidant, inflammation suppression, coronary heart disease prevention, blood vessel expansion, and heart failure reduction [2]. It is also used as treatment for bleary-eyed symptoms [3,4] and AIDS patients [5]. However, Chrysanthemum juice has a short shelf-life at room temperature, developing Chrysanthemum juice in the form of powdered drinks is another option to extend the shelf life, value added, easy to transport, and easy to use for consumer.

Spray drying is a production process that transforms food from a liquid state into a dry particle form [6]. Tonon et al. [7] studied the effects of drying process on the physicochemical properties of mulberry powder juice. It was found that increasing the inlet air temperature affects the moisture content, water activity value, and total phenolic content of the powder. Quek et al. [8] reported that the moisture content of watermelon powder decreases when the inlet air temperature increases. Abadio et al. [9] reported that increasing the air inlet temperature resulted in a decrease in powder density. However, spray dried powder faced flavor loss and clogging after storage. Food additives can be added to the liquid before processing to help trapping flavor and improve physicochemical properties of powder [10].

Maltodextrin is a polysaccharide derived from hydrolyzed starch. It is a white powder with no taste or odor, and it dissolves well in water [11]. Maltodextrin is commonly used in dried foods because it is cheap and helps improve the physicochemical properties of powders. Kha [12] found that an increase in maltodextrin content affects the moisture content, water activity, density, and total phenolic content of baby jackfruit powder. This is consistent with the research of Bae & Lee [13] which reported an increase in maltodextrin content resulted in a decrease in density, but an increase in wettability of avocado powder. Koca et al. [14] reported that maltodextrin-added cheese powder had the highest melting index and lowest coagulation index compared to control cheese powder and whey-added cheese powder. Mishra et al. [15] found that increases in maltodextrin content and air inlet temperature resulted in decreases in moisture content, water activity, and total phenolic content of Indian gooseberry powder.

The objective of this study is to investigate the effects of maltodextrin and air inlet temperature on the physicochemical properties of Chrysanthemum juice powder. The color value and antioxidant activity of reconstituted Chrysanthemum juice are analyzed.

2. MATERIAL AND METHODS

2.1 Chrysanthemum juice preparation

To 36-liter 95°C water, 154 grams (0.43% w/w) of dried Chrysanthemum flowers were added, heated for 5 min, filtered using cheesecloth, cooled to room temperature.

2.2 Spray drying process

The maltodextrin (MD) DE10 was added to Chrysanthemum juice at 20%, 25%, and 30% w/w to adjust the total soluble solid of juice, dissolved at 70-80°C, then cooled to room temperature. The total soluble solid was measured using a hand refractometer. The samples were subjected to a counter-current spray drying process using pressure nozzle with 180, 190, and 200°C air inlet temperature and 70-95°C air outlet temperature. The process condition was 25 Hz sample feed rate, 250 psi, and 42.50 Hz blower power. The Chrysanthemum juice powder was weighed and calculated % yield as

$$\%Yield = \frac{\text{powder weight}}{\text{raw material weight}} \times 100 \quad (1)$$

The samples were kept in a sealed container and stored in a dark place before analyzed.

2.3 Analytical methods

2.3.1 Chrysanthemum juice powder

2.3.1.1 CIE color value

The color value was measured using a TRI-STIMULUS COLORIMETER, JC 801, Japan. The color system obtained is the Hunter color system, consisting of 3 variables: L* for lightness ranging from 0 (black) to 100 (white), a* for indicating red (a+) to green (a-), and b* for indicating yellow (b+) to blue (b-).

2.3.1.2 Moisture content

The samples were analyzed using the modified method of AOAC. The 1g sample was weight in the moisture can and placed in a 105°C hot air oven for 4 hr or until a constant weight gained. The moisture content was calculated as

$$\%Moisture\ content = \frac{\text{weight of sample before baking} - \text{weight of sample after baking}}{\text{weight of sample before baking}} \times 100 \quad (2)$$

2.3.1.3 Water activity

The 3g samples were analyzed using AquaLab Series 3 TE machine.

2.3.1.4 Bulk density

The samples were analyzed using the modified method of Jangam & Thorat [16]. The powder was filled up in the measuring cylinder to reach 4 ml volume, then the cylinder was tapped 200 times. The bulk density was calculated as

$$Density\ (g/cm^3) = \frac{\text{mass of powder}}{\text{Volume of powder in measuring cylinder}} \times 100 \quad (3)$$

2.3.1.5 Wettability

The wettability was analyzed using the modified method of Vissotto et al. [17]. The 1g samples were poured into 400 ml of 25°C distilled water, then the time at all the powder was completely sunk under water was recorded.

2.3.1.6 Solubility

To 10 ml of 25°C water in centrifuge tube, the 1g sample was added, dissolved, and then centrifuged at 3000 rpm for 10 minutes. The supernatant was weighed in moisture can and placed in hot air oven at 105°C 24 hours. % Solubility was calculated as

$$\%\ solubility = \frac{\text{gram of soluble solids in supernatant}}{\text{gram of samples}} \quad (4)$$

2.3.2 Reconstituted Chrysanthemum Juice

The reconstituted Chrysanthemum juice was prepared based on soluble solid content in the juice equal to the original juice.

2.3.2.1 CIE color value

The color value was measured using a TRI-STIMULUS COLORIMETER, JC 801, Japan. The color system obtained is the Hunter color system, consisting of 3 variables: L* for lightness ranging from 0 (black) to 100 (white), a* for indicating red (a+) to green (a-), and b* for indicating yellow (b+) to blue (b-).

2.3.2.2 Total phenolic content

The total phenolic content was analyzed using the modified method of Yuan et al. [18]. To 200 µl of Folin-Ciocalteau reagent, 200 µl of reconstituted Chrysanthemum juice was added, mixed thoroughly, let it stand for 5 min, and then 7 ml of sodium carbonate was added and mixed thoroughly, placed in the dark for 60 min at room temperature. The absorbance at 765 nm was measured using a Spectrophotometer (RAYLEIGH, VIS723G). The gallic acid was used as a standard and the samples were reported as the concentration in gallic acid equivalent (GAE) per gram of sample.

2.3.2.3 DPPH radical scavenging assay

DPPH radical scavenging activity was analyzed using modified method of Yuan et al. [18]. The 3 ml of 0.02 mM DPPH solution in 95% ethanol and 0.2 ml of reconstituted Chrysanthemum juice

were mixed thoroughly, let it stand in dark at room temperature for 30 min. The absorbance of the solution at 517 nm was measured and calculated as

$$DPPH(\%) = \frac{1-OD_{sample}}{OD_{blank}} \times 100 \quad (5)$$

3. RESULTS AND DISCUSSION

3.1 % Yield

The spray-dried Chrysanthemum juice powder was dried, cream color without clogging. The percentage of yield lied between 12.70% and 20.18%. As shown in **Figure 1.**

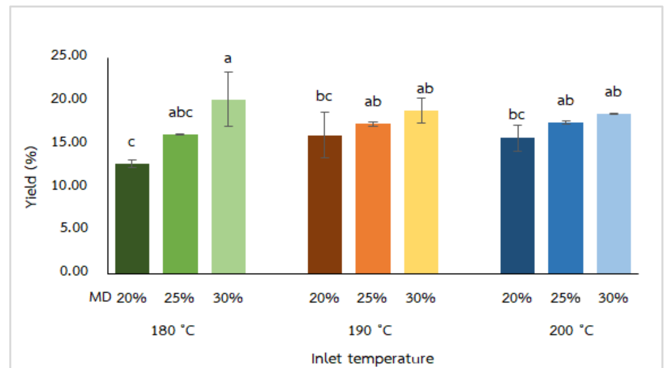


Figure 1. % Yield of spray-dried Chrysanthemum juice powder at 180, 190, and 200°C air inlet temperature and 20, 25, and 30% w/w maltodextrin at 180°C air inlet temperature, the more maltodextrin content, the more % yield because maltodextrin acts as soluble solids in the powder. However, at 190 and 200°C air inlet temperature, there was no significant difference of % yield at different maltodextrin contents ($p>0.05$). The air inlet temperature didn't significantly affect % yield which is consistent with the research findings of Quek et al. [8] that an increase in air inlet temperature didn't increase the steam to come out of spray-dried powder.

3.2.1 Effects of maltodextrin and air inlet temperature on the quality of Chrysanthemum juice powder

3.2.1 CIE color value

The L* color values (indicating brightness) of Chrysanthemum juice powder range from 87.54 to 88.94. When the amount of maltodextrin increases, the brightness value increases. The brightness of 30% w/w maltodextrin Chrysanthemum juice powder at 190°C shows the highest value as shown in **Figure 2**. The a* color values range from -1.64 to -2.54, indicating the intensity of the green color. Increases in the maltodextrin content and air inlet temperature significantly affect the green color of the powder ($p\leq0.05$) except at 190°C air inlet temperature. The b* color values range from 16.49 to 20.00, indicating yellow color. The more maltodextrin content and air inlet temperature, the less yellow color. The addition of maltodextrin results in an increase in brightness of foods which is consistent with the research of Quek et al. [8] and Paseephol et al. [19] reported that adding more than 10% maltodextrin increased the brightness of the powder.

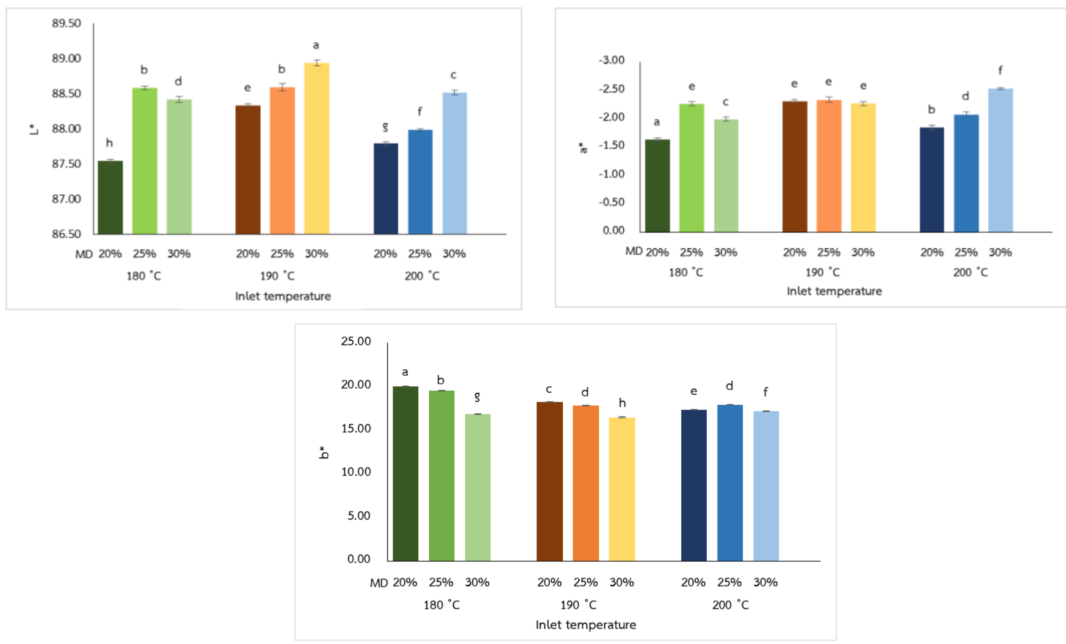
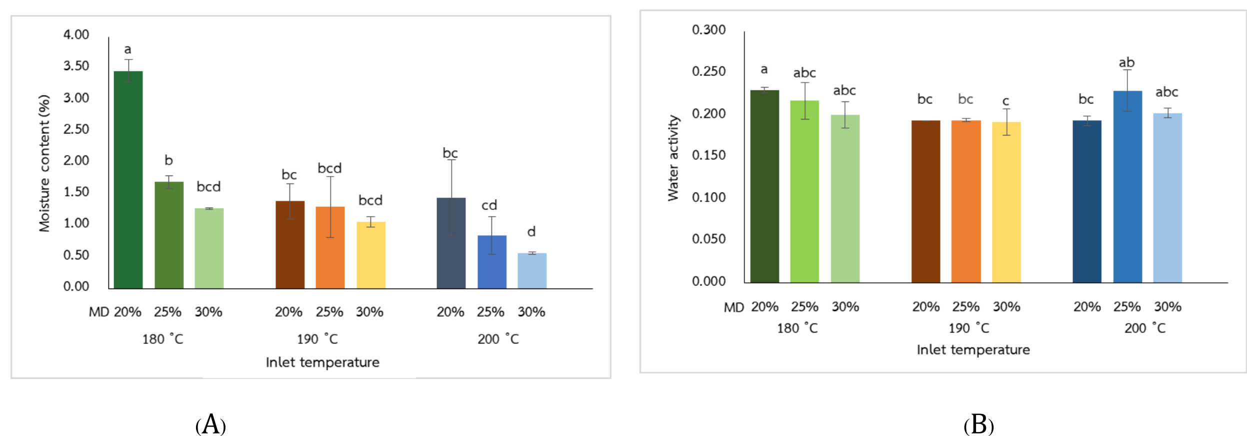


Figure 2 CIE color values of spray-dried Chrysanthemum juice powder at 180, 190, and 200°C air inlet temperature and 20, 25, and 30% w/w maltodextrin

3.2.2 Moisture content and Water Activity



(A) (B)

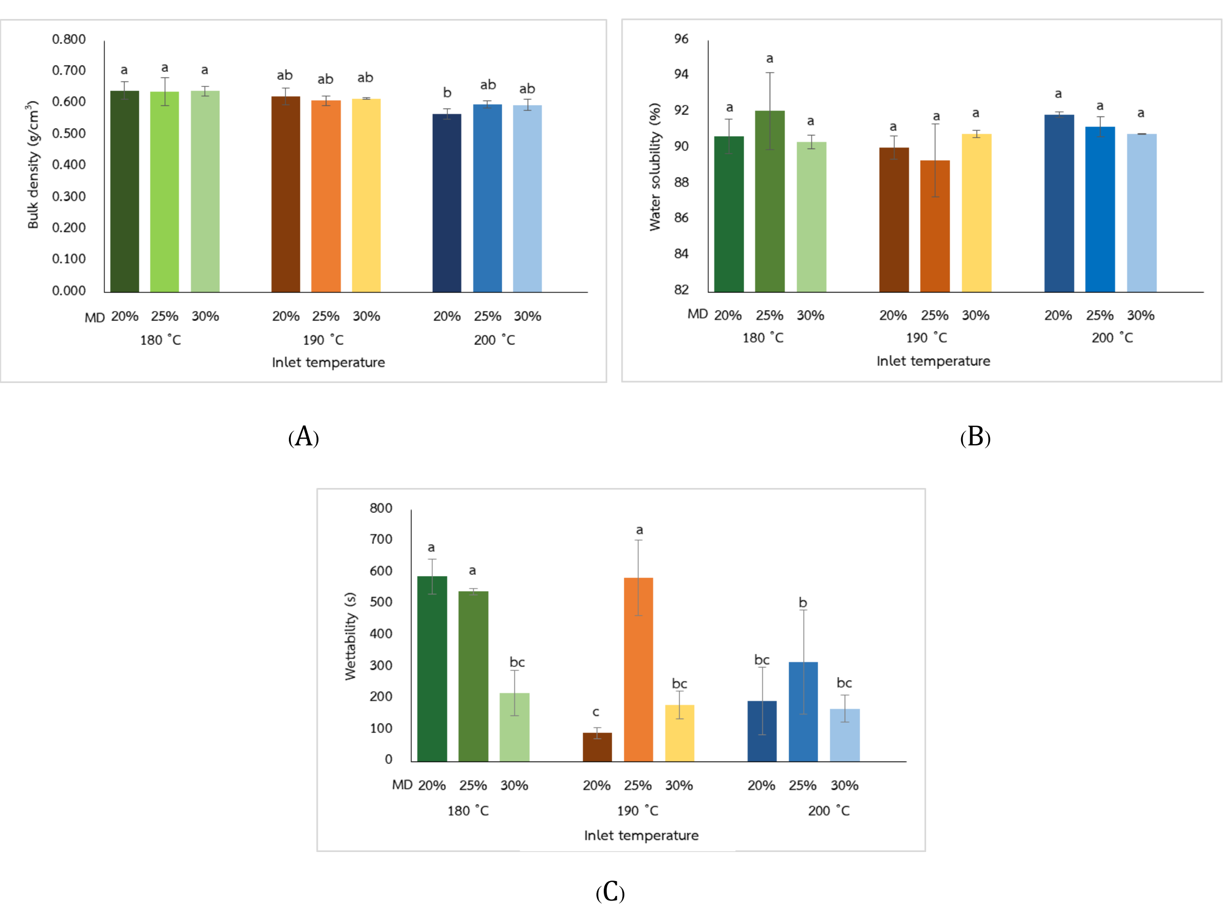
Figure 3. Moisture content and water activity of spray-dried Chrysanthemum juice powder at 180, 190, and 200°C air inlet temperature and 20, 25, and 30% w/w maltodextrin

As shown in **figure 3A**, increases in the maltodextrin content and air inlet temperature decrease the moisture content of Chrysanthemum juice powder. The higher the temperature of the air inlet, the faster the rate of water evaporation, resulting in a decrease in moisture content. Increasing the concentration of maltodextrin can reduce the moisture content of the powder because adding maltodextrin increases soluble solid content. Decreasing moisture content by increasing the

maltodextrin content and air inlet temperature can improve powder quality by prolonging the shelf-life [20]. Quek et al. [8] reported that watermelon powder showed a decrease in moisture content when air inlet temperature and maltodextrin content increased. Fazaeli et al. [21], Chegini & Ghobadian [22], Rodríguez-Hernández & Lecommandoux [23], Ersus & Yurdagel [24], and Kha [12] also reported that the moisture content of the powder decreased with an increasing of air inlet temperature.

As shown in **Figure 3B**, increases in the maltodextrin content and air inlet temperature does not significantly affect Chrysanthemum juice powder ($p>0.05$) except at 20 % w/w maltodextrin, indicating that air inlet temperature affects the water activity of powder. However, there is a slight trend that increasing air inlet temperatures can decrease water activity. The water activity of the samples ranges from 0.192 to 0.231 that can be observed that all values are less than 0.6, which is considered as a range where microorganisms cannot grow [25]. Goula & Adamopoulos [26], Masters & Jacob [27], and Fazaeli et al. [21] reported that when maltodextrin content and air inlet temperature increased, the water activity of powder decreased.

3.2.3 Bulk density, water solubility, and wettability



(A) (B)

(C)

Figure 4. Bulk density, water solubility, and wettability of spray-dried Chrysanthemum juice powder at 180, 190, and 200°C air inlet temperature and 20, 25, and 30% w/w maltodextrin

Bulk density is determined by the ratio of mass to unit volume, representing the space between particles. Large particles with many pores and voids give low density and allows water to penetrate easily, resulting in faster dissolving compared to small particle sizes. As shown in Figure 4A, an increase in maltodextrin content does not significantly affect the bulk density of Chrysanthemum juice powder while an increase in air inlet temperature decreases bulk density ($p \leq 0.05$) due to the higher air inlet temperature causes the faster water in the particles to evaporate and give more pores. Tamsama et al. [28], Masters et al. [29], Abadio et al. [9], and Fazaeli et al. [21] reported that an increase in air inlet temperature decreased bulk density of the powders.

Wettability indicates the ability of powder particles to absorb water on the particle surface. When the powder has a high-water absorption rate or large surface area for water absorption, it will have the ability to sink quickly into the water after absorbing water and have good dispersibility without clumping, making the powder dissolves well [30]. As shown in Figure 4C, at 180°C air inlet temperature, an increase in maltodextrin content decreases wettability of the Chrysanthemum juice powder. An increase in the air inlet temperature affects the wettability of powder. Wettability of the powder depends on several factors, especially the particle size and bulk density. Large particle size has more spaces between the particles, which loosely aggregate and are not uniform. This allows water to easily penetrate and has a high rate of water absorption, as a result, it has good rehydration properties [31]. Erbay et al. [32] reported that increasing the particle size increased the wettability of cheese powder.

As shown in Figure 4B, the water solubility of the spray-dried Chrysanthemum juice powder ranges between 90.06-92.09%. An increase in maltodextrin content and air inlet temperature shows no significant effect on solubility of Chrysanthemum juice powder ($p>0.05$). The air inlet temperature at 180-200°C is enough to evaporate all the water on sphere surface of particles, resulting in no clumping appearance and similar solubility. Tuyen et al. [33], and Sousa et al. [34] reported that the obtained powder products had a constant solubility when the maltodextrin content increased. Vongsawasdi et al. [35] reported that an increase in air inlet temperature showed no statistical difference on soymilk powder solubility ($p>0.05$).

3.3 Effects of maltodextrin and air inlet temperature on the quality of reconstituted Chrysanthemum juice

3.3.1 CIE color value

As shown in **Figure 5,** the color values of reconstituted Chrysanthemum juice have the same trend as Chrysanthemum juice powder (Figure 2).

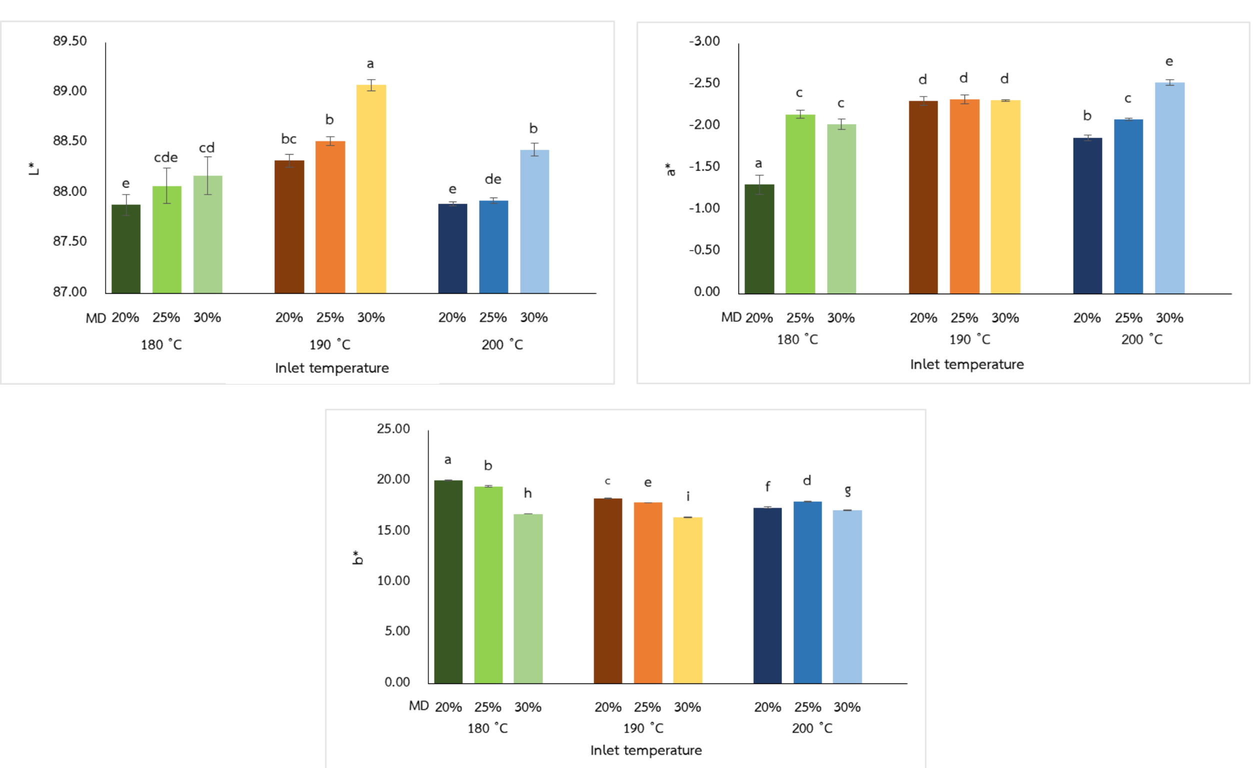


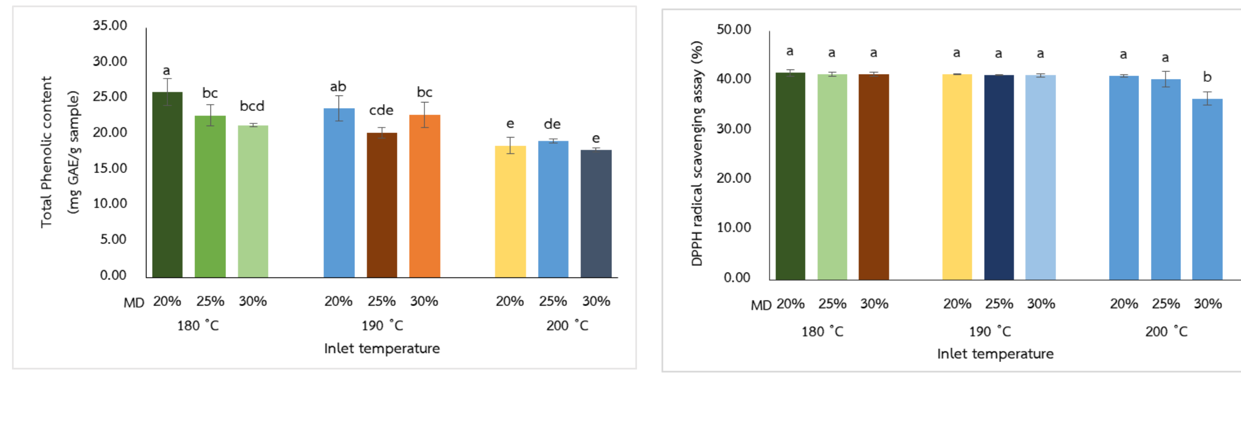
Figure 5. CIE color values of reconstituted Chrysanthemum juice from powder processed at 180, 190, and 200°C air inlet temperature and 20, 25, and 30% w/w maltodextrin

3.3.2 Antioxidant ability

Analysis of the antioxidant ability of reconstituted Chrysanthemum juice was conducted using two methods: total phenolic content and antioxidant capacity using 2,2- diphenyl- 1-picrylhydrazyl (DPPH). Phenolic compounds act as electron donors to inhibit active oxygen in the oxidation reaction [36]. DPPH plays as electron acceptor derived from antioxidant compounds and becomes stable as the color of reactant will change from purple to yellow [37].

As shown in **Figure 6A,** it was found that the total phenolic content of reconstituted Chrysanthemum juice tends to decrease when maltodextrin content and air inlet temperature increase. Spray drying process employed with high temperature to evaporate water from foods and high temperature can degrade phenolic compounds [38]. Mishra et al. [15] studied the effect of maltodextrin concentration and inlet temperature during spray drying on physicochemical and antioxidant properties of amla (*Emblica officinalis*) juice powder and concluded that high temperatures affected the phenolic structure by causing its breakdown or synthesis in various forms.

Antioxidant activity measured using DPPH shows no statistical difference among samples, except the sample at 200°C air inlet temperature and 30% w/w maltodextrin (**Figure 6B**). Maltodextrin has no effect on free radical inhibition, hence the more maltodextrin added, the less antioxidant activity [15]. Sonthong et al. [39] claimed that an increase in maltodextrin content decreased total phenolic content and DPPH radical scavenging activity of holy basil powder because maltodextrin increased total soluble solids of powder which maltodextrin itself didn't have any antioxidant activity.



(A) (B)

Figure 6. Total phenolic content and DPPH antioxidant capacity of reconstituted Chrysanthemum juice from powder processed at 180, 190, and 200°C air inlet temperature and 20, 25, and 30% w/w maltodextrin

4. CONCLUSIONS

Maltodextrin content and air inlet temperature affect the physicochemical properties of spray-dried Chrysanthemum juice powder and reconstituted Chrysanthemum juice. Increases in maltodextrin content and air inlet temperature increase color brightness but decrease moisture content of spray-dried Chrysanthemum juice powder. When air inlet temperature increases, bulk density, and wettability of spray-dried Chrysanthemum juice powder decrease, while solubility remains constant. Reconstituted Chrysanthemum juice from powder processed with high maltodextrin and high air inlet temperature gives less phenolic compounds. The reconstituted Chrysanthemum juice prepared from the spray-dried powder at 200°C air inlet temperature and 30% w/w maltodextrin had the lowest DPPH radical scavenging activity.

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Optimizing Food Gel Texture and Nutrition with Silkworm Pupae, Black Glutinous Rice, Sesame, and Brown Sugar: A Mixture Design Study

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ABSTRACT

Silkworm pupae offer a promising source of highly nutritious foods rich in protein. Processing them into powdered form extends their shelf life and versatility for incorporating into food products to enhance their nutritional profiles. This research aimed to prepare and investigate the potential of powdered silkworm pupae in premixed flours for microwaveable food gels. Powdered silkworm pupae were prepared by blending them with maltodextrin in a 1:1 ratio and drying in a hot air oven at 50°C for 48 h. Premixed flour formulations with varying proportions of black glutinous rice flour (50–60%), silkworm pupae powder (40–50%), black sesame seed powder (0–10%), and refined brown sugar (0–10%) were designed using a mixture design and surface response methodology. The optimal ingredient ratios for desired gel hardness (268–387 g) were determined to be 50–56.25% rice flour, 1.25–10% silkworm pupae powder, 1.25–10% black sesame seed powder, and 0–10% refined brown sugar. These formulations fall under the third category of Universal Design Foods' soft texture food standards, suitable for manipulation with the tongue instead of chewing. This finding suggests the potential of silkworm pupae powder as a novel and functional ingredient for convenient and nutritious food applications.

1. INTRODUCTION

The people, concerned about potential food shortages due to population growth and the decline in agricultural land from global warming, are seeking environmentally friendly alternatives to traditional meat production. Seeking alternatives to meat, scientists have explored various options, and insects have emerged as a promising new protein source that addresses these concerns. Insect farming is environmentally friendly, requiring less space for the protein produced. Insects, which also boast a better nutrient-to-meat ratio than other animals, can be easily raised due to their low position on the food chain (1). The silkworm (*Bombyx mori* Linn.) is a source of high-quality, highly nutritious protein and is popular in many Asian countries (2).

Thailand is considered as a country with a large silk industry. Silkworm pupae are obtained after silkworm processing and are often blended to make animal feed or eaten as roasted silkworm pupae, particularly in the north and northeast regions (3).

Black glutinous rice (*Oryza sativa* var. glutinosa) is characterized by its deep purple to almost black color. Research has shown that this type of rice contains several nutrients beneficial to the body (4, 5). Black sesame seed (*Sesamum indicum* nigrum) is highly valuable and nutrient rich. Sesame seeds contain an oil called sesame oil, rich in essential fatty acids (45–55%). Essential fatty acids are crucial for human health but cannot be synthesized by the body and must be obtained through the diet (6, 7, 8). Natural brown sugar is a fine powder, ranging from light brown to reddish-brown, with a distinctive burnt sugar aroma. Its main component is sucrose, the same as white sugar. Brown sugar retains slightly higher levels of minerals than white sugar, including calcium, magnesium, phosphorus, potassium, and sodium. Brown sugar is produced by simmering sugarcane juice until concentrated and crystallized, then ground into a coarse powder. The addition of molasses darkens the color further (9, 10).

Microwaves have long been utilized in household, owing to the desire for fast and quick meal preparation in modern life. Microwave heating offers efficient and uniform heating for precooked and premixed food products, making it ideal for semi-finished meals. However, numerous research reports suggest it alters the microstructure-level composition of foods, especially those containing proteins and polysaccharides (11, 12, 13). Therefore, understanding the properties of food gels formed from various ingredients via microwave heating is a crucial step in designing the final food product. This study aimed to analyze the chemical composition of silkworm pupae, black sticky rice, black sesame, and brown sugar. The goal was to then optimize their use in creating premixed flour for microwaveable food gels with a desirable texture and enhanced nutritional value.

2. MATERIAL AND METHODS

2.1 Material

Frozen silkworm pupae (*Bombyx mori* Linn.), with moisture content (MC) of 67.71 ± 0.22%, were obtained from Jim Thompson farms (Takhop Subdistrict, Pak Thong Chai District, Nakhon Ratchasima Province) and raised on organically grown mulberry leaves without pesticides or chemicals. Black glutinous rice flour (MC of 12.07 ± 0.25%), refined brown sugar (MC of 2.08 ± 0.03%; Wangkanai brand, Wangkanai Co., Ltd., Kanchanaburi, Thailand), and black sesame seed powder (MC of 1.39 ± 0.03; JM Cereal brand, Jaim Good-nature Co., Ltd., Chiang Mai, Thailand) were purchased from a local supermarket. All chemicals used were analytical grade.

2.2 Silkworm pupae powder preparation

Frozen whole silkworm pupae were processed into silkworm pupae powder (SWP) using a high-speed blender (e.g., Philips model HR2061-600W). A 1:1 ratio (100 g each) of silkworm pupae to maltodextrin was used for blending. The mixture was then dried in a hot air oven at 50°C for 48 hours. After drying, it was thoroughly blended, filtered through an 80-mesh sieve, and vacuum-packed in an opaque aluminum foil bag. The SWP samples were stored in a refrigerator until the experiment was conducted.

2.3 Chemical composition analysis

The moisture, fat, protein, ash, crude fiber, and carbohydrates of four ingredients: frozen silkworm pupae, black glutinous rice flour (BGR), black sesame seed powder (BSS), and refined

brown sugar powder (RBS) were determined using the proximate analysis technique according to the AOAC standard (14). MC was evaluated by drying 2 g of each sample in an oven at 105°C. The protein was assessed using the combustion technique (Leco, model EP 528, MI, USA), with nitrogen conversion factors of 6.25 for silkworm pupae, 5.3 for BSS, and 5.95 for BGR. Fat content was determined using a Soxhlet apparatus (Soxtec Avanti, model 2055, Tecator, Sweden) using n-hexane as the extraction solvent. A fiber digester (Fibertec 8,000, Scanco Analytical Instruments, Florida, USA) was used to measure crude fiber. The ash was measured by incinerating the samples at 550°C. Total carbohydrate content was determined by the difference (100% - sum of protein, fat, and ash).

2.4 Moisture analysis of premixed flour

The method determines the moisture content (MC) of premixed flour by accurately weighing 3 g of flour into an aluminum weighing dish and heating it to 105°C for 5 min. using an infrared moisture analyzer (Sartorius MA45).

2.5 Food gels preparation

Premixed powdered formulations with varying proportions of rice flour (50–60%), silkworm pupae powder (40–50%), black sesame seed powder (0–10%), and brown sugar (0–10%) were designed using an extreme vertices design with 3 replications at the center points for 12 runs. The food gel was prepared by mixing 10 g of premixed flour with 12.5 g of distilled water. The mixture was stirred well and transferred to a silicone mold before being microwaved (home microwave, LG model intellowave, South Korea) at 360 W for 1 min. After cooling down, each food gel was packed into a plastic can with a lid.

2.6 Texture profile analysis

Texture profile analysis (TPA) was conducted using a Texture Analyzer (Brookfield, CT3, USA) equipped with a TA 25/1000 probe. The test parameters were set as follows: pre-test, test, and post-test speeds at 5 mm/s, trigger force at 5 g, deformation at 50%, and a 2-second holding time between compressions. Food gels were prepared using 10 g of premixed flour and 12.5 g of distilled water per sample. Five samples of food gel (2 x 2 cm^2) were analyzed per run for hardness, cohesiveness, springiness, chewiness, and gumminess.

2.7 Calculate the recommended daily intake of nutrients

The nutrient content of premixed flours was calculated based on the Thai Recommended Daily Intakes (Thai RDIs) for carbohydrates, proteins, fats, and fiber.

2.8 Experimental design and statistical analysis

The MC and TPA of food gels were analyzed using Minitab 14.0 statistical software. A one-way analysis of variance (ANOVA) with Tukey's post-hoc test was performed to determine significant differences in moisture content between groups. Response surface methodology (RSM) with a mixture design was employed to analyze the TPA data.

3. RESULTS AND DISCUSSION

3.1 Chemical composition of ingredients and moisture content of mixed flour

Analysis of the chemical composition of four ingredients showed that the frozen silkworm pupae had an average protein content of 53.38%, a fat content of 35.78%, and a fiber content of 6.70% on a dr basis (Table 1). Hirunyophat et al. (15) reported that silkworm pupae powder from various varieties contained protein content ranging from 46.22 to 63.89% and fat content ranging from 17.21 to 34.69% on a dry basis. A review by Habeanu et al. (16) found that silkworm pupae contained protein in the range of 48–94.98%, fat in the range of 12.1–35.7%, and fiber in the range of 3.5–14% on a dry basis. Compared to other studies, silkworm pupae from Jim Thomson Farm were found to contain high levels of protein and fat, along with moderate fiber content. However, processing the SWP into powder by blending it with maltodextrin in a 1:1 ratio reduced its content of key nutrients like protein, fat, ash, and fiber by approximately 50% compared to the raw frozen silkworm pupae.

Table 1. Chemical components of ingredients (dry basis)

Ingredient	Carbohydrate (%)	Protein (%)	Fat (%)	Ash (%)	Fiber (%)
Frozen silkworm pupae	9.36±1.37	53.38±0.76	35.78±0.77	1.09±0.04	6.70±0.50
Black glutinous rice powder	91.79±0.10	7.11±0.23	0.89±0.26	0.22±0.02	1.65±0.03
Black sesame powder	17.35±1.20	24.60±0.55	44.68±0.81	13.37±0.38	21.65±0.14
Refined brown sugar powder	99.38±0.09	0.00±0.00	0.60±0.09	0.01±0.01	1.10±0.00

*The values shown in the table are the average ± standard deviation (n = 3). Protein and carbohydrate content of frozen silkworm pupae derived from two replications.

BGR had an average protein content of 7.11%, 0.89% fat, 0.22% ash, and 1.65% fiber, with 91.79% carbohydrate by dry basis. These analyzed values are similar to those reported by Itthivadhanapong & Sangnark (5), who found that black glutinous rice flour contained 8.0% protein, 0.4% fat, 1.5% ash, 0.9% (crude) fiber, and 89% carbohydrate by dry basis. However, the amount of ash BSS had an average protein content of 24.60%, 44.68% fat, and 21.65% fiber on a dry basis**.** These values are similar to those reported by Apichartsrangkoon et al. (17), who found 19.29% protein, 46.18% fat, 5.72% ash, 19.06% crude fiber, and 22.26% carbohydrate on a dry basis. However, the analyzed dietary fiber content (21.65%) appears higher than the calculated carbohydrate content (17.35%). This discrepancy could be due to limitations in the analytical method or the large fat content of BSS, which might hinder complete ash conversion during analysis. Similarly, the results of this analysis align with Plaitho et al. (6), who reported values for black sesame cake (a byproduct of sesame oil production) as 21.12% protein, 13.43% fat, 27.46% ash, and notable, 20.11% crude fiber, exceeding the carbohydrate content of 17.87% on a dry basis and fiber may vary depending on the growing source and the milling process.

This analysis showed that RBS contained 0.60% fat, 0.01% ash, 1.10% crude fiber, and 99.38% carbohydrate on a dry basis, which aligns with the findings of Azlan et al. (18), who reported brown sugar to contain 0.12% protein, 0.58% fat, 0.07% ash, 2.38% crude fiber, and 96.8% carbohydrate. Notably, the RBS used in the premixed flour contained slightly less protein, fat, and ash but slightly more carbohydrate.

Table 2 presents MC of the premixed flour samples. Analysis revealed significant differences ($p < 0.05$) in MC among the treatment groups. All 12 runs exhibited MC values within the range of 6.06–6.44%

Table 2. Moisture content of premixed flour.

Run	Black glutinous rice powder (%)	Silkworm pupae powder (%)	Black sesame powder (%)	Brown sugar powder (%)	Moisture content (%)
1	50.00	40.00	0.00	10.00	6.06±0.86b
2	60.00	40.00	0.00	0.00	7.44±0.40a
3	50.00	50.00	0.00	0.00	7.03±0.31ab
4	50.00	40.00	10.00	0.00	6.43±0.22b
5	52.50	42.50	2.50	2.50	6.23±0.27b
6	51.25	41.25	1.25	6.25	6.06±0.09b
7	56.25	41.25	1.25	1.25	6.47±0.22ab
8	51.25	46.25	1.25	1.25	6.52±0.22ab
9	51.25	41.25	6.25	1.25	6.87±0.12ab
10	52.50	42.50	2.50	2.50	6.57±0.28ab
11	52.50	42.50	2.50	2.50	6.43±0.10b
12	52.50	42.50	2.50	2.50	6.41±0.03b

*The values shown in the table are the average ± standard deviation from three replications.
a, b Different letters represent statistically significant differences ($p < 0.5$).

3.2 Texture profile analysis of food gels

The hardness of food gels was in the range of 268–387 g (67.00–96.75 g/cm3) (Table 3), falling within category 3 of Universal Design Food's (UDF) soft food standards (hardness < 101.97 g/cm^3), except run 2 (60% BGR, 40% SWP). These food gels have a soft texture, suggesting that tongues can be used instead of occlusion for consumption (19, 20). The food gel in run 2 (60% BGR, 40% SWP), with a hardness between 101.97 g/cm^3 and 409.86 g/cm^3 (UDF category 2), falls within the range suitable for gums that require occlusion. Therefore, the ingredients of all 12 runs can be targeted at the elderly or individuals with chewing difficulties, offering them soft-textured food options. The appropriate component proportions were 50.00–56.25% BGR, 1.25–10.00% SWP, 1.25–10.00% BSS, and 0.00–10.00% RBS

Table 3. Texture profile analysis of food gels.

Run	Hardness (g)	Cohesiveness	Springiness(mm)	Gumminess (g)	Chewiness(g)
1	280.00	0.373	6.70	104.50	8.17
2	610.00	0.334	6.71	185.00	10.62
3	366.25	0.378	7.42	135.75	11.80
4	314.00	0.352	6.90	110.80	7.46
5	323.00	0.414	11.89	134.80	16.42
6	295.00	0.350	12.13	103.25	12.32
7	357.50	0.402	5.60	132.20	8.82
8	331.00	0.366	8.35	121.60	10.52
9	387.00	0.362	6.89	142.00	10.72
10	312.00	0.375	4.36	112.80	6.48
11	376.25	0.390	8.32	136.00	9.18
12	268.00	0.415	9.49	114.20	11.36

*The values shown in the table are averages from five samples.

TPA is a widely used instrumental technique for characterizing the textural properties of food materials. It simulates the mastication process through two compression cycles, providing valuable insights into how a food product will behave when chewed. A high cohesiveness value indicates that the gel structure can effectively recover from the first bite, while a low value suggests greater susceptibility to breakdown. Springiness essentially measures the gel's ability to bounce back after the first bite. In food gels, gumminess relates to the perceived stickiness or adhesiveness when bitten or squeezed, while chewiness describes the effort required to chew and break down the gel before swallowing (21).

The textural properties of food gels prepared from 12 premixed flours varied considerably, which displayed significantly lower TPA values compared to glutinous rice gels in a study by Thongrod et al. (22). The previous study prepared rice gels from glutinous rice flour and hydrocolloids (gelatin, carrageenan, or xanthan gum) and displayed hardness ranging from 525.83 to 3066.67 g. The difference in TPA is likely attributable to the ingredients. BGR contained high levels of fiber, SWP contained high levels of fat, and BSS contained high levels of fat and fiber. Fat typically lubricates starch and protein structures in food gels and dietary fiber; conversely, it hinders the bonding of these structures, leading to significantly lower TPA values, particularly for hardness, gumminess, and chewiness. Notably, lower gumminess and chewiness may translate to a less sticky texture.

3.3 Estimated regression coefficients for texture profile analysis

Regression coefficient analysis using RSM identified the multiple regression equation for hardness and gumminess as the ideal model to describe the relationship between ingredient proportions and texture characteristics of food gels (Table 4). This choice is supported by its high adjusted R-square (>75%) and statistically significant lack-of-fit (>0.05). The results suggest a strong correlation between the equation's terms and the observed data. A positive coefficient of BGR (31126) and SWP (43458) in the hardness equation indicated a positive influence on hardness, while negative coefficients of BSS (-60000) and RBS (-9649) suggested a negative impact. The magnitude of the BSS coefficient (-60000) suggested a greater influence on reducing hardness compared to RBS (-9649). Notably, the negative coefficient of interaction terms showed that all three ingredients interacted with BGR, further decreasing hardness. Among these interactions, the coefficient of interaction between BGR and SWP (-147700) had the greatest negative impact. Interestingly, the coefficient of interaction between SWP and BSS was positive (93088), resulting in an increased hardness of food gels. A low cohesiveness value suggests a greater susceptibility to breakdown. If the value is too small, the food gel may crumble, which is undesirable. The interaction between BGR & SWP and BGR & BSS increased the cohesiveness value, while the interaction between BGR & RBS and SWP & BSS decreased it. Therefore, the next step in formulation development must consider optimizing or reducing specific ingredients. For example, if the amount of BSS is increased, the proportion of SWP should be reduced.

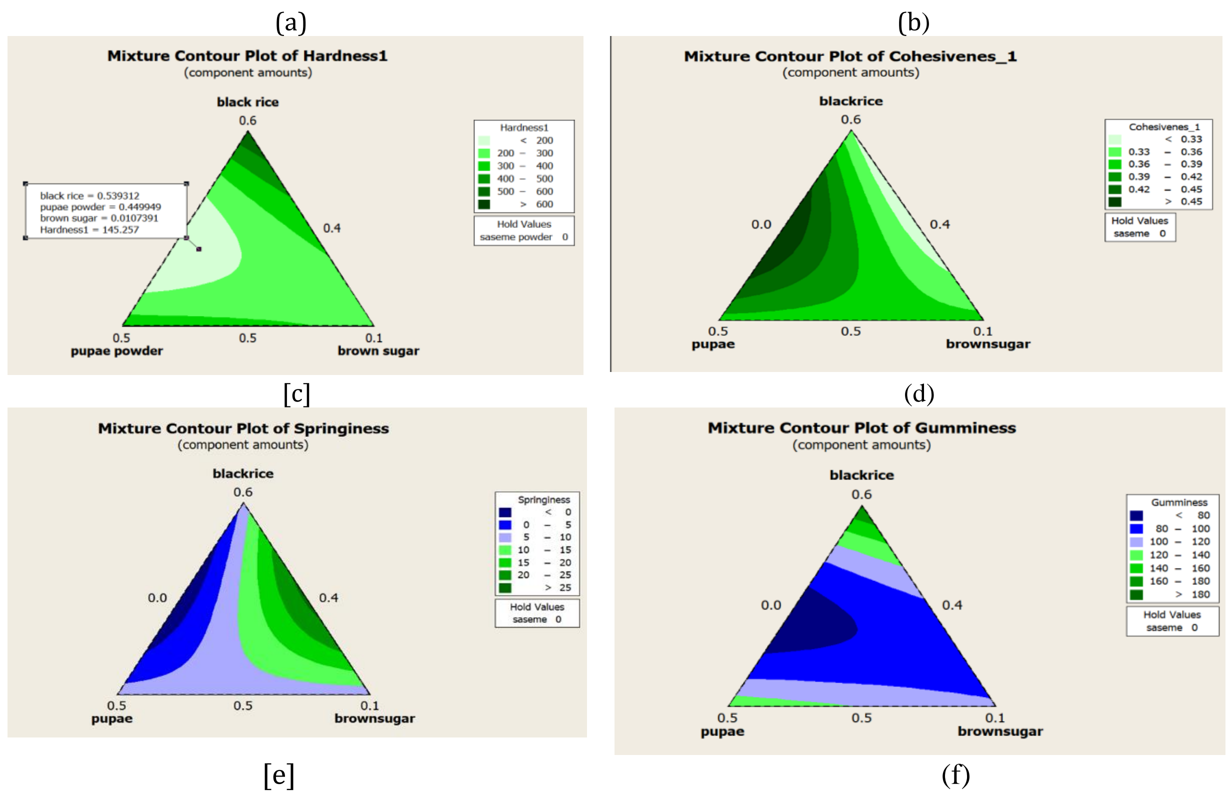
The gumminess of food gels increased when the proportion of BGR, SWP, and RBS increased. Conversely, gumminess decreased when the proportion of BSS increased. The interaction of BGR & SWP and BGR & RBS exhibited a synergistic effect in reducing gumminess, while the interaction of BGR & BSS and SWP & BSS had an antagonistic effect in increasing it. In the context of food gels, gumminess manifests as stickiness or adhesiveness when bitten, rendering swallowing difficult due to potential throat adherence. Considering the interaction effects observed in the combination trial plan, incorporating both SWP and BSS in food gel formulations might be less desirable due to their combined tendency to increase hardness and gumminess. Therefore, choosing only one of these ingredients may be preferable.

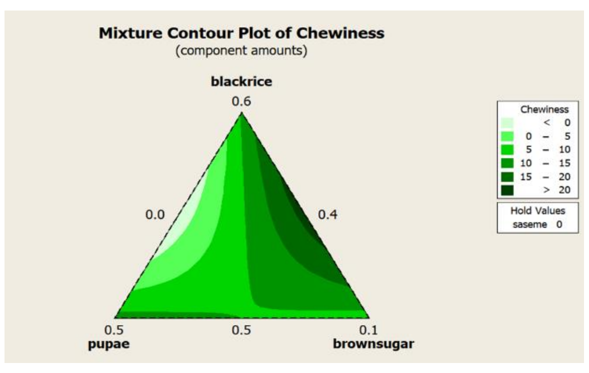
Table 4. Estimated regression coefficients for texture profile analysis.

Term	Coefficient of Parameter (Coded equation)		
	Hardness	Cohesiveness	Gumminess
BGR	31126	-9.84	7450
SWP	43458	-14.40	10491
BSP	-60000	-4.02	-24350
RBS	-9649	18.65	1994
BGR*SWP	-147700	50.00	-35340
BGR* BSP	-16300	64.40	17260
BGR* RBS	-43212	-16.20	-18972
SWP*BSP	93088	-44.60	20728
R-Sq (%)	93.22	72.13	91.14
R-Sq(adj)(%)	81.36	23.35	75.65
Lack-of-Fit	0.881	0.229	0.814

*BGR- Black glutinous rice, SPP- Silkworm pupae powder, BSS- Black sesame seed powder, RBS- Refined brown sugar

Figure 1 shows a mixture contour plot of the TPA of food gels. Color intensity gradients within the plot represent texture characteristics, with the darkest green hues indicating the highest values and the darkest blue hues indicating the lowest. Figure 1(f) shows the overlaid contour plot when setting target responses for the hardness, gumminess, and chewiness of food gels. The predicted ingredient proportions were 51.38% BGR: 48.07% SWP: 0.55% BSS: 0.00% RBS, with a predicted response of hardness 273.10 g, gumminess 111.96 g, and chewiness 7.07 g. This prediction was absent in the RBS proportion and had less content in the BSS.





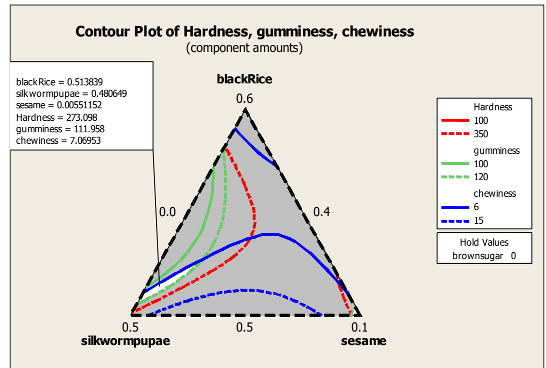


Figure 1. Mixture contour plot of texture profile analysis of food gels, (a) Hardness, (b) Cohesiveness, (c) Springiness, (d) Gumminess, (e) Chewiness, and (f) Overlaid contour plot

Table 5. Pearson's correlation coefficients of the component proportion and texture profiles of food gels.

Ingredient	Hardness	Cohesiveness	Springiness	Gumminess	Chewiness
Black glutinous rice powder	0.781**	-0.110	-0.206	0.758**	0.026
Silkworm pupae powder	-0.112	0.204	0.073	0.002	0.267
Black sesame powder	-0.196	-0.137	-0.095	-0.202	-0.236
Brown sugar powder	-0.474	0.044	0.229	-0.558	-0.057

**significant at $\alpha < 0.01$

The correlation coefficient analysis in Table 5 revealed that increasing BGR proportions positively correlated with both hardness and gumminess in food gels.

3.4 Estimation of nutrition values by calculation method

The estimated nutritional value of each premixed flour sample (10 g) was compared to the Thai RDI (Table 6), revealing protein content at 2% RDI, carbohydrate content between 2 and 3% RDI, and both fat and fiber content at 1% RDI.

Table 6. Estimation of nutrition values by calculation method (per 10 g of premixed flour)

Run	Total Protein (g)	% Thai-RDI	Carbohydrate (g)	% Thai-RDI	Fat (g)	% Thai-RDI	Fiber (g)	% Thai-RDI
1	0.89	2	7.68	3	0.41	1	0.16	1
2	0.96	2	7.60	3	0.41	1	0.17	1
3	1.02	2	7.21	2	0.49	1	0.17	1
4	0.91	2	6.86	2	0.85	1	0.27	1
5	0.95	2	7.34	2	0.54	1	0.19	1
6	0.92	2	7.51	3	0.47	1	0.18	1
7	0.95	2	7.47	2	0.48	1	0.18	1
8	0.98	2	7.27	2	0.52	1	0.18	1
9	0.93	2	7.10	2	0.69	1	0.23	1
10	0.95	2	7.34	2	0.54	1	0.19	1
11	0.95	2	7.34	2	0.54	1	0.19	1
12	0.95	2	7.34	2	0.54	1	0.19	1

Table 7. Estimated regression equation for calculating nutrition values of premixed flour

Nutrient	Equation
Total protein (g)	0.0711A+0.1334B+0.0246C
Total carbohydrate (g)	0.9179A+0.5234B+0.1735C+9.938D
Total fat (g)	0.0089A+0.08945B+0.4468C+0.006D
Total fiber (g)	0.0165A+0.01675B+0.12C+0.011D

*A denoted black glutinous rice flour content; B denoted silkworm pupae powder content; C denoted black sesame seed powder content; and D denoted refined brown sugar powder content.

Table 6 presents the multiple linear regression model for calculating the nutrient values of premixed flour. This equation can be a valuable tool for estimating the proportion of ingredients in formulations while optimizing their nutritional value. The recommended BGR proportion should not exceed 56.25% per serving due to concerns about hardness. The formula can be adjusted by increasing the proportions of SWP and RBS and reducing the proportion of BSS.

4. CONCLUSIONS

Silkworm pupae powder (SWP) is suitable for use in premixed flours for microwaveable food gels at proportions of 40.00–50.00%. Their combination with black glutinous rice powder (BGR) and refined brown sugar powder (RBS) reduced the hardness and gumminess of these gels. Black sesame seed (BSS) has interactions with SWP, which may increase the hardness and gumminess of food gels. The obtained food gels are intended for consumption with the tongue rather than chewing, according to Universal Design Foods' soft texture food standards. Nutrition value can be further increased adjusting ingredients by incorporating ingredients rich in protein such as pea protein, rice protein, heme protein, or egg white protein powder. Further development of the formulation may allow for an even higher proportion of SWP or RBS. This, if used in larger proportions, may reduce the hardness and gumminess of food gel in BGR-based formulations due to their interaction.

5. ACKNOWLEDGEMENTS

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Enhancing Consumer Satisfaction in Coconut Water Wine Products through an Analysis of Appropriate Characteristics Using the Kano Model

CP-P034

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ABSTRACT

Ratchaburi aromatic coconut is renowned for its sweet taste and unique aroma. However, currently, farmers are facing an issue with a significant amount of substandard aromatic coconuts. Therefore, the potential to reduce the problem of food loss and add value by developing these into coconut water wine products was recognized. This research aimed to identify appropriate characteristics for developing coconut water wine products from substandard aromatic coconuts that would satisfy the target consumer group. The target group included individuals aged 20 and older who can legally consume alcoholic beverages, have a preference for and experience in drinking fruit wine. The research began with in-depth interviews with 6 target consumers to initially determine the characteristics of coconut water wine that affect consumer satisfaction. Subsequently, a study using the Kano model was conducted through an online questionnaire via Google Form with a target group of 130 consumers. The results from the interviews informed the design of the questionnaire using the Kano model with 11 characteristics considered important by consumers, encompassing both positive and negative characteristics. It was found that the 'One-dimensional' characteristics, which increase satisfaction if present in coconut water wine, include having a sweet taste, being rich in antioxidants, vitamins and minerals, probiotics or prebiotics, and being packaged in a clear glass bottle. The 'Attractive' characteristic identified is the addition of collagen. Hence, these characteristics can be leveraged to develop coconut water wine products that enhance satisfaction and attract consumers.

1. INTRODUCTION

Aromatic coconut (Cocos nucifera L.) is a fruit that is popular among both Thais and foreigners due to its sweet taste and unique aroma in its coconut water. This refreshing beverage is renowned for its numerous health benefits, notably its richness in essential vitamins and minerals, particularly potassium, which aids in reducing blood pressure [1].

In 2022, Thailand's exports reached 416,808 tons with a value of 277.1 million US dollars and is expected to increase every year [2]. Moreover, aromatic coconuts have been registered as a Geographical Indication (GI) of Ratchaburi Province, with the most important aromatic coconut growing area in Thailand located in Damnoen Saduak District. Many farmers in the area, therefore, grow aromatic coconut as their main occupation, which involves meeting the standards of middlemen in grading the aromatic coconut products that are popularly sold in the form of fresh fruits. When the aromatic coconut is small, has bruises or defects that do not meet the standards, it will be classified as substandard and discarded, even though there is still sweet coconut juice inside. Farmers, therefore, have to sell them to buyers at prices below cost or dispose of them in large quantities, leading to the problem of food loss [3]. In addition, the problem is further aggravated by El Niño, resulting in smaller produce in less quantity [4]. From this information, the researchers observed that coconut water from substandard aromatic coconuts in Damnoen Saduak District, which still retains its distinctive sweet taste and unique aroma but has not been put to good use, has the potential to be developed into a wine product. This could be a new approach to reducing the issue of food loss, thereby increasing value and providing a way to generate additional income for affected farmers.

Figure 1. (a & b) Substandard Aromatic Coconuts in Damnoen Saduak District, Ratchaburi Province, Thailand

When considering the global fruit wine market, it is estimated to be valued at approximately 921.0 million US dollars in 2024 and is expected to reach 2.4867 billion US dollars by 2034, expanding at a Compound Annual Growth Rate (CAGR) of 10.4% during the forecast period from 2024 to 2034. This expansion is propelled by the rising popularity of craft beverages and the increasing demand for unique and flavorful alcoholic drinks. Factors such as the growing trend among consumers towards seeking healthy options, as well as the appeal of naturalness and variety, are key drivers of this market growth. Over the past decade, fruit wine production has experienced rapid development in numerous countries. It serves as an alternative that offers novelty through the use of various fruits as raw materials, thereby aiding in the reduction of perishable fruit wastage post-harvest. Additionally, fruit wines have proven to be rich sources of antioxidants, phytonutrients, and minerals, thereby contributing to improved health and reduced disease risks, appealing to health-conscious consumers seeking delicious alternatives to traditional wine. [5,6,7].

When developing new products for the market, the Kano model is often used. First developed by Dr. Noriaki Kano in 1984, the Kano model is considered an excellent tool for categorizing and prioritizing consumer needs by considering the relationship between product characteristics and consumer satisfaction levels using a questionnaire consisting of functional and dysfunctional questions. The functional questions reveal the feelings of the consumer when receiving that characteristic, while the dysfunctional questions reveal the feelings of consumers when they do not receive that characteristic. The answers obtained for each pair of questions were then analyzed to classify product characteristics

according to consumer satisfaction levels. This helps in gaining insights into consumer preferences and expectations, enabling the design of products that can meet or exceed consumer needs. In addition to creating products that are different from competitors, it also helps to utilize resources appropriately, avoiding wasting time and money on characteristics that consumers are not interested in or are not satisfied with [8,9]. Therefore, the objective of this research was to study appropriate characteristics for the development of coconut water wine products from substandard aromatic coconuts that satisfy the target consumer group using the Kano model.

2. MATERIAL AND METHODS

2.1 Sample Selection and Research Method

This study selected its sample group for research using non-probability sampling with the purposive sampling method. The Kano model tool was used with a target sample group of consumers aged 20 and older who can legally consume alcoholic beverages, have a preference for and experience in drinking fruit wine. The number of participants was over 100, surveyed using an online questionnaire via Google Form. This was designed using in-depth interviews regarding views, opinions, and expectations about coconut water wine, as well as factors that stimulate purchases among the targeted consumer group of 6 people, along with studying previous research on the characteristics consumers prioritize in fruit wines or fermented beverages. The questionnaire used in the research was divided into 5 sections, consisting of (1) screening questions to obtain study results from the target consumer group, (2) demographic data, (3) wine consumption behavior, (4) the Kano model regarding appropriate characteristics of coconut water wine, and (5) additional expectations or suggestions. The screening questions included queries to determine whether the respondent met all the criteria set for the target group as specified by the researchers. If not, they would not be able to complete the next section of the questionnaire.

2.2 Demographic Data and Wine Consumption Behavior

The demographic data section of the questionnaire consisted of closed-ended questions in a checklist format, allowing respondents to select one answer. This included gender, age, highest level of education or current education, occupation, and average monthly income. The wine consumption behavior section of the questionnaire comprised closed-ended questions in a checklist format with options for selecting one answer, including the frequency of wine consumption and where they purchase wines, as well as multiple answers, including favorite types of wines and factors influencing wine purchase decisions.

2.3 Kano Model

This part of the questionnaire is a study of consumers' satisfaction with the appropriate characteristics of coconut water wine products made from substandard aromatic coconuts using the Kano model. The characteristics tested were divided into 11 characteristics across 4 aspects: taste, feature, nutritional value, and packaging. It consisted of functional and dysfunctional questions. Functional questions assess the consumer's feelings when receiving a specific characteristic, while dysfunctional questions evaluate consumers' feelings when they do not receive that characteristic. Then, the sample was asked to select 1 out of 5 options: like, must, neutral, live with, or dislike.

Following that, each respondent's answers were used to determine the type of satisfaction level, categorized as A, M, O, I, R, and Q, as illustrated in Table 1. An 'Attractive' (A) characteristic is one that most consumers do not expect but, when present in a product, can greatly increase satisfaction. However, its absence doesn't cause dissatisfaction. 'Must-be' (M) is a basic characteristic that must be present in the

product; its absence will lead to great dissatisfaction. 'One-dimensional' (O) is a feature that, when present in the product, increases satisfaction, but its absence leads to dissatisfaction. 'Indifferent' (I) is a characteristic that consumers are not interested in; its presence or absence does not affect satisfaction levels. Any characteristic that is unwanted or contradicts expectations and reduces satisfaction when present in the product will be classified as 'Reverse'(R). If questions in the created questionnaire were worded incorrectly, respondents misunderstood them, or they accidentally provided incorrect answers, they were classified as 'Questionable' (Q). The result should contain no more than 2% Q.

Table 1. Kano Evaluation Table [10]

Customer requirements		**Dysfunctional**				
		Like	Must	Neutral	Live with	Dislike
Functional	Like	Q	A	A	A	O
	Must	R	I	I	I	M
	Neutral	R	I	I	I	M
	Live with	R	I	I	I	M
	Dislike	R	R	R	R	Q

Following that, the interpretable data from each respondent's table were used to count the frequencies and calculate the percentages for each characteristic. The percentages of M, O, A, and I for each characteristic were then used to calculate the customer satisfaction coefficient according to the following equation:

$$\text{Satisfaction coefficient (Sat)} = (A+O)/(A+O+M+I) \quad (1)$$

$$\text{Dissatisfaction coefficient (Dissat)} = (O+M)/((A+O+M+I)x(-1)) \quad (2)$$

The satisfaction and dissatisfaction coefficients of each characteristic were plotted on a graph, where the X-axis represents the dissatisfaction coefficient and the Y-axis represents the satisfaction coefficient to examine the distribution of various characteristics in the graph. Lines were drawn to demarcate the satisfaction levels of different characteristics using the A-Kano classification according to the method of Xu et al. [9], classifying all features into groups A, O, M, or I [9,11,12].

2.4 Statistical Analysis

Statistical data analysis was conducted in the form of finding frequencies and percentages. The demographic data and wine consumption behavior were analyzed using IBM SPSS Statistics 26. Regarding the Kano model, the types were identified, and the frequency of satisfaction levels, namely A, M, O, I, R, and Q, were counted. The customer satisfaction coefficient was calculated using Microsoft Excel.

3. RESULTS AND DISCUSSION

3.1 Demographic Data

From a survey of 130 target respondents, it was found that the majority were female, accounting for 50.8%, followed by males at 38.5% and LGBTQ+ individuals at 10.8%. The largest age group was 35-54 years, representing 46.9%, followed by 20-34 years at 45.4%, and only 7.7% were aged 55 years and above. The highest level of education or current education was predominantly a bachelor's degree at

74.6%, followed by a master's degree at 23.1%, a doctorate degree at 1.5%, and less than a bachelor's degree at 0.8%. The majority worked as private company employees at 40.8%, followed by business owners or self-employed individuals at 29.2%, government officials or state enterprise employees at 11.5%, students at 10.8%, freelance workers at 6.9%, and others, such as retirees, at 0.8%. The most common average monthly income was 15,000-30,000 baht at 32.3%, followed by 30,001-60,000 baht at 30.0%, 60,001-120,000 baht at 27.7%, less than 15,000 baht at 6.2%, and more than 120,000 baht at 3.8%.

3.2 Wine Consumption Behavior of the Target Group

From studying the wine consumption behavior of the target consumers who participated in the questionnaire, it was found that the majority had a frequency of wine consumption of less than 2 times per month at 43.1%, followed by 2-4 times per month at 40.8%, 5-7 times per month at 12.3%, and more than 7 times per month at 3.8%. The most popular types of wine were red wine at 28.4%, fruit wine at 23.6%, white wine at 21.7%, sparkling wine at 9.9%, rosé wine at 9.6%, and dessert wine at 6.7%. The most common places to purchase wine products were supermarkets such as Tops and Villa Market at 44.6%, wine shops at 23.8%, restaurants at 21.5%, convenience stores such as 7-11 and Lawson at 4.6%, online channels at 4.6%, and others, such as duty-free shops, at 0.8%. The most influential factors in wine purchase decisions were found to be aroma and taste at 27.6%, followed by price at 14.2%, brand name at 13.1%, packaging at 11.3%, grape variety or fruit used as raw material at 10.7%, novelty at 9.6%, country of origin at 8.0%, and nutritional value at 5.6%.

3.3 Appropriate Characteristics of Coconut Water Wine Based on the Kano Model

From studying the appropriate characteristics of coconut water wine made from substandard aromatic coconuts, analyzed using the Kano model, the results are shown in Table 2 and Figure 2.

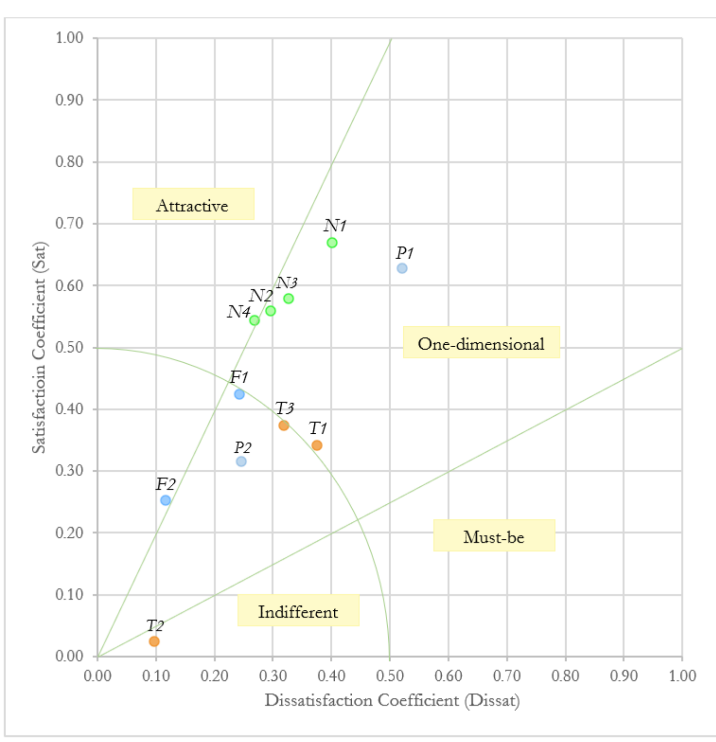

Figure 2. Classification of Satisfaction Levels Using the A-Kano Classification Method

Table 2. Percentage of Types of Satisfaction Levels and Customer Satisfaction Coefficient Values

Characteristic	A	O	M	I	R	Q	Sat	Dissat	Classification
Taste Aspect									
Sweet Taste (T1)	6.2	26.2	9.2	53.1	5.4	0.0	0.34	-0.37	O
Astringency (T2)	0.8	0.8	5.4	56.2	36.9	0.0	0.02	-0.10	I
Taste Similar to Aromatic Coconut (T3)	15.4	20.8	10.0	50.8	1.5	1.5	0.37	-0.32	I
Feature Aspect									
Having a High Alcohol Content)8-14%) (F1)	13.8	18.5	0.0	43.8	23.1	0.8	0.42	-0.24	I
Carbonated (is a Sparkling Wine) (F2)	13.1	5.4	3.1	51.5	25.4	1.5	0.25	-0.12	I
Nutritional Value Aspect									
Rich in Antioxidants (N1)	29.2	36.2	3.1	29.2	1.5	0.8	0.67	-0.40	O
Rich in Probiotics or Prebiotics (N2)	28.5	25.4	3.1	39.2	3.1	0.8	0.56	-0.30	O
Rich in Vitamins and Minerals (N3)	26.9	30.0	2.3	39.2	1.5	0.0	0.58	-0.33	O
Added Collagen (N4)	29.2	23.8	2.3	42.3	2.3	0.0	0.54	-0.27	A
Packaging Aspect									
Packaged in a Clear Glass Bottle (P1)	13.8	44.6	3.8	30.8	6.2	0.8	0.63	-0.52	O
Packaged in a Frosted Glass Bottle (P2)	9.2	14.6	3.8	47.7	23.1	1.5	0.32	-0.24	I

When considering each aspect’s characteristics, it was found that the taste characteristics in terms of having a ‘sweet taste' can be classified as 'One-dimensional (O).’ This means that if the coconut water wine has a sweet taste, it will increase consumer satisfaction. Conversely, if it lacks a sweet taste, it will cause consumer dissatisfaction. The characteristics of having ‘astringency' and a ‘taste similar to aromatic coconut' were classified as ‘Indifferent (I).’ This means that the presence or absence of these characteristics in coconut water wine does not affect the level of consumer satisfaction. However, when considering the satisfaction coefficient (Sat) and dissatisfaction coefficient (Dissat) values of the 'astringency' characteristic, it was found that the Sat value was 0.02 and the Dissat value was -0.10,

indicating that the value was not close to 1. Berger et al. [13] stated that if the Sat value is close to 1, it implies that such a characteristic in the product will significantly increase satisfaction. Conversely, if the Dissat value is relative to -1, the absence of such a characteristic in the product will substantially increase dissatisfaction. Therefore, it is possible that if astringency is present in coconut water wine, it may have the opposite effect; that is, consumer satisfaction may significantly decrease. Conversely, if there is no astringency, consumer dissatisfaction may significantly decrease. Similar to the findings of Zhu et al. [14] study, it was observed that the majority of consumers prefer wines that exhibit a harmonious balance of sweetness, sourness, and aroma, exemplified by blueberry wine. Conversely, fruit wines characterized by astringency, bitterness, and excessive sourness are generally less favored. Additionally, as indicated in a study by Merlino et al. [15], both neophiles—adventurous consumers open to novel experiences—and new entries—individuals with limited experience in fruit wine consumption—expect a certain degree of sweetness in the product. Consequently, sweet fruit wines are poised to attract a broad spectrum of consumers and demonstrate substantial potential for commercial success.

Feature characteristics in terms of 'having a high alcohol content (8-14%)' and being 'carbonated (is a sparkling wine)' were both classified as 'Indifferent (I)' or indifferent to the presence or absence of these characteristics in the product. Based on in-depth interviews with target consumers, it can be reasoned that the indifference towards these two characteristics was because, although consumers are familiar with aromatic coconut water, developing it into a coconut water wine product is considered a new concept. Consumers still can't imagine what characteristics or forms would make coconut water wine most satisfying. They may also not be able to imagine how their senses or feelings will respond after consuming coconut water wine in that form. All target consumers interviewed expressed similar opinions, aligning with the results from the research by Masamran and Supawong [16] on the 'Application of the Kano Model on Consumer Demand for Immune-Boosting Beverages.' They found that the appearance and flavor characteristics of the immune-boosting beverages were classified as 'Indifferent.' The reason given was that this may be due to consumers being unable to imagine the actual taste of the immune-boosting beverages and lacking experience in tasting certain flavors or forms of beverages.

Nutritional value characteristics in terms of being 'rich in antioxidants,' 'rich in probiotics or prebiotics,' and 'rich in vitamins and minerals' were classified as 'One-dimensional (O).' This means that if such nutritional values are present, they will increase consumer satisfaction. Conversely, their absence will decrease satisfaction or increase dissatisfaction. 'Added collagen' is classified as 'Attractive (A),' meaning that if collagen is added, it will attract consumers' attention, increasing satisfaction. However, if it is not included in the product, it will not cause dissatisfaction, as consumers did not expect collagen to be in the product. Zhu et al. [14] discovered that consumers tend to lean towards products perceived as healthier choices. Notably, when consumers were informed about the anthocyanin content in fruit juices, they were more inclined to buy the product with the highest anthocyanin content.

Regarding packaging characteristics, 'packaged in a clear glass bottle' was classified as 'One-dimensional (O),' and 'packaged in a frosted glass bottle' was classified as 'Indifferent (I).' These classifications align with the interview results regarding which coconut water wine packaging would most attract purchasers. Most target consumers preferred a clear glass bottle because seeing the wine's appearance inside the packaging is more appealing. If decorated with beautiful patterns indicating it's made from coconut or stands out from other wines, it will create even more demand.

Therefore, to make the most efficient use of consumer feedback and product development, it is crucial to maintain the Must-be (M) characteristics, add in 'One-dimensional' (O) and 'Attractive' (A) characteristics, avoid 'Indifferent' (I) characteristics as much as possible, and avoid 'Reverse' (R) characteristics [17]. When considering Figure 2, it was found that no characteristic was classified as 'Must-be' (M). This may be because wine has a variety of styles and flavors, and consumers are not attached to

any particular characteristic that must be present in the product [12]. The satisfaction levels O, A, and I can be arranged in order of importance as M>O>A>I to create wine products that provide the highest satisfaction and are in demand in the market while also enabling the most appropriate utilization of the producer's resources [18]. Therefore, it is possible to arrange the importance of these rules in conjunction with the satisfaction coefficient (Sat) values, from highest to lowest, for each characteristic. This begins with developing characteristics with a 'One-dimensional' (O) level of satisfaction, which will directly increase consumer satisfaction, and its absence will lead to dissatisfaction. This includes taste characteristics like having a 'sweet flavor'; nutritional value characteristics such as being 'rich in antioxidants,' 'rich in vitamins and minerals,' and 'rich in probiotics or prebiotics'; and packaging characteristics like being 'packaged in a clear glass bottle.' Subsequently, characteristics with an 'Attractive' (A) level of satisfaction should be developed to attract consumers and create a sense that the product exceeds expectations, namely the nutritional value of 'added collagen.

In addition, the researchers experimented with using substandard aromatic coconut water to ferment wine. This involved adjusting the Total Soluble Solid (TSS) and pH, along with modifying the coconut water conditions to be suitable for yeast growth. Three different strains of yeast, EC1118, 71B, and K1V-1116, were used to ferment the wine at room temperature for 6 days. Measurements were taken for Total Soluble Solid (TSS), pH, and alcohol concentration (%ABV). The results are shown in Figure 4. It was observed that all three yeast strains could initiate the alcohol fermentation process, yielding a final alcohol content within the range of 7-10%. This demonstrates the feasibility of producing coconut water wine products from substandard aromatic coconuts. Such products can be scientifically analyzed and developed to embody various characteristics based on the research findings, thereby creating coconut water wine products that can achieve maximum satisfaction for the target consumers.

Figure 3. Coconut Water Wine from Substandard Aromatic Coconuts

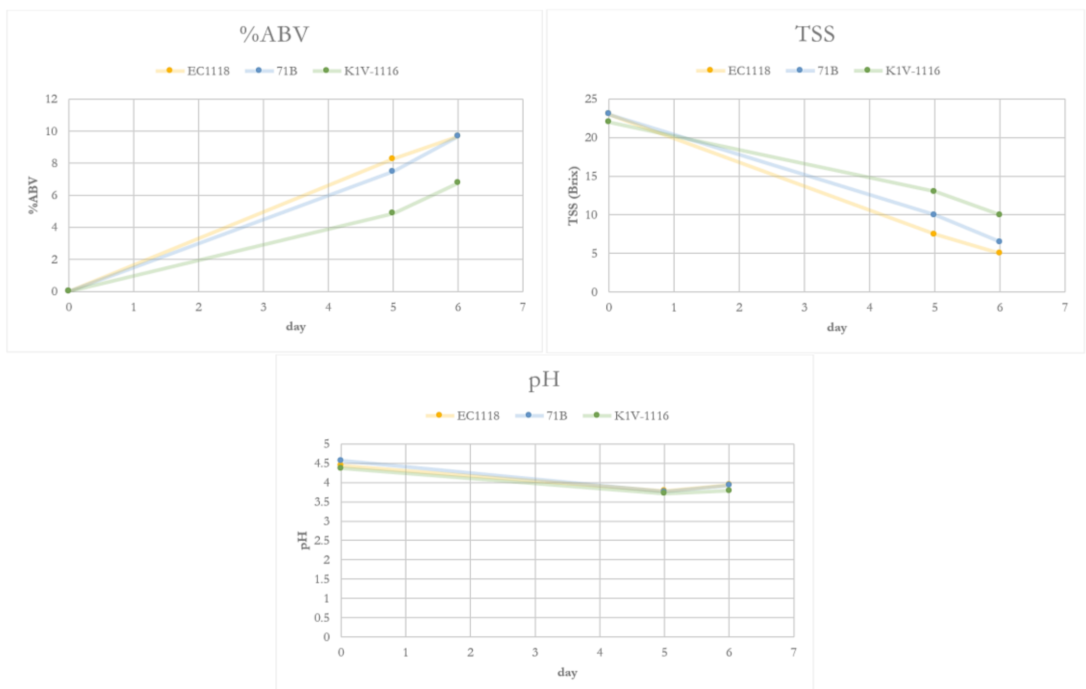

Figure 4. (a) Alcohol Concentration (%ABV), (b) Total Soluble Solid (TSS), and (c) pH of Coconut Water Wine Fermentation from Substandard Aromatic Coconuts For 6 Days

4. CONCLUSIONS

In summary, the characteristics most appropriate for developing coconut water wine products from substandard aromatic coconuts that the target consumer group is most satisfied with start with developing a coconut water wine that has a sweet taste, is rich in antioxidants, vitamins and minerals, and probiotics or prebiotics, and is packaged in a clear glass bottle. Additionally, avoid any astringency in the wine as much as possible. Subsequently, add in collagen to further attract consumers. Therefore, it is evident that the Kano model is a tool that can be used to identify product characteristics that most significantly influence consumer satisfaction in line with the research objectives.

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Design thinking for food product development fortified with microcapsulated Gourami fish oil extracted

CP-P035

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ABSTRACT

This research involves research and development (R&D) with the following objectives: (1) to study the behavior and needs of consumer groups towards fish oil products, and (2) to examine the feasibility of extracting oil from the intestines of gourami fish, which is a waste product produced through high-pressure technology. The design thinking process is employed for qualitative research. The sample was selected using a homogeneous sampling method, and data were collected from three groups. Data analysis aims to understand consumer behavior and needs through the value proposition canvas. Additionally, the study involves the idea generation process using the lotus blooming techniques tool to obtain ideas that can be developed into innovative gourami fish oil microcapsule dietary supplement products. The results of the group discussions revealed that all three consumer groups share similar perspectives regarding fish oil products available in the market. Specifically, there is a consensus that fish oil lacks variety in appearance, inducing a sensation of hard to swallow. Many consumers express hesitancy to bite into the capsules due to concerns about the associated fishy smell and taste. Additionally, capsules tend to clump together if left in the jars. The study suggests that developing gourami fish intestines oil microcapsules in powder form could elicit greater interest among consumers. The outcomes of utilizing the lotus blooming techniques tool for product design, encompassing a total of 64 potential products, indicate that consumers are most intrigued by salad dressing products. In parallel, a feasibility study on oil extraction from gourami fish intestines at pressures of 100 MPa and 300 MPa for 30 minutes yielded insightful results. Specifically, at 100 MPa, a higher oil yield was achieved, resulting in a lower peroxide value and a more vibrant yellow color. A suitable to be selected for use in the process of encapsulation technique developed for fish oil microcapsules from gourami fish intestines, extracted using high-pressure processing technology, resulting in a powder form that can be added to salad dressing products to meet consumers' needs.

1. INTRODUCTION

Gourami fish (*Trichogaster pectoralis*) is a freshwater species found in Thailand, commonly processed into dried or salted fish food. Due to its excellent taste, delectable meat, and aromatic fragrance, the current gourami fish processing industry in the country shows a tendency to expand internationally [1]. Consequently, gourami fish holds significance as a crucial economic aquatic resource in Thailand. The processing of gourami fish results in food waste, comprising the head, intestines, bones, and skin. formerly, these remnants were frequently utilized as raw materials for producing animal feed, such as fish meal and fish silage. Fish intestines, although possessing important nutritional value including protein and certain amino acids, also contain fats and unsaturated fatty acids in the omega-3 group [2]. However, they exhibit a distinctive odor and characteristics that render them challenging for use in the processing of animal feed raw materials. Consequently, it is deemed suitable to explore their development into an extracted fish oil product obtained from fish intestines.

According to Grand View Research [3], the current fish oil market is expected to exceed 137.5 million dollars USD by 2023, reflecting an increase in consumer adoption of fish oil from 2013. Fish oil, obtained through extraction, is rich in omega-3 fatty acids, specifically Eicosapentaenoic Acid (EPA) and Docosahexaenoic acid (DHA) [4]. These essential unsaturated fatty acids play a crucial role in reducing blood triglyceride levels, preventing fat accumulation in arteries, and inhibiting platelet adhesion. All significant factors contributing to heart disease and cerebral blockages [5]. However, fish oil is susceptible to deterioration due to oxidation reactions, resulting in the formation of odoriferous substances and an unusual taste, commonly referred to as rancidity. Selecting a method for extracting oil from gourami fish intestines involves utilizing high pressure processing technology without subjecting to the heat process. Subsequently, creating fish oil powder through the encapsulation technique, combined with the spray-drying process, emerges as a popular method. This approach is effective in preventing lipid oxidation in foods rich in omega fatty acids, and it also aids in masking the flavor more effectively than directly adding fish oil to the food. By encapsulating the gourami fish oil within a wall material or a substance that acts as a barrier, a biological substance (Bioactive) is formed. This enhances the efficiency of controlling the delivery and release of biological molecules, thereby reducing the reaction of the mixture. This method proves to be convenient to use and preserves the nutritional value, which may otherwise degrade due to various factors such as oxidation, temperature, pH and acidity [6].

Therefore, this research focuses on studying the behavior and needs of consumers towards fish oil products by employing design thinking tools in the development of oil microcapsule dietary supplements from gourami fish intestines. The primary objective is to enhance the value of waste in the industry. The study encompasses five main steps, commencing with the empathize step, which involves understanding the consumer group through focus group discussions. Subsequently, the define step identifies problems or issues using the value proposition canvas. The process then moves to the Ideate stage, where data is analyzed, and ideas are brainstormed using the lotus blooming techniques tool, resulting in the creation of 64 products. Following this, three products are selected based on the highest total score obtained through the checklist method for the target consumer group. Initially, one product is chosen that best meets the most desired features. Subsequently, the research delves into the feasibility of extracting oil from gourami fish intestines using high-pressure processing technology. This involves a comparison of yield percentage, peroxide value, and color value under conditions of 100 MPa and 300 MPa for 30 minutes. The information gathered from this study serves as a basis for making informed decisions regarding the further development of innovative food products.

2. MATERIAL AND METHODS

2.1 Study of consumer behavior towards interest in fish oil products

The researcher has established guidelines for focus group discussion, with questions for discussion divided into 4 parts for the group of consumers who consume fish oil and questions for the group of consumers who do not consume fish oil. The tools used to collect data are notebooks and voice recorders for recording conversations and retrieving information.

2.1.1 Demographic Information

The participants in the group discussions for this research are individuals of working age, both male and female, with an age range of 23-60 years. They reside and work in Thailand, specifically in Bangkok and surrounding areas. The selection of participants was purposive, considering the researcher's characteristics. The chosen groups were based on the research objectives and were divided according to the framework of experience and behavior in choosing to purchase or consume fish oil. The three groups, each with five participants, consist of 6 men and 9 women, include target consumers who regularly consume fish oil, consumers who occasionally consume fish oil, and consumers who do not consume fish oil.

2.1.2 Questions for consumers who consume fish oil

Section1: General information of the interviewee, covering gender, age, occupation, and province of residence

Section2: Open-ended questions regarding experiences and attitudes towards the product, including frequency of consumption of fish oil products, product brand and type of fish oil consumed, aroma, taste, and texture of the product, problems occurring during consumption, purchase channels, and factors such as price and variety of fish oil products currently sold

Section3: Open-ended questions regarding expectations and motivations for purchasing fish oil products. This includes the motivators that led to the desire to buy the product, as well as the properties expected in fish oil products and their shelf life.

Section4: Open-ended questions regarding suggestions and opinions on guidelines for developing fish oil products. This includes preferences for fish oil products that participants would like to see developed or modified, experiences in consuming gourami fish, interest in utilizing gourami fish intestines through the extraction of specific oil components using high-pressure processing technology, and thoughts on developing gourami fish oil microcapsules in powder form as an innovative product in the future. This section also covers any suggestions regarding the addition of gourami oil microcapsules to food products.

2.1.3 Questions for consumers who do not consume fish oil

Section1: General information of the interviewee, covering gender, age, occupation, and province of residence

Section2: Open-ended questions about experiences and attitudes towards the product include participants' experiences and perspectives on fish oil products, significant reasons for not currently taking fish oil, perceptions of the benefits of fish oil commonly sold in the market, factors influencing the decision to purchase fish oil, and the trend of turning to consume fish oil or acquiring knowledge about the benefits of fish oil in making purchasing decisions.

Section3: Open-ended questions regarding expectations and motivations for purchasing fish oil products include exploring the factors that would motivate this group of consumers to purchase such products and identifying the properties they believe fish oil products should possess.

Section4: Open-ended questions regarding suggestions and opinions on guidelines for developing fish oil products. This includes preferences for fish oil products that participants would like to see developed or modified, experiences in consuming gourami fish, interest in utilizing gourami fish intestines through the extraction of specific oil components using high-pressure processing technology, and thoughts on developing gourami fish oil microcapsules in powder form as an innovative product in the future. This section also covers any suggestions regarding the addition of gourami oil microcapsules to food products.

The group discussion constitutes a single focus group, fostering interactive discussions on the given issues. The interview style adopted is a Semi-Structured Interview. Throughout the conversation, permission will be sought to take notes and record audio for documentation purposes

2.2 The Value Proposition Canvas

The process of constructing a value proposition canvas adheres to a bifurcation into two integral segments: the customer segment and the product segment. Within the customer segment, the articulation of customer jobs delineates the specific desires and needs of customers, encapsulating features, functionalities, and emotional experiences pertinent to product utilization. Gains pertain to the identification of elements that augment or complement customer needs in their respective jobs, fostering heightened preferences, satisfaction, and an increased inclination to utilize the product. On the contrary, Pains revolve around the acknowledgment of aspects that customers wish to avoid. The value proposition canvas, situated within the product segment, encompasses three crucial components: Products and Services, epitomizing strengths and capabilities tailored to fulfill the needs of each customer job; gain creators, discerning features that resonate with and attract customers, honing in on features that effectively address and mitigate customer pain points

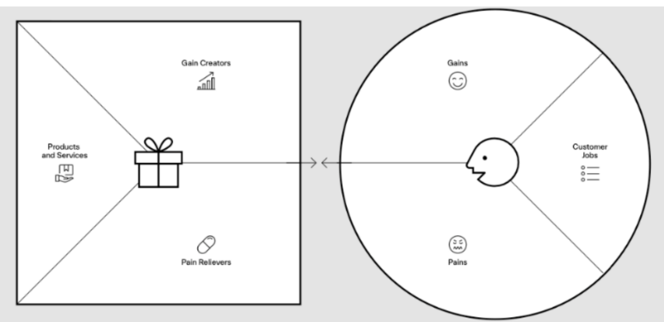

Figure 1. Value Proposition Canvas

2.3 Lotus Blooming Techniques

Design thinking initiates from the core of the narrative and radiates outward, employing expansive circles (lotus petals) to articulate the product concept, thereby generating numerous key ideas. These ideas are then distributed into sub-products based on specified categories. This process facilitates the broad expansion and dissemination of new ideas, as illustrated in Figure 2.

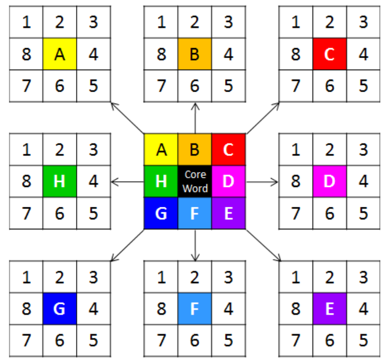

Figure 2. Lotus Blooming Techniques

2.4 Feasibility study on extracting oil from gourami fish intestines using high pressure processing technology

Clean the frozen gourami fish intestines and separate the portion containing fat lumps. Grind or blend this part finely into a uniform paste using a hand homogenizer. Transfer the paste into a 250 ml PET plastic bottle, and add water in an equal volume to that of the fish intestines. Place the mixture into a high pressure processing (HPP) machine under pressure conditions of 100 and 300 MPa for 30 minutes. Afterward, centrifuge the resulting fish oil at 4,000 rpm for 25 minutes at a temperature of 25 °C, and then extract the separated substances. The clear portion, which is the oil, will emerge. The extracted oil will undergo further purification through filtration, using a 50micron filter paper. Finally, calculate the percentage yield and peroxide value. The experiment was independently triplicated (n=3). Data were subjected to one-way analysis of variance (ANOVA). The data are reported as mean values ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1 Results of the study on thought processes

Adhering to the principle of considering two main consumer groups, a set of 10 consumers are selected, consist of 5 men and 1 women, comprising 5 who regularly consume fish oil and 5 who occasionally consume it. Additionally, the perspectives and opinions of another group of 5 consumers who do not consume fish oil are taken into account, aiming to gather insights through group interviews. The intention is to utilize the ideas garnered from these group discussions to address issues and enhance the development of fish oil products, ensuring they genuinely fulfill the needs of consumers. This is achieved by implementing the product design process in accordance with the design thinking method.

3.1.1 Understanding the Target Group (Empathize)

A group study of consumers who consume fish oil

During the group interviews (Focus Group), two target consumer groups, each consisting of five individuals selected through specific targeting, revealed that consumer groups share similar experiences and attitudes towards fish oil products. The preferred form of purchased fish oil is clear soft capsules, packaged in jars with visible yellow fish oil. There are variations in brand and price preferences, including popular choices like EVERFRAME, Black More Fish Oil, ZEAVITA, MEGA We Care, and Vistra fish oil. Consumers indicated purchasing these fish oil products from stores, pharmacies, department stores, and general online channels. A common observation among the majority of consumers was the fishy smell experienced while consuming fish oil, irrespective of the brands. The capsules, available in both soft and hard textures, were noted to cause a feeling of throat

stiffness, necessitating the intake of water. Additionally, consumers mentioned the limited variety of product formats and the tendency for the capsules to develop a rancid smell and clump together over time.

Upon examining expectations and purchasing motivations, it was discovered that consumers are prompted to increase their consumption of fish oil due to a desire for enhanced self-care. This inclination stems from advice received from various sources, including friends, family, doctors, and online platforms. The anticipated characteristics of fish oil involve a lack of fishy smell, ease of consumption, and the nutritional benefits of omega groups, including DHA and EPA. Additionally, a longer shelf life is identified as a factor that increases interest, making it more appealing than brands with similar attributes. In terms of suggestions and opinions on guidelines for developing fish oil products, it was observed that consumer groups express a desire to diversify eating styles, preferring smaller sizes that are easy to consume and devoid of any fishy smell. Additionally, there is a preference for products that are easily portable. This aligns with the findings of Suriyan's research [7], which highlighted that the majority of Thai consumers of fish oil recommend improvements specifically in terms of the product's scent. However, in the present day, the necessity to purchase these products persists because omega-3 fatty acid products are more convenient to consume in powder form than in pill form. Additionally, there are only a few brands available in the market, limiting the options for new products that are responsive to consumer needs. To illustrate, when presenting an example of extracting oil from Gourami fish intestines using high pressure processing technology and subsequently developing it into microcapsules in powder form, consumers showed keen interest.

A group study of consumers who do not consume fish oil

Based on interviews conducted with 5 consumers who currently do not consume fish oil, utilizing a specific target group selection through the focus group interview method, the study revealed their experiences and attitudes towards the product. Among these consumers, 3 out of 5 had previously taken fish oil in both liquid and capsule forms, but all 5 individuals have chosen not to take it presently. The primary reason cited was the issue of the fishy smell associated with fish oil. Despite being aware of the benefits of fish oil, the unappealing portability and appearance of capsule products deterred their consumption. This finding aligns with various influencing factors in the purchasing decision, including price, appearance, flavor, nutritional value, production source, and certification standards.

When considering expectations and purchasing motivation, it was identified that consumers are driven to buy fish oil due to a desire to take care of themselves, recommendations from close acquaintances, and influences from trends and social media. This motivation is further amplified by research or test results that have received certification. Consumers anticipate specific characteristics in fish

3.1.2 Data Synthesis (Define)

Based on the data acquired through understanding the target group (Empathize), as illustrated in Figure 3, the utilization of this data aims to identify patterns and relationships within the consumer group data. This process facilitates the summarization of needs, the explicit specification of problems and issues requiring resolution, and the identification of the actual problem. This analysis is conducted using the value proposition canvas diagram.

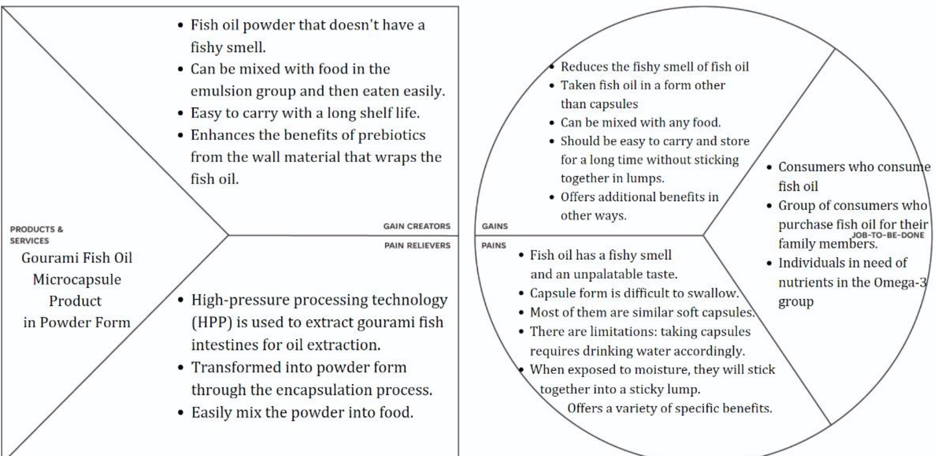

Figure 3. Value Position Canvas diagram derived from consumer interviews on fish oil

3.1.3 Creative brainstorming (Ideate)

Following the data synthesis in the Define step, the analysis of the data is conducted to choose ideas for developing products that align with the needs of the target consumers. Specifically, the aim is to create products that can be seamlessly incorporated into food, aligning with the innovative concept of introducing fish oil microcapsules in powder form into food. This concept serves as a prototype for testing. Consequently, the researcher opted to utilize the lotus blooming techniques tool, as illustrated in Table 1.

Table 1. The results of brainstorming food products suitable for adding fish oil microcapsules using the Lotus Blooming Techniques

Milk	Milk Tablet	Drinking Yogurt	Soy Milk	Energy Drink	Coffee	Cream of Mushroom Soup	Butternut Squash Soup	Cream of Spinach Soup
Frozen Yogurt	Dairy Products	Stirred Yogurt	Fish Soup	Drink	Milk Tea	Cream of Yam Soup	Cream Soup	Cream of Chicken Soup
Sweetened Condensed Milk	Greek Yogurt	Set Yogurt	Chicken Soup	Cocoa	Jelly Carrageenan	Cream of Ginger Soup	Cream of Corn Soup	Cream of Tuna Soup
Jelly	Gummy	Candy	Dairy Products	Drink	Cream Soup	Donut	Pudding	Shu Cream
Chocolate Bar	Snacks	Cookies	Snacks	Fish oil microcapsule powder supplement product	Desserts	Chiffon Cake	Desserts	Waffle
Snack Bar	Ice Cream	Biscuits	Main dishes	Condiments	Processed foods	Brownies	Scones	Pie
Thai Green Curry	Massaman Curry	Panang Curry	Salad dressing	Chili Paste	Peanut Spread	Meatball	Fried Fish Patty	Chicken Nuggets
Hang-le Curry	Main dishes	Black Sesame Congee	Mala Powder	Condiments	Mayonnaise	Tapioca Ball	Processed foods	Instant Noodles
Fermented Fish Entrails Soup	Tuna Salad	Oatmeal Congee	Furikake Rice Seasoning	Non-dairy Creamer	Mustard	Tofu	Rice Porridge Powder	Sausage

Next, choose 10 products that align with the fundamental consumer needs and employ the quantitative screening method, specifically the checklist method, to select the top 3 products with the highest scores from Table 2. These scores are assigned based on criteria such as the procurement of raw materials, production methods, attractiveness, and shelf life. The specified criteria are fundamental factors or preliminary methods for considering the selection of product ideas suitable for future development. For example, raw materials should be easily available in the market and have a reasonable price and nutritional value. Additionally, the production process should have sufficient machinery and manpower to support it and should be able to attract markets for the products in the food group that will be developed. It was observed that stirred yogurt products, salad dressings, and cream soups received the highest overall scores, ranking as the top 3. When 10 consumers from the same group of fish oil consumers were asked to choose 1 product that best addressed their most desired properties, salad dressings received the highest acceptance scores. This prompted the decision to develop salad dressings into an innovative food product enhanced with gourami fish oil microcapsules. This finding aligns with the research of Ratchadaporn [8], where a survey of 400 consumers revealed that 93% of respondents had previously purchased salad dressing, primarily for its perceived health benefits, reaching as high as 97.5%. If the product can incorporate a beneficial formula rich in essential fatty acids such as omega-3 and omega-6, it is expected to serve as a significant selling point, capturing the interest of consumers.

Table 2. Check list method ideas for developing fish oil microcapsule powder dietary

Product idea	Procurement of raw materials (25)	Production methods (25)	Attractiveness (25)	Shelf life (25)	Overall (100)
1. Stirred Yogurt	25	23	25	22	95
2. Milk	25	23	10	22	80
3. Candy	15	15	18	25	73
4. Gummy	20	22	15	20	77
5. Salad Dressing	25	25	25	22	97
6. Cream Soup	25	23	23	25	96
7. Milk Tea	15	23	20	15	73
8. Ice Cream	15	20	18	15	68
9. Furikake Rice Seasoning	22	20	20	25	87
10. Peanut Spread	20	15	15	25	75

Table 3. Characteristics of oil extracted from gourami fish intestines using

High Pressure Processing (HPP) Conditions	Oil Yield (%w/w)	Peroxide Value (mEq/kg)	Picture the appearance of Gourami fish oil extracted
100 MPa 30 min	38.383 ± 0.3889	1.437 ± 0.0458	
300 MPa 30 min	36.299 ± 0.3073	4.451 ± 0.3018	

3.1.4 The Feasibility of Extracting Gourami Oil Using High-Pressure Processing (HPP) Technology for the Creation of Product Prototypes

The experiment results, presented in Table 3, reveal that gourami fish intestines subjected to oil extraction through high pressure processing (HPP) technology at 100 MPa for 30 minutes yielded a higher oil quantity compared to those processed at 300 MPa. The percent yield

was 38.383, accompanied by a lower peroxide value of 1.437 mEq/kg and a fresher, brighter color. In contrast, gourami fish intestines processed at 300 MPa extracted only 36.299 percent of the oil, resulting in a higher peroxide value of 4.451 mEq/kg and a more opaque, darker color. According to the research conducted by Kim and Barbara [9], newly extracted fish oil should not surpass a peroxide value of 10 mEq/kg. The Peroxide Value serves as a metric for gauging the rate of lipid oxidation reactions, a key factor contributing to odor and rancidity [10]. This value signifies the deterioration of the oil. The study suggests that utilizing high-pressure technology can yield high quality oil from gourami fish intestines. gourami fish oil extracted at a pressure of 100 MPa for 30 minutes is deemed suitable for further exploration and development in the process of creating oil microcapsules from gourami fish intestines

4. CONCLUSIONS

The results obtained through the application of design thinking tools revealed consumer interest in gourami fish oil extracted using high-pressure processing technology. This interest stems from the potential to enhance the value of gourami fish intestines, a byproduct of the industry, through the encapsulation process. This process transforms the oil into microcapsules in powder form, addressing the issues of fat oxidation in omega-rich foods. Furthermore, it serves to mask the fishy odor associated with fish oil, making it convenient for storage, transport, and easy incorporation into various foods. These insights were derived from the analysis of consumer needs, identified through the value proposition canvas diagram. Notably, the identified problems included the fishy odor of fish oil and the limited variety of capsule textures, making it challenging to integrate with other foods and prone to clumping. During the brainstorming session for product development

using the lotus blooming techniques tool, it was observed that consumers expressed the highest interest in fish oil microcapsule salad dressing products. The feasibility of extracting fish oil using high-pressure technology was explored, and gourami fish oil extracted at 100 MPa for 30 minutes emerged as a promising candidate for further research and development. This innovative salad dressing product, enriched with microcapsule powder, showcases favorable characteristics such as a high oil yield, low peroxide values, and well preserved freshness of colors. This aligns seamlessly with the identified needs of the target consumers, providing a basis for creating prototype products that are not only accepted but also offer a new, more convenient alternative for incorporating fish oil into one's diet. It serves as a valuable guideline for the future development of healthy food products.

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Effect of Holy Basil By-Product Fiber as a Fat Replacer on the Physicochemical and Sensory Quality of Pork Frankfurters

CP-P084

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ABSTRACT

Sausages, particularly frankfurters, are popular, but their high fat content raises concerns for health-conscious consumers. Utilizing dietary fiber, particularly from food industry by-products, presents a promising fat replacement strategy. This study investigated the potential of holy basil powder (HBP), derived from holy basil by-products, as a fat replacer and functional ingredient in fiber-fortified frankfurters. Pork back fat was partially substituted with HBP at varying levels (0%, 9%, 12%, and 15%). Frankfurters with 9-15% HBP exhibited significantly reduced fat content and increased dietary fiber (total, insoluble, and soluble) compared to the control ($p<0.05$). This was accompanied by enhanced antioxidant activity and total phenolic compound content. Importantly, sausage pH remained within the acceptable range (5.40-6.00) for typical fermented sausages, which helps inhibit the growth of many pathogenic microorganisms. HBP inclusion improved product characteristics by significantly decreasing cooking loss due to its water-holding capacity. While lightness (L*) and redness (a*) values decreased, likely due to lower fat content and HBP's antioxidant properties, the 9% HBP substitution was the most preferred by consumers, indicating a successful balance between nutritional benefits and sensory acceptability. This study highlights the potential of holy basil by-products to create healthier frankfurter options without compromising consumer appeal.

1. INTRODUCTION

Pork sausage, a globally popular and versatile food, is traditionally formulated with ground pork, fat, spices, and other flavorings. While fat contributes to texture, juiciness, and flavor, its high saturated fat content raises health concerns (1). Increasing consumer demand for healthier options necessitates alternatives that prioritize both taste and nutritional value.

Dietary fiber emerges as a promising fat replacer in meat products (2). It can reduce fat while potentially enhancing texture, water-holding capacity, and providing benefits like increased satiety and improved digestion (3,4). While various plant-based by-products have been explored, there's

room to investigate herb-based by-products.

Utilizing dietary fiber as a fat substitute in food products is a burgeoning area of research. Dietary fiber offers numerous health benefits and can reduce calorie content. However, challenges like impacts on taste, texture, and cost require further study. Research into developing suitable dietary fibers and processing techniques is ongoing. Numerous studies have explored using dietary fiber to replace fat in sausages, increasing fiber content and reducing fat. Examples include wheat fiber (5), apple pomace (2), hemp seed dregs (6), mango peel (7), citrus peel fiber (8), and pineapple pomace fiber (3). These fibers impart desirable physicochemical properties to meat products.

Holy basil (*Ocimum sanctum* L.), widely consumed in Thailand and globally, presents a promising source of dietary fiber. Research highlights its nutritional and bioactive properties, including high phenolic compound content with potent antioxidant activity (9). These antioxidants offer potential benefits like cellular protection and reduced chronic disease risk. While leaves and shoots are widely used, stems and flowers are often underutilized by-products, despite their richness in beneficial compounds.

This study aims to valorize holy basil by-products by converting them into a powder for use as a fat substitute in pork sausage. Specifically, we investigated the effects of incorporating varying ratios (0%, 9%, 12%, and 15%) of holy basil powder on the sausage's chemical, physical, and sensory properties.

2. MATERIAL AND METHODS

2.1 Raw Material

Holy basil by-products (stems, leaves, and flowers) were kindly supplied by CP FoodLab Co., Ltd., Bangkok, Thailand. Lean pork (moisture 65.65%, protein 18.11%, fat 15.73%) and pork back fat (moisture 9.90%, protein 1.65%, fat 86.73%) were purchased from a local market in Bangkok, Thailand. Additional ingredients for sausage preparation included salt, sodium nitrite, sodium erythorbate, sodium tripolyphosphate, soy protein isolate, smoke flavor, monosodium glutamate, sugar, seasoning (ground black pepper, ground nutmeg and cloves, garlic powder, paprika powder, and majoram).

2.2 Holy Basil Powder (HBP) Preparation

Fresh holy basil by-products were cleaned, cut into small pieces (20-30 mm), and dried in a tray dryer (Frecon ix, Japan) at 60°C for 18 hours. The dried material was ground using a hammer mill (AP-S, Hosokawa Micron, Japan) and sieved (100-mesh) to produce a fine powder. The powder was stored in an aluminum bag for further analysis. Proximate composition and properties of holy basil powder are presented in Table 1.

2.3 Pork Sausage Preparation

This study investigated the effects of substituting pork back fat with holy basil powder (HBP) at four levels (0%, 9%, 12%, and 15%). A completely randomized design (CRD) was used for chemical and physical analyses, while a randomized complete block design (RCBD) was employed for sensory evaluation, where panelists served as blocks. Table 2 presents the basic pork sausage formulation with varying levels of HBP.

For each treatment, 3-kg batches of meat batter were prepared according to the following protocol: First, pork back fat and HBP were premixed and frozen at -20°C for 30 minutes. Next, frozen minced lean pork was processed in a silent cutter (CM-14, Mainca, Spain) with curing salts (sodium nitrite, sodium erythorbate) and sodium tripolyphosphate for 3 minutes with intermittent additions of ice. Subsequently, frozen pork back fat, HBP (according to treatment ratios), and remaining ingredients were added and blended for a total of 6 minutes (4 minutes with ice, 2 minutes without). The sausage mixture was then stuffed into 22 mm diameter collagen casings (PTK Solution and Supplies Ltd., Thailand) using a stuffer machine (FC-12, Mainca, Spain). Sausages were cooked and smoked in a smoke chamber (CS700, Kerres, Germany) with oak wood, reaching an internal temperature of 80°C for 1 hour. Finally, samples were vacuum-sealed and stored at 4°C until further analysis.

Table 1. Proximate composition (%wet basis) and properties of holy basil powder

Chemical composition and properties	Holy basil powder
Moisture (%)	5.94 ± 0.05
Ash (%)	11.72 ± 0.04
Fat (%)	2.34 ± 0.04
Crude fiber (%)	30.28 ± 0.08
Protein (%)	22.21 ± 0.11
Carbohydrate (%)	25.38 ± 0.12
Insoluble dietary fiber (IDF, %)	46.61 ± 0.18
Soluble dietary fiber (SDF, %)	6.26 ± 0.19
Total dietary fiber (TDF, %)	52.87 ± 0.21
Antioxidant activity (mg Trolox/ g sample)	2.74 ± 0.35
Total phenolic compound (mg GAE/g sample)	70.15 ± 1.13
L*	61.23 ± 0.05
a*	0.85 ± 0.01
b*	18.05 ± 0.04
Water solubility (%)	76.21 ± 0.69
Water binding capacity (g/g)	5.36 ± 0.04
Oil binding capacity (g/g)	19.23 ± 0.02

Table 2. Pork sausage recipes with varying levels of fat substitution using holy basil powder (HBP)

Ingredients (g/100g)	Treatments			
	0%HBP	9%HBP	12%HBP	15%HBP
Pork back fat	45.00	40.95	39.60	38.25
Holy basil powder	0.00	4.05	5.40	6.75
Lean pork	55.00	55.00	55.00	55.00
Ice	18.52	18.52	18.52	18.52
Salt	0.65	0.65	0.65	0.65
Sodium nitrite	0.40	0.40	0.40	0.40
Sodium erythorbate	0.27	0.27	0.27	0.27
Sodium tripolyphosphate	0.40	0.40	0.40	0.40
Soy protein isolated	1.30	1.30	1.30	1.30
Seasoning	2.00	2.00	2.00	2.00

2.4 Physicochemical Analysis of Sausages

2.4.1 Proximate Composition

Compositional analysis of sausage samples was performed according to AOAC (10) to determine moisture, fat, ash, crude fiber, protein, and carbohydrate content. Moisture content was analyzed via oven drying (105°C, 12 hours, FD 115, Binder, Germany). Fat content was determined using an automated Soxhlet extraction unit (E-816, Buchi, Switzerland). Crude fiber content was measured using an automated fiber analyzer (Fibertec 2010, Foss, Sweden). Protein content was assessed using a Kjeldahl nitrogen analyzer (KjeltecTM 9, Foss, Sweden) with a 6.25 conversion factor, and carbohydrate content was calculated by difference.

2.4.2 pH

The pH of the sausages was determined according to AOAC (10). A 10 g sample of sausage was homogenized with 100 mL of distilled water. The pH of the filtered homogenate (Whatman No.1 filter paper) was measured using a pH meter (C1020, Consort, Belgium) at 25°C.

2.4.3 Total Dietary fiber (TDF), Insoluble Dietary Fiber (IDF), Soluble Dietary Fiber (TDF)

Total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) content were determined according to AOAC (10). The process involved initial enzymatic digestion with thermostable α-amylase to remove starch, followed by IDF isolation through filtration and drying. SDF was determined after further enzymatic digestion and precipitation. Finally, TDF was calculated as the sum of IDF and SDF.

2.4.4 Antioxidant Activity

The antioxidant activity of sausage samples was determined using a modified DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Sausage samples were homogenized with methanol to extract antioxidants, and the homogenate was incubated with a DPPH solution. DPPH, a stable free radical, reacts with antioxidants causing a color change and a decrease in absorbance at 517 nm. This decrease in absorbance, measured spectrophotometrically, was used to calculate the antioxidant activity as follows: Antioxidant Activity (%) = [1 - (A sample / A control)] x 100, where A sample is the absorbance of the sample-DPPH mixture and A control is the absorbance of the DPPH solution.

2.4.5 Total Phenolic Compounds

Total phenolic compounds (TPC) were extracted from homogenized sausage samples using a mixture of water, methanol, and acid (1:10) according to Gong et al. (11). After centrifugation (6800 rpm, 10 minutes), the soluble phenolic fraction was analyzed using the Folin-Ciocalteu method. The reaction with Folin-Ciocalteu reagent, followed by sodium carbonate addition, produced a colored complex. Absorbance at 760 nm was measured using UV-Vis spectrophotometry (LM 7-2000, Harikul, Thailand) and compared to a gallic acid calibration curve. TPC was expressed as mg gallic acid equivalents (GAE) per gram of sample.

2.4.6 Cooking loss

Cooking loss, an important quality parameter in sausage, was assessed using a modified method. Raw sausages were weighed (W), cooked in boiling water (75°C) for 30 minutes, and then cooled to room temperature (25°C) for 3 hours. The cooked sausages were weighed again (W1), and cooking loss was calculated as follows: Cooking loss (g/100g) = [(W - W1) / W] x 100, where: W = Weight of raw sausage (g) and W1 = Weight of cooked sausage (g).

2.4.7 Water activity

Water activity (a_w) of sausage samples was assessed using a Water activity meter (Series 3 TE model, Aqualab, USA). Homogenized sausage was packed into cups and analyzed for a_w at 25°C.

2.4.8 Color Analysis

Sausage color properties were assessed using a spectrophotometer (Ultrascan pro, Konica Minolta, United Stage) according to Pathare et al. (12). The CIE Lab* system was used under D65 illumination with a 10° observer angle. Color parameters, including lightness (L*), redness/greenness (a*), and yellowness/blueness (b*) were recorded.

2.4.9 Texture Analysis

Sausage texture was analyzed using a TA-XT Texture Analyzer (Stable Micro System Ltd., Godalming, UK) with Texture Exponent (version 32) analysis software. A modified TPA method adapted from Choi et al. (13) was employed to measure hardness (N), springiness (mm), cohesiveness, adhesiveness, and chewiness (Nmm) of sausage samples. Four samples (20 mm height and 22 mm diameter) from each formulation were analyzed under the following conditions: pretest and test speed of 2.0 mm/s, post-test speed of 5.0 mm/s, and 50% strain.

2.5 Sensory Analysis

A 9-point hedonic sensory test was conducted with 50 untrained panelists who were regular sausage consumers. Sausage samples were steamed at 100°C for 5 minutes, cut into 15 mm pieces, and placed in coded plastic cups with lids. The order of presentation was randomized. Panelists evaluated the samples for color, appearance, flavor, texture, juiciness, and overall liking.

2.5 Statistical Analysis

Analysis of variance (ANOVA) and Duncan's multiple range test were used to determine significant differences ($p \leq 0.05$) among treatments for chemical, physical, and sensory data. Data analysis was performed using IBM SPSS Statistics (Version 29).

3. RESULTS AND DISCUSSION

3.1 Chemical Qualities

Table 3 presents the chemical composition of sausages with varying levels of holy basil powder (HBP) substitution. As expected, increasing HBP led to a significantly higher moisture content ($p < 0.05$) compared to the control. This is likely due to the higher water-holding capacity of HBP, a phenomenon observed in studies using makgeolli lees fibers in frankfurters (10). HBP did reduce fat content in sausages. The control (0% HBP) contained 31.74% fat, while the highest HBP level (15%) resulted in a 29.23% fat content. While this is a modest reduction of approximately 2.5%, it aligns with findings from studies using other plant-based fibers as fat substitutes, such as brown rice fiber and apple pomace fiber (13, 14). However, this reduction may not be substantial enough to warrant health claims like "low-fat" or "reduced-fat" according to specific regulatory standards. These claims often require a more significant fat reduction compared to a reference product. Nevertheless, the observed fat reduction, though small, is still a positive outcome from a nutritional standpoint. It contributes to a lower overall saturated fat intake, which is associated with a reduced risk of chronic diseases. The addition of dietary fiber from HBP further enhances the nutritional profile of the sausage.

The addition of HBP significantly increased crude fiber content ($p < 0.05$), as expected due to its high dietary fiber content. Sausage pH remained within the typical range (5.0-5.8) across all HBP levels, consistent with reports using oat fiber as a fat replacer (17). Maintaining a suitable pH is crucial for sausage quality, ensuring microbial stability, proper protein functionality, and flavor development. Holy basil's antioxidant properties and phenolic compounds were successfully incorporated into the sausages. Increasing HBP led to significantly higher ($p < 0.05$) total dietary fiber (TDF), insoluble dietary fiber (IDF), soluble dietary fiber (SDF), antioxidant activity, and total phenolic compounds. The increase in TDF aligns with studies like Alves et al. (18) on green banana flour in bologna-type sausage. Holy basil's insoluble fiber components likely account for the IDF increase, while smaller amounts of soluble fibers contribute to SDF (19). Enhanced antioxidant activity and phenolic content could lead to improved shelf life and reduced formation of harmful by-products during cooking (20), similar to findings using olive by-products (21) and carrot fiber extract (22).

Table 3. Chemical qualities of fiber-fortified sausages with varying holy basil powder (HBP) as a fat replacer

Chemical qualities	Treatments			
	0%HBP	**9%HBP**	**12%HBP**	**15%HBP**
Moisture (%)	48.85 ± 0.03^{d}	48.90 ± 0.08^{c}	49.00 ± 0.04^{b}	50.30 ± 0.07^{a}
Ash (%)	1.92 ± 0.01^{d}	2.24 ± 0.01^{c}	2.36 ± 0.01^{b}	2.49 ± 0.01^{a}
Fat (%)	31.74 ± 0.08^{a}	31.33 ± 0.09^{b}	30.40 ± 0.08^{c}	29.23 ± 0.09^{d}
Crude fiber (%)	1.47 ± 0.08^{d}	3.64 ± 0.04^{c}	3.90 ± 0.08^{b}	4.44 ± 0.02^{a}
Protein (%)	11.60 ± 0.04^{d}	12.15 ± 0.08^{c}	12.20 ± 0.06^{b}	12.45 ± 0.07^{a}
Carbohydrate (%)	4.42 ± 0.11^{a}	1.74 ± 0.11^{c}	2.14 ± 0.05^{b}	1.09 ± 0.09^{d}
pH	5.50 ± 0.05^{a}	5.60 ± 0.05^{a}	5.50 ± 0.06^{a}	5.40 ± 0.01^{a}
TDF (%)	0.56 ± 0.01^{d}	2.46 ± 0.03^{c}	3.06 ± 0.01^{b}	3.58 ± 0.01^{a}
IDF (%)	0.31 ± 0.01^{d}	2.03 ± 0.04^{c}	2.61 ± 0.01^{b}	3.13 ± 0.02^{a}
SDF (%)	0.33 ± 0.05^{b}	0.43 ± 0.12^{a}	0.45 ± 0.14^{a}	0.46 ± 0.05^{a}
Antioxidant activity (mg Trolox/g sample)	0.33 ± 0.03^{b}	0.37 ± 0.01^{ab}	0.39 ± 0.01^{a}	0.40 ± 0.02^{a}
Total phenolic compound (mg GAE/ g sample)	0.60 ± 0.04^{b}	0.61 ± 0.01^{b}	0.72 ± 0.05^{a}	0.78 ± 0.03^{a}

*Different superscript letters within a row indicate significant differences among treatments ($p<0.05$)

3.2 Physical Qualities

Table 4 indicates a significant decrease in cooking loss ($p<0.05$) from 9.36% to 7.92% with increasing HBP levels. This reduction is likely due to HBP's soluble dietary fiber, which improves water-holding capacity within the sausage (23). Similar findings were reported in burger patties using brewer's spent grain fiber (24). Water activity (a_w), a crucial factor in sausage safety, quality, and shelf life, was not significantly affected by HBP levels ($p>0.05$). The observed a_w range (0.84-0.86) falls within the ideal range for intermediate-moisture foods (0.60-0.90), indicating that HBP substitution maintains desirable water activity (25).

The incorporation of HBP influenced sausage color (Figure 1). HBP-containing sausages exhibited significantly lower L* (lightness) and a* (redness) values compared to the control ($p<0.05$) (Table 4), likely due to the lower fat content, higher water content, and HBP's potential antioxidant properties, protecting oxymyoglobin from oxidation to metmyoglobin (26). Similar observations were reported with brown rice and pineapple fibers in fat-reduced frankfurters (3). Conversely, b* values (yellowness) significantly increased ($p<0.05$). This is likely due to two combined influences: (i) the reduction in the white/yellowish fat content, allowing naturally occurring chromophores in the sausage to become more prominent, and (ii) the potential contribution of yellow-brownish pigments from the holy basil powder itself (27).

Texture Profile Analysis (TPA) revealed changes in textural properties with HBP addition. Table 4 shows a significant increase ($p<0.05$) in hardness. This could be because replacing fat with dietary fiber often strengthens texture (3,16). The smaller particle size of HBP might also facilitate its integration into the meat protein matrix, enhancing gel strength and overall hardness (28). Chewiness increased similarly ($p<0.05$), potentially due to HBP's gel-like properties and viscosity, mimicking the texture of fat. Conversely, springiness, cohesiveness, and adhesiveness decreased significantly ($p<0.05$). This could be because HBP's gel-like nature increased sausage viscosity, reducing the ability to return to the original state and resist secondary compression. HBP integration into the meat protein network might also increase rigidity, further affecting springiness and cohesiveness (29).

Table 4. Physical qualities of fiber-fortified sausages with varying holy basil powder (HBP) as a fat replacer

Physical qualities	Treatments			
	0%HBP	9%HBP	12%HBP	15%HBP
Cooking loss (%)	9.36 ± 0.15^{a}	9.14 ± 0.19^{b}	8.64 ± 0.14^{c}	7.92 ± 0.15^{d}
Water activity	0.86 ± 0.01^{a}	0.85 ± 0.01^{a}	0.86 ± 0.01^{a}	0.84 ± 0.01^{a}
L*	59.65 ± 0.61^{a}	49.08 ± 0.50^{b}	47.48 ± 0.54^{c}	43.29 ± 0.50^{d}
a*	5.81 ± 0.08^{a}	2.23 ± 0.10^{b}	2.13 ± 0.09^{c}	1.95 ± 0.08^{d}
b*	21.20 ± 0.31^{d}	21.52 ± 0.19^{c}	22.27 ± 0.14^{b}	22.73 ± 0.17^{a}
Hardness (N)	68.99 ± 1.29^{d}	76.29 ± 1.25^{c}	80.27 ± 1.12^{b}	83.85 ± 1.32^{a}
Springiness (mm)	0.93 ± 0.01^{a}	0.94 ± 0.03^{a}	0.88 ± 0.02^{b}	0.85 ± 0.06^{c}
Cohesiveness	0.79 ± 0.18^{a}	0.78 ± 0.13^{a}	0.74 ± 0.17^{b}	0.66 ± 0.16^{c}
Adhesiveness	4.64 ± 0.89^{a}	3.86 ± 0.83^{b}	3.80 ± 0.89^{c}	3.46 ± 0.85^{d}
Chewiness (Nmm)	33.03 ± 1.85^{d}	39.44 ± 1.65^{c}	46.46 ± 1.68^{b}	49.08 ± 1.57^{a}

*Different superscript letters within a row indicate significant differences among treatments ($p<0.05$)

Figure 1. Fiber-fortified sausages with varying holy basil powder (HBP) as a fat replacer

3.3 Sensory Qualities

Sensory evaluation results (Table 5) indicate a progressive decline in overall liking scores with increasing holy basil powder (HBP) levels. Samples with 15% HBP received significantly lower scores than the control. This could be due to HBP's impact on sensory characteristics. Higher HBP content might lead to firmer, darker, and potentially coarser sausages, influencing mouthfeel and overall acceptability (30). Interestingly, sausages with 9% HBP exhibited sensory scores similar to the control (0% HBP). This suggests the potential for successful partial fat substitution using HBP without significantly compromising consumer acceptance.

Table 5. Sensory liking scores of fiber-fortified sausages with varying holy basil powder (HBP) as a fat replacer

Attributes	Treatments			
	0%HBP	9%HBP	12%HBP	15%HBP
Color	7.88 ± 1.39^{a}	7.04 ± 1.43^{b}	6.78 ± 1.24b^{c}	6.09 ± 1.34^{d}
Appearance	7.65 ± 1.12^{a}	6.89 ± 1.11^{b}	6.53 ± 1.26^{c}	6.12 ± 1.21^{c}
Flavor	7.84 ± 1.22^{a}	7.01 ± 1.32^{b}	6.80 ± 1.28^{b}	6.00 ± 1.32^{b}
Texture	7.89 ± 0.96^{a}	7.51 ± 0.91^{b}	6.66 ± 0.99^{c}	6.00 ± 1.11^{d}
Juiciness	7.81 ± 0.99^{a}	6.82 ± 1.01^{b}	6.58 ± 1.05^{b}	6.10 ± 1.03^{b}
Overall liking	7.82 ± 1.11^{a}	7.36 ± 1.08^{b}	6.58 ± 1.13^{c}	5.88 ± 1.10^{d}

*Different superscript letters within a row indicate significant differences among treatments ($p<0.05$)

4. CONCLUSIONS

Incorporating holy basil powder (HBP) as a fat replacer in pork sausages successfully increased fiber content, antioxidant activity, and phenolic compounds, while maintaining consumer acceptance at the 9% HBP level. This demonstrates HBP's potential for creating healthier sausages. To further enhance HBP's efficacy as a fat substitute, future research should optimize HBP preparation methods (e.g., particle size reduction, pre-treatment), explore synergistic effects with other ingredients, and address consumer preferences regarding color and texture. This multifaceted approach can unlock the full potential of HBP as a functional ingredient in developing healthier and more appealing meat products.

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CP-P097

Production and Quality of Sweet Fish Sauce (Nam Pla Wan) Whipping Cream

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ABSTRACT

Sweet fish sauce is a sweet dipping sauce usually served with sour fruit such as green mango and pineapple. The production of whipping cream products with sweet fish sauce was studied according to the needs of the Phuket Kaew entrepreneur to develop a new product that can be sold in the market. The optimal amount of sweet fish sauce for producing whipping cream was 15% (w/w of total ingredient weight). The foam capacity and foam stability of the sweet fish sauce whipping cream were gradually increased with sweet fish sauce concentration from 5% to 15% then declined when reached 20% ($p \leq 0.05$). On the other hand, the overrun value was not significantly different from the control ($p > 0.05$) but decreased when adding sweet fish sauce up to 20%. The addition of 15% sweet fish sauce received the highest liking scores and showed higher values in the textural characteristics including hardness, adhesiveness, cohesiveness, and chewiness than the control. The chemical compositions of the sweet fish sauce whipping cream resulted in significantly more ash, carbohydrate, and energy than the control ($p \leq 0.05$). In terms of shelf life, the sweet fish sauce whipping cream can be kept in the refrigerator for at least 15 days.

1. INTRODUCTION

Sweet fish sauce or "Nam pla wan" is a famous dipping sauce in Thailand that is sweet, sticky, slightly salty, and a hint spicy. The main ingredients are palm sugar, shrimp paste, fish sauce, shallots, dried shrimp, and chili. It is usually served with a green mango or sour fruits. It perfectly combines green mango and sweet fish sauce [1].

Whipping cream is cream that can be whipped from a liquid form into little peaks of creamy goodness[2]. Whipped cream, a popular dairy product, is cream already whipped and widely used for topping desserts, ice cream, cake, and some beverages [3,4]. Whipping cream is an emulsion-based structure that contains water, sugars, fat, protein, stabilizers, and emulsifiers and is stable during frozen storage [4,5]. A source of fat preparation for whipping cream is dairy milk. The two-phase oil-in-water emulsion is transformed into a three-phase system when the cream is whipped. The gathered air bubbles are trapped in a network of fat globules, including some proteins that will be denatured [6]. Whipped cream is an air-in-water foam that is a film containing fat droplets surrounding air cells and is stable by a protein film. There is some clumping of the fat globules in the cell walls of the foam, and the fat is partially solidified, preventing the collapse of the cell walls [7].

Desirable properties of whipped cream are a short whipping time, a high overrun, sufficient stiffness in the whipped cream to allow shaping, and lack of syneresis. Overrun is the percentage increase in volume when cream is whipped. The desirable overrun is greater than 80% [8].

The Phuket Kaew entrepreneur producing sweet fish sauce, beverages, and ice cream from sour mango wanted to use whipped cream with sweet fish sauce flavor as a topping. Therefore, this research aims to develop sweet fish sauce whipping cream including studying quality and shelf life as part of providing academic services to entrepreneurs in Phuket, Thailand.

2. MATERIAL AND METHODS

2.1 *Material and Chemicals*

The sweet fish sauce obtained from the Phuket-Kaew entrepreneur was blended with a blender and filtered to separate the liquid parts for the production of sweet fish sauce whipping cream (SFW). All chemicals used in the investigation were analytical grade.

2.2 *Preparation of Sweet Fish Sauce Whipping Cream*

The ingredients used for making SFW based on the recipe [9] are shown in Table 1. Sweet fish sauce was added to whipping cream making in the following percentages: 0%, 5%, 10%, 15%, and 20% (w/w of total ingredient weight). The whipping cream process was started by mixing the sterilized milk with bloomed gelatin, then sugar was added and mixed evenly. It is pasteurized at 72 ^{o}C for 15 seconds, packed in aluminum foil bags then cooled at 4 ^{o}C, and stored in the refrigerator (10 ^{o}C) until use.

Table 1. Formulation of SFW with five different percentages of sweet fish sauce.

Ingredients	**Quantities (g)**				
	Control 0%	**5%**	**10%**	**15%**	**20%**
Milk	200	200	200	200	200
Gelatin	5	5	5	5	5
Sugar	2.80	2.80	2.80	2.80	2.80
Water	50	50	50	50	50
Sweet fish sauce	0	12.89	25.78	38.67	51.56

2.3 *Determination of foaming properties*

In the study of the foaming properties of the SFW, 100 ml of each sample was poured into a 1,000 ml beaker and whipped with a hand-blender at the highest level speed for 5 minutes. The foaming capacity (FC) and foaming stability (FS) were measured by observing the volume of the whipped creams in the beakers for 60 minutes [10,11] and calculated with Eq(1), Eq(2) and Eq(3) [11].

$$\%\ \text{Foam capacities} = \frac{V2-V1}{V1} \times 100 \quad (1)$$

$$\%\ \text{Foam stabilities} = \frac{V60}{V1} \times 100 \quad (2)$$

$$\%\ \text{Overrun} = \frac{(MUW-MW)}{MW} \times 100 \quad (3)$$

while, V1: Volume of foam before homogenization (mL); V2: Volume of foam after homogenization (mL); V60: Volume of foam after 60 minutes (mL); MUW: mass of unwhipped cream with a certain volume; MW: mass of whipped cream with a certain volume.

2.4 *Sensory evaluation*

Thirty panelists evaluated the sensory properties (appearance, foam stability, flavor, texture, and overall liking) according to a 9-point hedonic scale.

2.5 *Textural characteristics analysis*

The textural characteristics of SFW were investigated by filling SFW into the cylindrical mold and adjusting the height of SFW foam to have an equal height of mold [12]. The texture profile analysis was measured. Foam structure properties were performed by hardness index. In addition, the hardness (g), adhesiveness (mJ), cohesiveness, and chewiness (mJ) were determined.

2.6 *Chemical analysis*

Proximate composition i.e. moisture, protein, fat, ash, carbohydrate, and energy was determined using the standard method of AOAC [13].

2.7 *Determination of microbial quality of SFW*

The samples of SFW were placed in aluminum foil bags, and stored in the refrigerator (10 °C). The microbiological tests were evaluated every 3 days for 15 days to determine the total plate count (TPC) [13].

2.8 *Statistical analysis*

The experiments were performed in triplicates and all results were expressed as mean ± standard deviation. The study's data were subjected to an analysis of variance (ANOVA) using SPSS. Significant differences among mean values were determined by Duncan's multiple range tests (DMRT), while pairwise comparison using the Least significant difference (LSD) with a 95% confidence interval that was significantly different ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1 *Foaming properties*

The various amounts of sweet fish sauce in SFW showed the effect on their FC, FS, and overrun in Table 2. The results showed that the FC of SFW at 5%, 10%, 15%, and 20% (w/w of total ingredient weight) were lower than the control (0%). The FS at 10% and 15% were higher than control. As the sugar, the main ingredient in sweet fish sauce increased, the soluble solids content, interface protein concentration, and fat coalescence increased so a more effective structure of whipped cream was formed to stabilize the aerated system, thereby leading to higher firmness [14]. The overrun values of 5%, 10%, and 15% showed no significant difference from the control ($p>0.05$) except at 20%. It was observed that at 20% the values of FC, FS, and overrun were the lowest because of the increase of stickiness and thus inhibited the formation of air bubbles in the structure of the whipped cream in the whipping process to create air bubbles [15].

Table 1. Effect of sweet fish sauce amount on foam properties of SFW.

Sweet fish sauce (%)	FC (%)	FS (%)	Overrun (%)
0	490.22 ± 0.11[a]	96.64 ± 1.74[b]	83.05 ± 0.40[a]
5	476.89 ± 0.62[b]	96.96 ± 1.33[b]	82.97 ± 0.43[a]
10	483.89 ±0.01[ab]	98.26 ± 1.99[a]	82.86 ± 0.31[a]
15	488.44 ± 0.34[ab]	98.77 ± 1.47[a]	83.10 ± 0.28[a]
20	467.89 ± 0.20[b]	95.15 ± 1.55[b]	81.64 ± 0.37[b]

FC, foaming capacity; FS, foaming stability; Data are expressed as means ± SD value. Different letters within the same column are significantly different at $p \le 0.05$.

3.2 *Sensory evaluation*

The appearance of SFW is shown in Figure 1, where it can be observed that the color of the whipped cream becomes darker as the percentage of sweet fish sauce increases. The sensory evaluation results of SFW are shown in Table 3. SFW with 5% sweet fish sauce added received a likeability score that was no different from the control ($p > 0.05$). While, increasing sweet fish sauce up to 15% showed the highest likability scores in foam stability, flavor, and overall liking ($p \le 0.05$), but decreased at 20% because of the too-strong flavor of sweet fish sauce and poor texture of SFW.

Figure 1. SFW was added with different percentages of sweet fish sauce.

Table 3. Sensory evaluation of SFW.

Sweet fish sauce (%)	Sensory attribute				
	Appearance	Foam stability	Flavor	Texture	Overall liking
0	7.12 ± 1.17[b]	7.01 ± 1.19[b]	6.71 ± 1.14[b]	6.93 ± 1.11[ns]	7. 11 ± 1.03[b]
5	7.30 ± 1.19[b]	7.12 ± 1.01[b]	6.67 ± 1.13[b]	7.10 ± 1.14	6.92 ± 0.44[b]
10	7.71 ± 1.07[a]	7.80 ± 0.98[a]	7.74 ± 0.88[a]	7.13 ± 0.20	7.94 ± 0.98[a]
15	7.71 ± 1.19[a]	7.92 ± 1.08[a]	7.89 ± 1.03[a]	7.10 ± 0.11	7.98 ± 1.05[a]
20	7.22 ± 1.05[b]	5.91 ± 1.20[c]	6.08 ± 1.20[c]	6.99 ± 1.05	7.28 ± 0.14[b]

Data are expressed as means ± SD values obtained from 30 panelists. Different letters within the same column are significantly different at $p \le 0.05$. ns indicates not significantly different at $p > 0.05$.

3.3 *Textural characteristics analysis*

The result of the textural characteristics analysis of SFW is shown in Table 4. The SFW with 15% sweet fish sauce showed more hardness, adhesiveness, cohesiveness, and chewiness than the control ($p \le 0.05$). Because the sugar in the sweet fish sauce affects the foam properties such as the whipping index, the index of foam durability, and specific density gradually increases with sugar concentration [16].

Table 4. Texture profile analysis (TPA) of SFW compared with control.

TPA	Sweet fish sauce (%)	
	0%	15%
Hardness (g)	25.00 ± 1.00[b]	29.83 ± 1.04[a]
Adhesiveness (mJ)	0.64 ± 0.10[b]	0.82 ± 0.02[a]
Cohesiveness	1.17 ± 0.10[b]	1.57 ± 0.14[a]
Chewiness (mJ)	0.46 ± 0.09[b]	0.88 ± 0.10[a]

Data are expressed as means ± SD values obtained from 3 replications. Different letters within the same row are significantly different at $p \leq 0.05$.

3.4 *Chemical analysis*

The chemical composition of SFW is shown in Table 5. The addition of sweet fish sauce reduced the content of moisture and protein but ash, carbohydrate, and energy increased significantly ($p \leq 0.05$) compared to the control. While the fat content was not significantly different ($p > 0.05$). Normally dairy cream contains 16 to 40 % fat content [17]. SFW has approximately 24% more energy than the control because the main component of sweet fish sauce is sugar.

Table 5. The chemical composition of SFW compared with control.

Chemical composition (%)	Sweet fish sauce (%)	
	0%	15%
Moisture	70.32 ± 0.18[a]	58.69 ± 0.09[b]
Protein	4.15 ± 0.34[a]	3.55 ± 0.03[b]
Fat	16.18 ± 0.05[ns]	16.92 ± 0.11
Ash	0.52 ± 0.04[b]	0.84 ± 0.07[a]
Carbohydrate	8.83 ± 0.10[b]	20.01 ± 0.14[a]
Energy (Kcal)	197.54 ± 0.09[b]	246.52 ± 0.10[a]

Data are expressed as means ± SD values obtained from 3 replications. Different letters within the same row are significantly different at $p \leq 0.05$. ns indicates not significantly different at $p > 0.05$.

3.5 *Microbial quality of SFW*

Table 6 shows the effect of the storage period on total bacterial count. There showed no evidence of microbes (colony/gram) in both of control and SFW during storage, indicating that the SFW could be kept for more than 15 days.

Table 6. The effect of the storage period on total bacterial count of SFW.

Storage time (days)	Total bacterial count (cfu/g)	
	0%	15%
0	Not found	Not found
3	Not found	Not found
6	Not found	Not found
9	Not found	Not found
12	Not found	Not found
15	Not found	Not found

4. CONCLUSIONS

The optimum amount of sweet fish sauce used in SFW production was 15%, which gave the best FC, FS, and overrun values similar to the control. It also received the highest liking score from 30 panelists. The shelf-life of SFW was satisfactory in a refrigerator for more than 15 days. Therefore, it is suitable for Phuket Kaew entrepreneur to produce for commercial.

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Effect of Starch-Protein Composite Modified by High Pressure Processing on the Characteristics of Gluten Free Bread

CP-P126

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ABSTRACT

Developing a good quality gluten free bread (GFB) is a challenge due to lacking of viscoelastic proteins, gluten. High pressure processing (HPP) is non-thermal processing that can be used to alter functional properties of food biopolymers such as starch and protein. The aim of this study was to evaluate the effect of substitution tapioca starch in mixed gluten free flour by starch-protein composite composed of tapioca starch and whey protein isolate, modified by HPP at 500 MPa for 20 min at the levels of 0, 2.5, 5.0, 10.0, and 20.0% w/w on the characteristics of GFB. Results showed that the characteristics of GFB were affected by the level of starch-protein composite replacement. Specific volume decreased with increasing levels of composite substitution level, although no significant different ($p>0.05$) between the sample at 2.5% substitution and control. Hardness, springiness, and chewiness of GFB crumb increased ($p\leq0.05$) with increasing levels of the substitution from 0 to 10%, then decreased at 20% of substitution. The composite replacement 0-20% had no effect on the cohesiveness of GFB crumb ($p>0.05$). The substitution also increased b^* of bread crumb but decreased L^* of that. The results indicate that starch-protein composite could be used as a potential ingredient to improve the quality of GFB.

1. INTRODUCTION

Bread is the staple food of many area in the world, typically made from a dough of wheat flour, water, yeast and other ingredients such as salt and sugar. Gliadin and glutenin are major proteins in wheat that can form a gluten network supporting dough elasticity and retain fermentation gases (CO_2) [1].

However, gluten is one of the proteins that trigger an immune reaction in the small intestine called celiac disease that is a serious public health problem worldwide and found more than 1.4% of the world's population [2]. Gluten contamination in food or bakery products, leading to malnutrition, diarrhea, growth retardation, anemia, and fatigue [3]. Therefore, avoiding gluten containing products is a necessary decision.

Gluten free (GF) bakery products are typically produced from combining of several native starches and grains such as, rice, corn, oat, buckwheat or millet. However, a bread lacking in gluten often has a poor overall appearance, taste, and low nutritional value [4]. To enhance the quality and nutritional value of GF products, several researchers have attempted to supplement with various GF cereals [5,6], proteins [7,8], dietary fibers [9], hydrocolloids [10,11], emulsifiers [12], and enzymes [13]. Moreover, studies on modification processes, including chemical, enzymatic, and physical modifications of native starch and protein have gained great interest in the literature. These modifications may be applied to gluten free bread (GFB) to solve the problem and improve properties of starches and proteins. Aungsuttivong [14] demonstrated that enzymatic hydrolysis can improve the functional properties of egg white protein and can be used as a food ingredient to enhance the GFB quality.

High pressure processing (HPP) is an attractive option for physical modification, as it does not involve harmful chemicals. HPP selectively affects non-covalent bonds, causing structural damage to biopolymers like starch and protein [15]. This structural damage affects starch gelatinization [16], increasing ability to hold water at low temperatures and increasing viscoelasticity [17]. Moreover, HPP can induce protein denaturation, exposing hydrophobic regions and sulfhydryl groups to the surface, which can affect their functional properties, including emulsification and rheological properties [18]. According to early studies, HPP treatment improves the functional properties of GF cereals [19] enhances baking performance by increasing batter elasticity [20] and reduce retrogradation rate [21]. Thus, the utilization of HPP on starch-protein composites might enhance their functional properties and improve the GFB quality. Therefore, the objective of this study was to use starch-protein composite (tapioca starch: TS-whey protein isolate: WPI) modified by HPP at 500 MPa for 20 min by replacing TS in mixed flour with the composite at various levels, including 0.0, 2.5, 5.0, 10.0, and 20.0% to improve quality of GFB.

2. MATERIALS AND METHODS

2.1 Materials

Rice flour (Erawan Brand) was purchased from Cho Heng Rice Vermicelli Factory Co., Ltd., Nakhon Pathom, Thailand. Tapioca starch (Newgrade) was purchased from Thai Wah Co., Ltd., Bangkok, Thailand. Job's tears flour (B-natural) was purchased from Bangyai Supply Ltd., Nonthaburi, Thailand. Honey, apple sauce, apple cider vinegar, salt, soybean oil, sugar and instant dry yeast were purchased from local supermarkets in Pathum Thani, Thailand. Whey protein isolate was purchased from Matell Intertrade Co., Ltd., Khonkaen, Thailand. Xanthan gum and guar gum were purchased from Chemipan Corporation Co., Ltd. Bangkok, Thailand.

2.2 Starch-protein composite preparation

Starch-protein composite modified by high pressure processing (HPP) was prepared by mixing 10% tapioca starch (TS) and 5% whey protein isolate (WPI) (w/w) in distilled water for 2 h with moderate stirring at room temperature (25±5 ˚C), then 10 g of the mixture was poured into 7cm×10cm polyethylene bag and hermetically sealed. The suspension immediately was kept at 4 ˚C for 24 h to ensure full hydration. The suspension was treated with HPP (HPP-600 MPa/5.0L, KeFa High Pressure Food Processing Co., Ltd., Baotou, China) at 500 MPa for 20 min. Then, the sample was

freeze-dried using aDL-0.5 Freeze Dryer (Kinetic Engineering Co., Ltd., Thailand), ground and sieved (100 mesh or 149 µm screen), then packed in aluminum foil laminated polyethylene bag and stored at -18 ˚C for further use.

2.3 *Gluten free bread (GFB) preparation*

The formulation of GFB was slightly modified from Aungsuttivong [14]. Composition of batters in percentage of total weight was shown in Table 1. The process was as follows: Instant dry yeast was added into 40˚C sugar solution followed by gentle stirring and stand for 5 min. Then the yeast suspension and dried ingredients were mixed for 4 min in a bowl of mixer equipped with a flat beater (BM6178, China) at medium speed (speed 3 from 6). Next, honey, apple sauce and cider vinegar were added and mixed at the same speed for 0.5 min, then adjusted to high speed (speed 6 from 6) and continued mixing for 2.5 min. After that, the soybean oil was added and mixed at medium speed for 0.5 min and adjusted to high speed for 5.5 min. Then the batter was proofed at 35 ˚C for 30 min and mixed again at low speed (speed 2 form 6) for 20 s. After that, seventy grams of homogeneous batter was placed into an aluminium pan (70x54x35 mm) and applied a thin layer of soybean oil on the surface, then proofed at 35 ˚C for 15 min, baked at 180 ˚C for 20 min in the domestic oven (HÄFELE, HBO-9FUN70L, German) and cooled at room temperature for 1 h, then packed in a low-density polyethylene (LDPE) bag and stored at room temperature for 24 h before analysis.

Table 7. Composition of gluten free bread (GFB) batters (in percentage of total weight)

Replacement of composite (%)	Tapioca starch	Starch-protein composite	Other ingredients*
0.0 (Control)	15.00	0.00	85.00
2.5	14.62	0.38	85.00
5.0	14.25	0.75	85.00
10.0	13.50	1.50	85.00
20.0	12.00	3.00	85.00

* Composed of 30% water, 16% rice flour, 13% honey, 11% apple sauce, 8% Job's tears flour, 2.55% soybean oil, 1.5% sugar, 1.2% instant dry yeast, 0.7% salt, 0.5% cider vinegar, 0.3% xanthan gum and 0.25% guar gum.

2.4 *Specific volume measurement of bread*

The specific volume of the GFB was determined by rapeseed displacement method according to AACC [22].

2.5 *Texture profile analysis of bread*

Texture profile analysis (TPA) of the GFB was measured by using a texture analyzer (TA-XT Plus, Stable Micro System, UK). Sample was cut from the center of the loaf into a cubic shape (20x20x20 mm^3) and compressed to reach 40% deformation by a P/50 aluminum cylinder probe. The test condition for TPA was: 2 cycles with 5 s delay, pre-test speed 1.0 mm/s, test speed 5.0 mm/s and post-test speed 5.0 mm/s. Hardness, cohesiveness, springiness and chewiness were reported [23].

2.6 *Crumb color analysis of bread*

The color of the GFB was evaluated by colorimeter (CX2687, Color Flex, USA). Color analysis was performed on three different samples taken from the center of the crumb. The measured parameters were L^* (brightness), a^* (red-green) and b^* (yellow-blue

2.7 *Statistical analysis*

Experiments were performed triplicate. Data were analysed by analysis of variance (ANOVA) using the SPSS for windows version 26.0 (SPSS Inc., USA). Duncan's multiple range test were used to compare means with significance determined at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 *Effects of starch-protein composite on gluten free bread (GFB) quality*

The effect of replacing tapioca starch with composite on the specific volume of GFB is showed in Table 2. The replacement of 0.0% composite (control) and 2.5% composite resulted in the highest specific volume, with no significant difference ($p>0.05$). Whereas, replacing composite more than 2.5% (5.0, 10.0 and 20.0%) significantly decreased ($p \leq 0.05$) in the specific volume from control sample. This decrease may be attributed to HPP treatment at 500 MPa inducing starch gelatinization, which interrupts gas expansion of dough. Consequently, there was a limited rise of dough, and small gas cells merged into larger and irregular gas cells, resulting in a decreased specific volume. A similar effect was reported by Roman et al. (2019), who found that the specific volume decreased with the addition of extruded banana starch [24]. This is consistent with the findings of Roman et al. (2020) and Trappey et al. (2015) [25,26].

Moreover, HPP can change the structure of protein molecules by exposed more surface hydrophobic regions. As a result, proteins can interact with neighboring proteins and form thicker, more flexible films around the air bubbles, which can benefit the retention of gas cells [27]. This is related to the high specific volume of replacing at 2.5% composite, which created small, uniform air cells as shown in Figure 1B. However, the results of the experiment showed that replacing at higher level of composite resulted larger air cells in GFB, as shown in Figure 1C-E. This suggests that excessive composite could interrupt the expansion of GF dough, which is related to the decrease in specific volume.

Table 2. Effect of starch-protein composite on specific volume of gluten free bread

Replacement of composite (%)	Specific volume (cm^3/g)
0.0 (Control)	$2.55^{ab} \pm 0.04$
2.5	$2.61^{a} \pm 0.05$
5.0	$2.46^{c} \pm 0.01$
10.0	$2.42^{c} \pm 0.04$
20.0	$2.49^{bc} \pm 0.02$

*Means within a column with different superscripts are significantly different ($p \leq 0.05$)

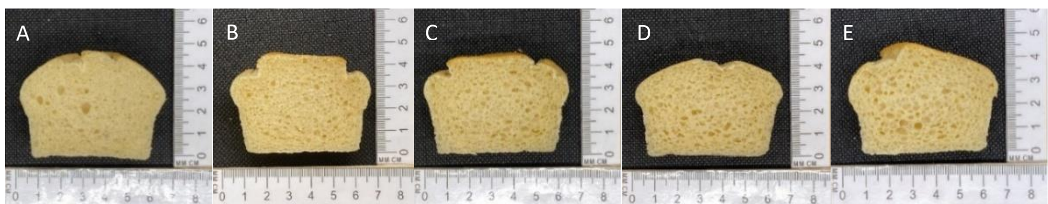

Figure 1. Images of cross-section and air cell of gluten free bread at various levels of starch-protein composite (A: 0.0% (control), B: 2.5%, C: 5.0%, D: 10.0%, and E: 20.0%)

The texture profile analysis of GFB is showed in Table 3. An increase in crumb hardness was associated with a decrease in specific volume, as shown in Tables 2 and 3. Replacing the composite at different levels significantly increased (p≤0.05) crumb hardness compared to the control sample. The highest hardness was observed at 10.0% composite. In terms of cohesiveness, all GFB samples showed no significant difference (p>0.05) from the control sample. However, replacement at 5.0% and 10.0% composite showed a tendency toward increased springiness and chewiness compared to the control sample. This suggests that the composite could improve the strength of the structure due to the exposure of hidden hydrophobic regions after HPP. This exposure induces proteins to interact with nearby proteins, thus providing high foaming stability that can form thicker, more cohesive, and viscoelastic film around each air cell [27]. Whereas, replacing at 20.0% composite exhibited larger air cells (Figure 1E), indicating a weak structure, resulting in the lowest springiness and chewiness.

Table 3. Effect of starch-protein composite on texture profile of gluten free bread crumb

Replacement of composite (%)	Hardness (g)	Springiness	Cohesiveness^{ns}	Chewiness
0.0 (Control)	349.65^{d} ± 2.26	0.27^{b} ± 0.01	0.16 ± 0.01	16.11^{b} ± 0.18
2.5	364.69^{c} ± 3.92	0.23^{c} ± 0.02	0.16 ± 0.01	14.30^{c} ± 0.66
5.0	375.60^{b} ± 5.36	0.32^{a} ± 0.00	0.16 ± 0.00	18.98^{a} ± 0.08
10.0	399.89^{a} ± 0.21	0.31^{a} ± 0.01	0.16 ± 0.00	19.84^{a} ± 0.91
20.0	373.04^{b} ± 0.40	0.19^{d} ± 0.00	0.17 ± 0.01	11.69^{d} ± 0.51

*Indicate no significant difference between means (p>0.05)
Means within a column with different superscripts are significantly different (p≤0.05)

Bread color is one of the most important indicators of its quality. The L^*, a^*, and b^* values of the bread crumb is showed in Table 4. Replacement with the composite decreased in L^* value (lightness) and increased in b^* value (yellowness), significantly different (p≤0.05) from the control sample. This is a consequence of the replacement with the composite promoting the Maillard reaction between amino acids from WPI and reducing sugars in the GFB recipes, leading to a golden brown color, which is accepted by consumers [28]. However, there were no significant differences (p>0.05) in a^* value among different replacement levels.

Table 4. Effect of starch-protein composite on color parameters of gluten free bread crumb

Replacement of composite (%)	L^*	$a^{*\,ns}$	b^*
0.0 (Control)	56.47^{a} ± 0.52	1.15 ± 0.06	14.43^{b} ± 0.01
2.5	53.33^{b} ± 0.19	1.21 ± 0.06	15.02^{a} ± 0.13
5.0	53.45^{b} ± 0.30	1.14 ± 0.05	15.05^{a} ± 0.14
10.0	53.97^{b} ± 0.45	1.17 ± 0.05	15.02^{a} ± 0.01
20.0	53.23^{b} ± 0.21	1.16 ± 0.04	15.07^{a} ± 0.07

* Indicate no significant difference between means (p>0.05)
Means within a column with different superscripts are significantly different (p≤0.05)

4. CONCLUSIONS

The objective of this research was to overcome the limitations of gluten free bread (GFB) by using high pressure processing (HPP). The results showed that the replacement of the composite modified by HPP at 500 MPa for 20 min in GFB demonstrated that replacing at a suitable level (10.0% composite) had the potential to improve the quality of GFB by enhancing the strength of the structure with higher springiness and chewiness. Additionally, it also improved color of GFB. Whereas,

excessive replacement of the composite was weakened the bread structure, leading to negative effect in terms of decreasing specific volume and increased larger pore size. Therefore, an optimum level of replacement with starch-protein composite can improve the quality in GFB. However, detailed studies should be conducted on consumer acceptability and shelf life.

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Effect of Amaranth (*Amaranthus viridis*) Enriched White Bread Product on Nutritional Values and Antioxidant Capacity

CP-P210

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ABSTRACT

White bread is a popular food that is high in energy but low in other nutrients. This research aims to increase the nutritional value of white bread by using amaranth (*Amaranthus viridis*), a local plant with high nutritional value. Production of amaranth powder by blanching amaranth leaves at 80 °C for 2 min, then blending thoroughly and drying at 60 °C for 3 h. Amaranth powder supplementation in bread ranges from 0% (control), 2.5%, 5%, 7.5%, and 10% (w/w of flour weight). The results showed that bread fortified with 5% amaranth powder received the highest acceptance score, especially in the attributes of appearance, color, taste, and overall preference ($p \leq 0.05$). Fortification with amaranth powder significantly decreased L*, a*, and cohesiveness however it increased b*, hardness, springiness, and chewiness significantly compared to the control group. The addition of amaranth powder resulted in bread having higher nutritional values than the control, including protein, fat, ash, and crude fiber, while the carbohydrate content was reduced ($p \leq 0.05$). The antioxidant capacity of bread supplemented with amaranth powder by DPPH and ABTS assays was 8.34 and 2.71 TEAC (mg TE/g sample (dry basis)), respectively, and were greater than the control.

1. INTRODUCTION

Amaranth (*Amaranthus viridis*) is an herb with an upright, light green stem that is eaten as a vegetable in many parts of the world [1] including Thailand. Amaranth leaves and stems are good sources of proteins (about 35%), especially of essential amino acids for adults. It has been reported that 17 amino acids were detected in amaranth leaves from 20 standard amino acids that are components of proteins such as methionine, lysine, and asparagine [2]. In addition, it is also a source of dietary fiber and rich in minerals, i.e. magnesium, calcium, potassium, copper, phosphorus, zinc, iron, and manganese [2-4] and it has many potential pigments such as chlorophylls and carotenoids [5]. Moreover, amaranth leaves and seed extracts contain varied types of pharmacologically active compounds with antioxidant and antimicrobial activities such as vitamin C, flavonoids, phenolics, and tannins [2-5]. The results from several studies indicate that amaranth leaves have the potential to be used as a nutrient source to alleviate macro- and micronutrient deficiencies [7].

Several researchers reported the phytochemicals and antioxidants of amaranth leaves and stems. These natural antioxidants act as types of active oxygen (ROS) scavengers in the human body [8] and reactive nitrogen species produced as a result of oxidation are linked with different degenerative disorders such as cardiovascular diseases, cancer, cataracts, atherosclerosis, retinopathy, arthritis, emphysema, and neurodegenerative diseases [6,9].

White bread is generally considered less healthy than brown or whole wheat bread because it is made from refined flour, which has been stripped of its fiber, vitamins, and minerals during processing. Even though people generally know that whole wheat or whole grain bread has higher nutritional value. However, white bread is popular for consumption because it has a good taste, odor, and softness. Including, bread is one of the most versatile foods that can be personalized and fortified, responding to the needs of consumers from those with gluten intolerance to those with diabetes or cardiovascular disease. The addition of vegetable powders into bread can improve nutritional values such as fiber, polyphenol, antioxidant capacity, and rheological properties [10]. Therefore, this research aims to increase the nutritional value of white bread with amaranth, which are vegetable with high nutritional value that are easily available and inexpensive.

2. MATERIAL AND METHODS

2.1 Material and chemicals

The amaranth leaves were obtained from the local market. Other ingredients for the bread recipe were bread wheat flour, yeast, sugar, salt, milk powder, butter, and water. All chemicals used in the investigation were analytical grade.

2.2 Preparation of amaranth powder

The amaranth leaves were washed, cut into pieces, blanched at 80 °C for 2 min then dried at 60 °C for 3 h, ground into a fine powder, and stored in zipper bags for further use.

2.3 Preparation of white bread supplemented with amaranth powder

The ingredients used for making bread are shown in Table 1. Amaranth powder was added to white bread in the following percentages: 0% (control), 2.5%, 5%, 7.5%, and 10% (w/w of flour weight). The typical bread process involves mixing ingredients until the flour is converted into a dough, followed by baking the dough into a loaf.

Table 1. Five formulations of white bread supplemented with amaranth powder.

Ingredients	Quantities (g)				
	Control 0%	2.5%	5%	7.5%	10%
Bread flour	200	200	200	200	200
Yeast	1	1	1	1	1
Sugar	25	25	25	25	25
Salt	3	3	3	3	3
Milk powder	10	10	10	10	10
Butter	20	20	20	20	20
Water	130	130	130	130	130
Amaranth powder	-	5	10	15	20

2.4 Sensory evaluation

The bread of each sample was sliced 12 mm in thickness and ready to eat, without prior heating requirements. The sensory method was a 9-point hedonic scale with 30 untrained consumers (1= dislike extremely, 9= like extremely). The sensory aspects that were used in the evaluation were appearance, color, flavor, taste, texture, and overall preference.

2.5 Physicochemical properties analysis

Proximate composition i.e. moisture, protein, fat, ash, crude fiber, and carbohydrate was determined using the standard method of AOAC [11]. The color of bread samples was analyzed with a colorimeter (Hunter Lab), where L*, a*, and b* values were recorded. The texture profile analysis (TPA) of bread was evaluated by using a texture analyzer in the attributes of hardness, cohesiveness, springiness, and chewiness.

2.6 Antioxidant activity analysis

2.6.1 Extraction of bread for antioxidant activity analysis

The extraction of bread sample to determine the antioxidant activity including DPPH and ABTS assay. Briefly, 1 g of bread was ground and mixed with 1 ml of solvents acetone/water mixture (70:30 v/v); agitation with a vortex for 5 min at room temperature. Later on, the mixture was centrifuged at 800× g for 10 min [12]. Afterward, the supernatant was collected to analyze the antioxidant capacity following the procedure of Shimamura et al. and Sui et al. with some modifications [13,14].

2.6.2 DPPH radical scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical cation method was determined with the partial modification procedure of Shimamura et al. as follows: 100 μl of bread extract and 3.9 ml of DPPH solution (60 μM) were mixed in a cuvette and then incubated in the dark for 13 h at room temperature. Afterward, the absorbance of the mixture solution was measured at 515 nm using a UV-spectrophotometer. Trolox was used as the standard. The DPPH radical scavenging activity of bread was reported as Trolox equivalent antioxidant activity (TEAC) in mg Trolox equivalent (TE)/g sample (dry basis) [13].

2.6.3 ABTS radical scavenging activity

The ABTS (2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)) radical cation method was determined with the partial modification procedure of Sui et al. as follows: 100 μl of bread extract and diluted ABTS radical cation (ABTS•+) solution (3.9 mL) were mixed in a cuvette and then kept in the dark at room temperature for 30 min. Afterward, the absorbance of the mixture solution was measured at 734 nm using a UV spectrophotometer. Trolox was used as the standard. The ABTS value was reported as TEAC in mg TE/g sample (dry basis) [14].

2.7 Statistical analysis

The experiments were performed in triplicates and all results were expressed as mean ± standard deviation. The data were subjected to an analysis of variance (ANOVA) using SPSS. Significant differences among mean values were determined by Duncan's multiple range tests (DMRT), while the pairwise comparison of the mean using the Least significant difference (LSD) with a 95% confidence interval was significantly different ($p \leq 0.05$).

3 RESULTS AND DISCUSSION

3.1 *Sensory evaluation of bread*

The sensory evaluation results of bread supplemented with amaranth powder are shown in Table 2. Bread fortified with 5% amaranth powder showed significant differences in appearance, color, taste, and overall preference (p≤0.05). It was observed that when the amount of amaranth powder added to bread was higher than 5%, the liking scores of all attributes decreased with scores in the range of 5.04-7.11 (neither liked nor disliked to moderately liked). Therefore, 5% amaranth powder is the appropriate amount for adding to white bread.

Table 2. Sensory evaluation of white bread supplemented with different percentages of amaranth powder.

Sensory attribute	Amaranth powder substitution (%)				
	0	2.5	5	7.5	10
Appearance	7.12 ± 0.16[b]	6.84 ± 0.07[b]	7.46 ± 0.08[a]	6.51 ± 0.15[bc]	6.22 ± 0.28[c]
Color	7.44 ± 0.05[b]	7.42 ± 1.02[b]	7.61 ± 0.05[a]	7.02 ± 0.19[c]	6.47 ± 0. 08[d]
Flavor	7.36 ± 1.15[a]	7.34 ± 0.18[a]	7.40 ± 1.01[a]	6.87 ± 1.20[b]	6.11 ± 0.06[c]
Taste	7.43 ± 0.03[b]	7.48 ± 0.38[b]	7.77 ± 0.07[a]	7.11 ± 1.08[c]	6.72 ± 0.04[d]
Texture	7.21 ± 1.03[a]	7.17 ± 0.17[a]	7.28 ± 0.11[a]	6.50 ± 0.06[b]	5.46 ± 0.05[c]
overall preference	7.45 ± 0.15[b]	7.34 ± 0.08[b]	7.76 ± 0.08[a]	6.53 ± 0.47[c]	5.04 ± 0.09[d]

Data are expressed as means ± SD values obtained from 30 untrained consumers. Different letters within the same row are significantly different at p≤0.05.

3.2 *Physicochemical properties of bread*

The appearance of white bread supplemented with different percentages of amaranth powder is shown in Figure 1. Different amounts of amaranth powder affected the appearance of bread. The color of the bread with amaranth powder added was green which is color-based of amaranth. In addition, the color of the bread gets darker when more amaranth powder is added.

Figure 1. White bread supplemented with different percentages of amaranth powder.

The result of the color and texture analysis of bread fortified with 5% amaranth powder compared with the control shown in Table 3. The addition of amaranth powder resulted in a decrease in the L* and a* values of the bread, while the b* value increased. Where the L* represents lightness from black to white on a scale of zero to 100, while a* and b* represent chromaticity with no specific numeric limits. Negative a* corresponds with green, positive a* corresponds with red, negative b* corresponds with blue and positive b* corresponds with yellow [15].

The texture profile analysis of bread fortified with 5% amaranth powder compared with control showed that the hardness, springiness, and chewiness increased when added amaranth powder, while cohesiveness decreased (Table 3). The amaranth bread had lower cohesiveness than

the control due to lower intermolecular force [10] while cohesiveness indicates the intensity of internal bonds of the structure that can be changed due to the addition of amaranth powder to the bread. While an increase in hardness was related to springiness and chewiness: hardness is the maximum force of food after compression that can represent the response of the bread during mastication compression between molars; springiness is the ability of a product to return to the original shape after initial compression that indicates the response of the structure when the bread is tested by hand compression; chewiness represents the energy needed for the destruction of a solid sample giving useful information on the consumer's perception during mastication.

Table 3. Color and texture profile analysis (TPA) of bread fortified with 5% amaranth powder compared with control.

Physical properties	Amaranth powder substitution (%)	
	0%	**5%**
L*	74.80 ± 1.68[a]	59.18 ± 2.80[b]
a*	8.36 ± 0.48[a]	-7.83 ± 1.45[b]
b*	25.28 ± 1.05[b]	31.52 ± 5.16[a]
Hardness (g)	59.33 ± 20.15[b]	81.33 ± 46.73[a]
Cohesiveness	0.97 ± 0.01[a]	0.80 ± 0.64[b]
Springiness (mm)	0.24 ± 0.15[b]	0.35 ± 0.82[a]
Chewiness (mJ)	10.64 ± 9.14[b]	46.59 ± 37.09[a]

Data are expressed as means ± SD value. Different letters within the same row are significantly different at $p \leq 0.05$.

The chemical composition of bread fortified with 5% amaranth powder compared with the control shown in Table 4. The addition of 5% amaranth powder increased the moisture, protein, fat, ash, and crude fiber content of bread ($p \leq 0.05$). Because the proximate composition of the amaranth leaves on a dry weight (DW) basis consists of 35% protein, 5.26 % fat, 21% ash, and 14% crude fiber [2].

Table 4. The chemical composition of bread fortified with 5% amaranth powder compared with the control.

Chemical composition (%)	Amaranth powder substitution (%)	
	0%	**5%**
Moisture	32.58 ± 0.07[b]	34.57 ± 0.16[a]
Protein	7.40 ± 0.23[b]	9.98 ± 0.13[a]
Fat	5.02 ± 0.05[b]	6.10 ± 0.12[a]
Ash	1.34 ± 0.02[b]	1.62 ± 0.01[a]
Crude fiber	0.00 ± 0.01[b]	2.83 ± 0.12[a]
Carbohydrate	53.65 ± 0.27[a]	44.98 ± 0.06[b]

Data are expressed as means ± SD values obtained from 3 replications. Different letters within the same row are significantly different at $p \leq 0.05$.

3.3 Antioxidant activity of bread

The antioxidant activity of bread is shown in Table 5. The DPPH and ABTS activity of amaranth powder before adding it to bread were 56.10 and 34.22 mg TE/g sample (dry basis) respectively. The bread fortified with 5% amaranth powder had significantly higher antioxidant capacity than the control both in the DPPH and ABTS assay ($p \leq 0.05$).

Table 5. The antioxidant activity of bread fortified with 5% amaranth powder compared with the control.

Antioxidant activity TEAC (mg TE/ g sample (dry basis))	Amaranth powder substitution in bread (%)		Amaranth powder
	0%	5%	
DPPH	0.53 ± 0.01[b]	8.34 ± 0.06[a]	56.10 ± 0.02
ABTS	0.26 ± 0.21[b]	2.71 ± 0.02[a]	34.22 ± 0.01

Data are expressed as means ± SD values obtained from 3 replications. Different letters within the same row are significantly different at $p \leq 0.05$.

4 CONCLUSIONS

The finding of this research was used as a guide to design a recipe for bread or other food with the addition of amaranth powder. This is because amaranth leaves are a source of many beneficial nutrients. Bread recipes containing 5% amaranth powder can be used to develop functional breads because they are sensory accepted as well as adding nutritional value that is beneficial to the health of consumers.

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Influence of Emulsifiers on the Characteristics of Eggless Mung Bean Protein Sponge Cake

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ABSTRACT

Driven by the growing popularity of plant-based diets, researchers are seeking innovative ways to adapt traditional recipes while promoting sustainability. This study investigated the potential for completely replacing egg proteins with mung bean protein (MBP) in sponge cakes. The impact of different emulsifiers, namely glycerol monostearate (GMS), lecithin (LC), and their combined blend (LC-GMS), on the quality of these eggless cakes were evaluated. The findings demonstrated that both individual and combined use of emulsifiers significantly improved the properties of the eggless cakes. Compared to the egg-based control, cakes formulated with MBP alone exhibited batter with higher viscosity, lower density, and a reduced specific volume. However, the addition of emulsifiers, particularly the LC-GMS blend, significantly increased the specific volume of the eggless cakes. While MBP alone resulted in a softer and less chewy texture compared to the control, the incorporation of emulsifiers further reduced hardness and chewiness. Proximate composition analysis revealed that eggless cakes made with LC and GMS displayed superior chemical properties ($p<0.05$) compared to traditional egg-based cakes, indicating a potential nutritional advantage. In conclusion, this study suggests that mung bean protein, when combined with a blend of emulsifiers, shows promise as a viable egg substitute for cake production.

1. INTRODUCTION

The demand for eggless cakes is on the rise, driven by various factors. These include dietary restrictions, such as those followed by vegetarians and individuals with allergies, as well as ethical considerations related to animal welfare. Additionally, health-conscious consumers, particularly those concerned about heart disease, are increasingly choosing egg-free alternatives. Furthermore, the growing trend towards plant-based diets, driven by sustainability concerns and environmental awareness, has encouraged the search for suitable egg alternatives in baked products [1].

In sponge cakes, eggs play a vital role in creating and maintaining the foam structure in the batter. They facilitate the incorporation of air during mixing and contribute to gel formation, crucial for achieving specific volume, texture qualities, and flavor [2]. The development of eggless cake formulations not only enhances dietary inclusivity but also aligns with the evolving preferences of consumers seeking healthier and more sustainable food options. Several studies have suggested that proteins from plant sources like soy, pea, chickpea, lentil, and sunflower cake [1,3,4] can potentially substitute for eggs.

Mung bean protein, a byproduct generated during vermicelli production, is attracting global attention due to its high nutritional value and functional properties. This underutilized resource presents an opportunity for the food industry to add value and reduce waste. Previous study indicate that mung bean protein concentrate can be used in sponge cake recipes, but it tends to result in inferior crumb structures [5]. To overcome this limitation, emulsifiers are usually employed, as they serve an important role in eggless baking by stabilizing air bubbles, improving texture, and extending shelf life [1]. Research has shown that incorporating various emulsifiers, such as polyglycerol ester, lecithin, and distilled monoglyceride significantly improves the characteristics of eggless cakes. These improvements include increased porosity, volume, and overall sensory acceptance [6-8]. Soy lecithin contains phospholipids with both hydrophilic and lipophilic properties, making it effective for stabilizing oil-water mixtures in batter system. Being plant-derived, it is suitable for vegan-friendly product. Glycerol monostearate (GMS) is a synthetic emulsifier with amphiphilic properties similar to lecithin, enabling it to stabilize oil-in-water (O/W) emulsions. Additionally, GMS acts as a softening agent by forming complexes with starch, which helps delay the retrogradation process of baked goods during storage.

Therefore, the objectives of this study were to explore the impact of various emulsifiers including GMS and LC on the attributes of eggless sponge cakes formulated with mung bean protein, in comparison to conventional egg-based sponge cakes. Gaining insights into the interaction between emulsifiers and mung bean protein will guide the creation of sustainable and nutritious baked goods that meet the increasing demand for plant-based alternatives.

2. MATERIAL AND METHODS

2.1 Raw Materials

Split mung bean (Raithip brand) and additional ingredients for sponge cake preparation included cake flour, baking soda, sugar, almond milk, margarine, vanilla, salt, egg, were purchased from hypermarket in Bangkok, Thailand. Food grade emulsifiers including glycerol monostearate (INS471) and soy lecithin (INS322) were obtained from local supplier.

2.2 Preparation of mung bean protein

The mung bean protein was extracted following the modified method of alkaline extraction and acid precipitation [9]. In brief, split mung beans were blended with water at a 1:1 ratio, then the pH was adjusted to 9.0 using baking soda before centrifugation (4000 rpm, 10 min) (Thermo sorvall legend XRT centrifuge, Massachusetts, USA) to obtain the supernatant. The pH of the extracts was subsequently adjusted to 4.5 with vinegar to precipitate the protein, which was then recovered through centrifugation (4000 rpm, 15 min) (Thermo sorvall legend XRT centrifuge, Massachusetts, USA). Subsequently, it was dried at 55°C for 12 hours (SOV420A drying oven, Beijing, China) to obtain mung bean protein powder.

2.3 Eggless sponge cake preparation

The cake formulation consisted of the following ingredients for the control cakes: 17% wheat flour, 14% sugar, 14% shortening, 12% almond milk, 0.5% salt, 0.5% baking powder, 2% vanilla, and 38% whole egg. For the eggless cakes, a 20% mung bean solution, equivalent to the proportion of whole egg in term of moisture content, was used. The treatments involved varying concentrations of emulsifiers (w/w) added to the sponge cake at 1%. Treatments include the following: control (egg cake), MBP (Mung bean protein cake), LC (1% Lecithin), GMS (1% glycerol monostearate), and LC-GMS (0.5% lecithin + 0.5% glyceryl monostearate).

The preparation process for the sponge cake entailed sifting wheat flour with baking powder and salt. Eggs or mung bean protein, sugar, and emulsifiers were combined in a mixing bowl (Philips mixer, 350W, Canada) and whipped at high speed for 7 min. Flour was then added and mixed until homogeneous at a speed of 1 for 2 min, followed by the addition of almond milk and another mixing cycle. Subsequently, melted shortening was slowly added at low speed for 1 min. The batter was poured into a prepared mold and baked at 180°C for 30 min (Chaichana, CBG-40SD, Thailand).

2.4 Analysis of control and eggless cake

2.4.1 Batter measurements

The Batter density was measured by filling it into an aluminium cup immediately after removal from the mixer, levelling it off with a rubber spatula and then weighing it. The density of batter was calculated by dividing the weight of the batter (W1) by the weight of distilled water (W2) that filled the same cup.

Batter viscosity was measured using a Rapid Viscoanalyser (RVA-4) (Newport Scientific model 4-SA, Warriewood, Australia). A batter sample (28 g) were placed in an RVA aluminum canister with a plastic paddle and submitted to a viscosity analysis (160 rpm at 30 °C).

Batter emulsion stability was examined by placing batter in cylindrical plastic tubes and centrifuging at 5,000 rpm for 15 min (Thermo sorvall legend XRT centrifuge, Massachusetts, USA). The percentage of emulsion stability in cake batters was calculated by dividing the height of the stable emulsion left in the tube after centrifugation by the initial height of the batter that was transferred.

2.4.2 Cake quality determination

Specific volume or final cake volume was obtained using the rapeseed displacement method [10]. The cake was cut into 25 × 25 × 25 mm cubes. Then, one piece of cake was weighed (Wo), placed in a container and the rest of the container volume was filled with rapeseed (V2). The volume of the empty container (V1) was calculated by filling with rapeseed. Both V1 and V2 were later determined by a graduated cylinder and the difference between V1 and V2 was defined as the cake volume (Vo). The specific volume was then calculated as the ratio of the volume to weight (Vo/Wo).

Texture characteristics of cake crumb (firmness, cohesiveness, springiness and chewiness) were evaluated using a TA-XT texture analyzer (Stable Micro System Ltd., Surrey, UK). Crumb of the cake samples were cut into cube shapes with 2.5 cm diameter and they were compressed to 25 % of

their original height at a speed of 100 mm/min using a cylindrical probe (35 mm diameter) and a 5 kg load cell (11).

The crumb color of cake was determined by the colorimeter (Color Flex EZ, Hunter's color lab, Virginia, USA). The CIE Lab* system was used under D65 illumination with a 10° observer angle.

Color parameters, including lightness (L*), redness/greenness (a*), and yellowness/blueness (b*) were recorded.

The chemical analysis of cake samples followed AOAC recommendations (12). Moisture content, crude fat, crude protein, crude fiber, and ash were all evaluated. The total carbohydrate content was calculated by subtracting the moisture, ash, crude fat, and crude protein percentages from 100.

2.5 Statistical Analysis

The results were expressed as mean ± S.D. of three replicates. The experiment was conducted in a Completely Randomized Design (CRD). One-way analysis of variance (ANOVA) and Duncan's new multiple range test were carried out at a confidence level of 95%, utilizing the XLSTAT statistical software (Addinsoft, New York, USA).

3. RESULTS AND DISCUSSION

3.1 Batter characteristics

Batter characteristics are shown in Table 1. Batter viscosity is important in determining cake quality during baking since excess viscosity can prevent the cake from rising properly. We observed lower viscosity when comparing the control batter to eggless batters, which is consistent with previous research that used soy milk as an egg substitute in cakes [13]. This difference could be related to eggs' inherently lower viscosity compared to mung bean protein. The eggless cakes with mixed emulsifiers—lecithin (LC), and glycerol monostearate (GMS)—had the highest viscosity, which was consistent with prior research results [7]. The rise in viscosity of eggless cakes described by Turabi, Sumnu, and Sahin [14] could be attributed to the synergistic interaction between different emulsifiers in the batter. Overall, the results indicate that eggless cakes made with LC or GMS had slightly lower viscosity compared to those made with MBP and egg. However, no significant differences were observed among these samples ($p>0.05$). Therefore, it could be suggested that mung bean protein has the potential to be used as an egg replacer.

Low density is desirable in cake batter because it indicates that more air is incorporated into the batter. Results showed that all eggless batters had a better positive effect on batter density and aeration than the control egg cake. This result could be interpreted as an increase in batter viscosity caused by mung bean protein and emulsifiers. The high viscosity of the batter helps to retain more air bubbles during mixing and whipping, and it retards the rise of bubbles to the surface. Among eggless cakes, the density values ranged slightly from 0.45 to 0.49 g/cm^3. The formation and stabilization of foams benefit from the addition of emulsifiers, which aid aeration by lowering the surface tension between the liquid and gas phases, as reported in previous works [1,7,15].

The batter stability test results indicated lower stability for all of the eggless batters. However, no significant differences from the control egg cake were observed ($p > 0.05$). These findings are consistent with previous studies [1,7]. Since egg protein possesses high foaming ability and contributes to air bubble formation, it appears that in the control batter, egg components primarily

stabilize air within the water system. However, in eggless batters, the oil-in-water emulsion was stabilized by mung bean protein, while air bubbles were stabilized by the emulsifiers employed. Previous research [6] has suggested that the use of emulsifiers can enhance air bubble stability by aiding in the entrapment of small bubbles within the batter.

Table 1. Characteristics of batters and sponge cake elaborated with different emulsifiers

Sample	Batter viscosity (cP)	Batter density (g/cm^3)	Batter stability^{ns} (%)	Cake specific volume (cm^3/g)
Control	902.67 ± 52.0^b	0.52 ± 0.00^a	74.97 ± 1.97	3.28 ± 0.49^a
MBP	957.00 ± 49.43^b	0.46 ± 0.01^c	71.50 ± 0.98	2.15 ± 0.87^b
MBP-LC	917.67 ± 47.6^b	0.49 ± 0.01^b	71.45 ± 1.67	2.44 ± 0.41^b
MBP-GMS	921.00 ± 11.53^b	0.49 ± 0.00^b	71.24± 1.59	2.75 ± 0.42^a
MBP-LC-GMS	1063.67 ± 20.03^a	0.46 ± 0.00^c	71.88 ± 0.83	2.98 ± 1.01^a

*Results were expressed as mean ± SD. Means in the same column with different superscript letters are significantly different ($p \leq 0.05$). ns: Means within the same column were not significantly different ($p > 0.05$)

3.2 Cake characteristics

Consumers prefer high-volume cakes. The volume of a cake is influenced by protein stabilization, as it forms a strong thermal gel that preserves the cake's structure while baking [16, 17]. Specific volume measurements indicate that the control egg cake achieved the highest volume (Table 1), suggesting that egg proteins facilitate optimal gel formation. However, all eggless cakes displayed lower specific volumes due to the inferior foam stability and gelling properties of mung bean protein, as noted by Lin et al. [18], who utilized soy protein in their eggless yellow cakes. Furthermore, our results indicate that eggless cake batters had lower density and viscosity, theoretically yielding larger baked cakes. Surprisingly, this was not observed in our study. This discrepancy may be attributed to the reduced batter stability, which failed to retain air bubbles during mixing and baking, resulting in smaller baked cakes.

Results revealed that among eggless cakes, those prepared with mixed emulsifiers (LC-GMS) exhibited a specific volume value not significantly different from that of the control egg cake ($p > 0.05$) (Table 1). Furthermore, eggless cakes utilizing emulsifiers like LC or GMS displayed a higher specific volume compared to MBP cake without emulsifier. This phenomenon stems from the emulsifiers' ability to bind ingredients together, create oil-in-water emulsions, and aid in air entrapment within the batter. The use of blended emulsifiers could enhance cake structure, as previously observed in eggless cakes employing monoglyceride and emulsifier blends in rice cake [14, 19]. Additionally, it has been reported that emulsifiers can prolong wheat flour starch gelatinization during the baking process. These effects promote greater expansion of the batter, resulting in increased final cake volume. These findings align with previous research, where the combination of soy protein isolate with monoglyceride and soy lecithin elevated the specific volume of eggless cakes [18, 20].

3.3 Color attributes of cake

The color attributes of cakes are shown in Table 2. Brightness is represented by the value L*, redness by the value a*, and yellowness by the value b*. The addition of mung bean protein reduced the lightness, redness and yellowness values, giving rise to darker cakes than control egg cake. This is because whole eggs produce a brighter hue than mung bean protein, which has a milky green shade. This result aligns with other studies related to the addition of plant proteins in cakes [20,21]. Among eggless cakes, it was found that the type of emulsifier had no effect on the color appearance, as there were no significant differences between the values.

Table 2. Crust color parameters of sponge cakes elaborated with different emulsifiers

Sample	L*	a*ns	b*
Control	52.49 ± 1.35a	14.39 ± 0.47	38.26 ± 0.21a
MBP	40.19 ± 3.63b	12.46 ± 2.58	31.30 ± 1.32c
MBP-LC	40.43 ± 1.35b	12.75 ± 0.64	31.79 ± 0.73c
MBP-GMS	44.40 ± 2.57b	13.14 ± 0.87	34.84 ± 1.01b
MBP-LC-GMS	39.60 ± 3.67b	13.20 ± 3.60	32.31 ± 2.35c

*Results were expressed as mean ± SD. Means in the same column with different superscript letters are significantly different (p≤0.05). ns: Means within the same column were not significantly different (p>0.05)

3.4 Texture attributes of cake

The results of texture measurements are presented in Table 3. The hardness and chewiness of eggless cakes were found to be significantly lower than those of the control egg cake ($p < 0.05$). This finding is consistent with previous studies indicating that cakes incorporating egg white protein tend to exhibit higher hardness compared to those incorporating plant proteins from rice and pea [20]. Among the eggless cake samples, it was observed that the inclusion of emulsifiers led to a decrease in the hardness and chewiness of the cakes. However, the type of emulsifier used did not have a significant effect on these values ($p > 0.05$). Similar results regarding the use of emulsifiers in cakes have been reported, indicating that lecithin or a blend of lecithin with glycerol monostearate can effectively reduce the hardness of eggless cakes [6,21,22]. Generally, emulsifiers function by inhibiting the swelling of starch granules, thereby preventing amylose from leaching out of the granule and forming a film around it. This process leads to an increase in the gelatinization temperature of starch, which in turn reduces the firming effect of retrogradation [23]. Additionally, the helical structure of amylose, with its hydrophobic inner surface, allows emulsifiers to insert themselves into the helix and form a complex. Emulsifiers that readily form insoluble complexes with amylose demonstrate the most pronounced anti-firming and shelf-life-extending effects [24].

Cohesiveness and springiness were unaffected by the types of emulsifiers used in the formulation or by the addition of mung bean protein. This finding is in line with previous studies on the use of lentil protein in angel cake [22]. Research indicates that cohesiveness and springiness are directly impacted by the elastic network the proteins in the dough structure. A less elastic and cohesive crumb will have a poorly developed gluten network. The results indicate that the addition of emulsifiers or mung bean protein did not significantly affect the functionality of gluten.

Overall, the texture evaluation in Table 3 indicates a decrease in hardness in the eggless formulation, which may affect consumer acceptability. Therefore, further sensory evaluation is necessary to understand how this change impacts consumer preferences and to ensure the eggless formulation meets consumer expectations.

Table 3. Textural characteristics of sponge cakes elaborated with different emulsifiers

Sample	Hardness (g)	Springinessns	Cohesivenessns	Chewiness (g)
Control	342.81 ± 26.09a	0.93 ± 0.05	0.70 ± 0.03	206.34 ± 29.50a
MBP	196.43 ± 26.05b	0.98 ± 0.01	0.70 ± 0.02	134.92 ± 20.63b
MBP-LC	138.45 ± 24.44c	0.95 ± 0.03	0.67 ± 0.04	82.39 ± 13.54c
MBP-GMS	159.43 ± 43.66b	0.95 ± 0.01	0.68 ± 0.01	104.61 ± 30.42b
MBP-LC-GMS	130.06 ± 20.45c	0.92 ± 0.02	0.68 ± 0.02	81.54 ± 14.05c

*Results were expressed as mean ± SD. Means in the same column with different superscript letters are significantly different (p≤0.05). ns: Means within the same column were not significantly different (p>0.05)

3.5 Chemical composition

The proximate composition of egg cake (control) and eggless cakes with various emulsifiers is displayed in Table 4. The cake enriched with mung bean protein shows a slight decrease in protein and fat contents, whereas ash and fiber content increase, thereby enhancing the nutritional value of the cake. In terms of moisture content, the results indicated that both eggless cakes, with or without emulsifiers, had higher moisture content compared to the control with egg. This increase can be attributed to the emulsifiers, which are known to aid in retaining moisture by binding with water molecules [25]. Similar findings were observed in another study, where the use of emulsifiers such as lecithin and DMG, in combination with WPC as an egg replacer, resulted in increased water-holding capacity, a characteristic typically enhanced by such emulsifiers [6,26]. There were no statistically significant differences in moisture content values between eggless cakes, which ranged between 29-33% ($p > 0.05$). This suggests that the soft texture of eggless cakes, utilizing mung bean protein as an egg replacer, is facilitated by emulsifiers.

In terms of protein content, it was observed that eggless cakes contained approximately 5% protein, while control cakes with eggs contained about 6%. These values did not differ greatly. Additionally, fat plays a crucial role in enhancing texture, rheology, and overall quality of the product. The results regarding fat content indicated significant differences between the samples, with eggless cakes exhibiting significantly lower fat content compared to the control cakes ($p < 0.05$). Eggs are recognized as a rich source of lipids, including cholesterol, whereas plant proteins such as mung bean contain no cholesterol. Consequently, reducing fat content in eggless cakes carries health benefits.

Furthermore, the analysis of ash and fiber content revealed no significant differences among cakes prepared with various emulsifiers, suggesting minimal variance in these contents after the addition of emulsifiers. However, incorporating mung bean protein as an egg substitute resulted in higher ash and fiber content compared to control cakes with eggs, thereby enhancing the nutritional value of the cake.

Table 4. Proximate composition of sponge cakes elaborated with different emulsifiers

Sample	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Carbohydrate (%)
Control	25.34 ± 0.45^{b}	6.62 ± 0.53^{a}	28.86 ± 0.13^{a}	0.32 ± 0.04^{b}	4.73 ± 0.39^{c}	34.13 ± 0.77^{b}
MBP	29.03 ± 0.59^{a}	5.27 ± 0.45^{b}	22.96 ± 0.45^{bc}	0.42 ± 0.1^{a}	5.61 ± 0.09^{a}	36.71 ± 1.82^{a}
MBP-LC	33.13 ± 0.35^{a}	5.75 ± 0.36^{ab}	23.11 ± 0.05^{b}	0.45 ± 0.07^{a}	5.44 ± 0.18^{ab}	32.42 ± 0.15^{b}
MBP-GMS	31.52 ± 0.93^{a}	5.36 ± 0.16^{b}	22.83 ± 0.24^{c}	0.45 ± 0.07^{a}	5.44 ± 0.10^{ab}	35.30 ± 0.91^{a}
MBP-LC-GMS	32.40 ± 0.94^{a}	5.24 ± 0.12^{b}	23.89 ± 0.01^{b}	0.42 ± 0.04^{a}	5.60 ± 0.05^{b}	32.47 ± 0.84^{b}

*Results were expressed as mean ± SD. Means in the same column with different superscript letters are significantly different ($p \leq 0.05$)

4. CONCLUSIONS

In conclusion, the study found that cakes made with mung bean protein and emulsifiers, instead of eggs, had higher viscosity, lower density, and a lower specific volume. However, the addition of emulsifiers improved the volume of the eggless cakes. Additionally, mung bean protein as an egg substitute resulted in darker cakes with decreased lightness, redness, and yellowness

values. The eggless cakes were softer and less chewy compared to the control egg cake, and emulsifiers further softened and reduced chewiness. Analysis of the cakes' composition revealed that eggless versions had slightly lower protein and fat content, but higher ash and fiber content, making them potentially more nutritious. To summarize, this study suggests that mung bean protein can be a promising egg substitute in cakes, especially when combined with emulsifiers. Further research is needed on how consumers perceive these eggless cakes through sensory evaluation.

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Investigating the Utilization of Konjac Glucomannan for Synbiotic Drinking Jelly Development

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ABSTRACT

Konjac Glucomannan (KGM) constitutes a naturally water-soluble dietary fiber with thickening properties, enabling the formation of a soft gel that is conducive to applications such as producing drinking jelly. In addition, probiotics are beneficial microorganisms throughout the immune and gastrointestinal systems. Accordingly, merging natural fiber as a prebiotic with probiotics in a synbiotic system can enhance consumer health while safeguarding probiotic viability against unfavorable environmental factors. The purpose of this research was to develop synbiotic drinking jelly. The probiotic bacteria used in this experiment is *Lactobacillus paracasei* LYO 150. The ability of probiotic bacteria to use prebiotic substances was compared between commercial prebiotics, including Galactooligo-saccharide (GOS), Highly soluble inulin (HSI) and konjac glucomannan (KGM). The best pair of probiotics and prebiotics found in *L. paracasei* LYO 150 with GOS had the highest prebiotic score (0.1669). Konjac glucomannan was used to develop drinking jelly at different concentrations of 0.5, 0.75 and 1% w/v. The effects of alkaline solution were studied with potassium hydroxide (KOH) or sodium hydroxide (NaOH) at 0.14% v/v. The physical properties of drinking jelly were analysed, including viscosity, water holding capacity (WHC), pH, colour index and sensory evaluation. The result showed that the concentration of KGM increases, resulting in higher viscosity. The viscosity properties of the drinking jelly containing 0.75% w/v konjac glucomannan and sodium hydroxide closely resembled those of commercial products. In addition, *L. paracasei* LYO 150 can survive in synbiotic drinking jelly at a higher rate (8.35 Log CFU/g) during storage (21 days), greater than 6 Log CFU/g according to the requirement for viable probiotic cells in final products regulations. For sensory evaluation, it was found that synbiotic drinking jelly is similar to commercial products in appearance, viscosity and texture. Therefore, this synbiotic drinking jelly has the potential to be applied as a health food product for consumers.

1. INTRODUCTION

Konjac Glucomannan (KGM) is a naturally occurring water-soluble dietary fiber derived from the roots of the konjac plant, also known as *Amorphophallus konjac* [1]. KGM is a high molecular weight, water-soluble and neutral polysaccharide [1,2]. The molecules are rich in hydroxyl groups, making them easily dissolved in water, leading to high viscosity that forms a thick hydrocolloid even when used in low concentrations [3]. This property makes it one of the most versatile and economically functional hydrocolloids with industrial applications, including manufacturing foods, pharmaceuticals, and chemicals [4,5]. Thailand is both a centre of diversity for Amorphophallus and an emerging producer of economically significant Amorphophallus products. Among the Thai Amorphophallus are *A. muelleri*, can produce significant amounts of KGM while *A. konjac*, the primary species used for KGM production in China, does not occur in Thailand. Therefore, other native species may be more suitable as sources of KGM in the region [6]. KGM gained popularity in the food industry due to its unique properties, including its ability to form a gel when mixed with water. One of the typical applications of KGM is in producing drinking jelly [7,8]. When KGM is dissolved in water, it forms a soft gel that can be flavoured and shaped into various forms, including a jelly-like consistency suitable for drinking. This makes it a popular ingredient in low-calorie and low-sugar food products, particularly those targeted towards health-conscious consumers. Probiotics are live microorganisms that confer health benefits on the host. These beneficial microorganisms, often strains of bacteria such as *Bifidobacterium* and *Lactobacillus*, primarily affect the gastrointestinal tract. Those probiotics contribute to gut health by modulating the composition and activity of the gut microbiota and enhancing immune function [9]. However, the challenge of probiotic survival under adverse environmental conditions was previous studies; the unsuitable condition can compromise their viability and reduce their effectiveness in health benefits to the host. [10]. Prebiotics, such as inulin, oligofructose, and fructooligosaccharides (FOS), are non-digestible dietary fibers that selectively stimulate the growth and activity of probiotic [11]. In addition, KGM is increasingly recognised for its role as a prebiotic. KGM undergoes fermentation in the colon by probiotics, producing short-chain fatty acids (SCFAs) like butyrate, acetate, and propionate, which have various health-promoting effects [12]. Certainly, a synbiotic system refers to a combination of probiotics and prebiotics that work synergistically to confer health benefits on the host [13]. Synbiotics also can serve a protective role for probiotics by fostering the proliferation of probiotics in the gut; prebiotics contribute to their survival and persistence in the gastrointestinal tract, thus enhancing their potential health benefits. Moreover, the synergistic action of probiotics and prebiotics in synbiotics enhances their individual effects and promotes the survival and efficacy of probiotics, ultimately benefiting host health [14]. This research aimed to develop drinking jelly with synbiotic properties of konjac glucomannan (KGM). Synbiotic drinking jelly is a new food product that combines the concepts of probiotics and prebiotics with the format of a gelatinous jelly, offering a convenient and enjoyable way to contribute benefits to consumers. The gel-like consistency of the jelly provides an appealing texture, while the probiotics and prebiotics work together as potential health benefits into a single product.

2. MATERIAL AND METHODS

2.1 Material

Probiotic bacteria used in this experiment is *Lactobacillus paracasei* LYO 150 (Chr. Hansen, Denmark) and a cocktail of *Escherichia coli* ATTC strains isolated from the intestinal of Thai people (*E.coli* TISTR 073, *E. coli* TISTR 074 and *E. coli* ATCC25922) from Department of Medical Sciences, Thailand, aim to represent the microorganisms in digestive tract. Commercial prebiotic substances include Galactooligosaccharide (GOS) (CHEM POINT, Thailand) and highly soluble inulin (Orafti®HSI,

PDO, Thailand). Konjac glucomannan (KGM), produced from Thailand's raw material, was purchased from Yunnan Konjac Resources Developing Co., Ltd. (Kunming, China).

2.2 Determination of Prebiotic Activity Score (PAS)

The investigation aimed to assess the capability of commercial probiotic microorganisms, *Lactobacillus paracasei* LYO 150, to utilise prebiotics, employing a prebiotic cocktail of *Escherichia coli*. Probiotic microorganisms were cultured in De Man Rogosa (MRS) medium, while M9 minimal medium was used for *E.coli* cocktail. For *E.coli* cocktail strains (TISTR 073, TISTR 074, and ATCC25922), a ratio of 1:1:1. Konjac Glucomannan (KGM) Galactooligosaccharides (GOS), highly water-soluble inulin (Orafti®HIS), and glucose (Control) were used for prebiotic activity determination at 1% w/v. Initial microbial counts were standardised at 6.0 log CFU/mL, followed by a 24-hour incubation period at 37°C. Initial microbial counts were documented at 0 hours, followed by incubation at 37°C for 24 hours. To identify the potential synbiotic combination, the prebiotic activity score of each prebiotic-probiotic pair was determined by the method described previously [15,17]. The viable cells at 0 h and 24 h were determined and expressed as colony forming unit per mL (CFU/mL). The prebiotic activity score was calculated as shown in Equation 1:

$$\mathrm{PAS} = \left(\frac{\log P_x^{24} - \log P_x^{0}}{\log P_g^{24} - \log P_g^{0}} \right) - \left(\frac{\log E_x^{24} - \log E_x^{0}}{\log E_g^{24} - \log E_g^{0}} \right) \quad (1)$$

where Px and Pg are the probiotic CFU/mL when grown on test substrates and glucose, respectively; Ex and Eg are the enteric CFU/mL when grown on test substrates and glucose, respectively, and the superscript numbers represent the culture time (0 and 24 h). Suitable Probiotic-prebiotic pairs exhibiting the highest prebiotic activity scores were identified for further development in drink jelly with synbiotic properties.

2.3 Preparation of Synbiotic Drinking Jelly

Synbiotic Drinking Jelly was produced using glucomannan konjac powder, with variations in the concentration of KGM (0.5, 0.75, and 1% w/v) in combination with alkaline solutions of potassium hydroxide (KOH) and sodium hydroxide (NaOH). The mixture was stirred continuously at low speed for 1 hour at room temperature. Alkaline solutions of 0.5N KOH and 0.14% v/v NaOH were added, and concentrated fruit juice was used for flavour enhancement. The solutions were stirred for 5 minutes to form a soft gel and then pasteurised. Initial probiotic strains were standardised at 6.0 log CFU/mL with suitable prebiotic (1%w/v) by aseptic filling. The physical properties of the synbiotic drink jelly were determined for illuminant color using a Hunter Lab colorimeter (HunterLab, Reston, Virginia, USA). The viscosity was measured with a Brookfield viscometer (Model DV-II, Brookfield Engineering Laboratories, Massachusetts, USA). Additionally, pH value and water holding capacity were determined, and sensory testing with a 9-point hedonic score (70 persons) was used to evaluate product acceptance relative to commercial drinking jelly products. The sensory participation in the sensory testing were selected based on criteria including age, gender, dietary restrictions, random selection, and informed consent.

2.4 Viability of L. paracasei LYO 150 in synbiotic drink jelly during storage

The viability of probiotic in different drinking jellies, including the control set (CMC + probiotic), KGM drinking jelly (KGM + probiotic) and GOS-KGM drinking Jelly (GOS+KGM+probiotic), were evaluated over a period of 21 days (day 0, 1, 2, 3, 5, 7, 9, 14 and 21) by following a method modified

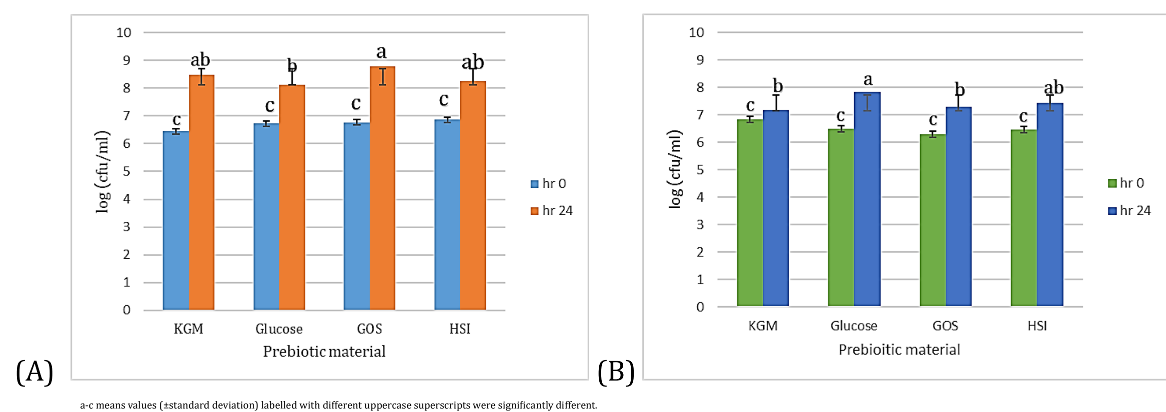
[16]. A drinking jelly sample (25 g) was dissolved in 225 mL of 0.85%(w/v) NaCl solution. The prepared suspension was then diluted, and the appropriate dilution ranges were plated out using the pour plate technique in MRS agar and incubated under anaerobic conditions at 37 °C for 72 h. Viability was reported as log CFU/ml.

2.5 Statistical analysis

All experiments were conducted in triplicate. Data were reported as means ± standard deviations (SD). Statistical analysis was performed using SPSS (version 22.0 software, IBM SPSS, Chicago, IL, USA). Statistical differences were determined using the general linear model (GLM), followed by Duncan's new multiple range test comparison of the means ($P < 0.05$).

3. RESULTS AND DISCUSSION

In the prebiotic activity score assay, cell density of all test probiotics and enteric mixture at 0 h did not differ among the broths added with different carbohydrates with an average concentration of 6.31±0.16 log CFU/mL and 6.60±0.07 log CFU/mL, respectively. The result is shown in Figure 1. On average, the density of probiotic cells increased by 3.04±0.20 log CFU/mL, while enteric bacteria grew less with an increase of 1.92±0.26 log CFU/mL (Figure 1). As for the growth of enteric bacteria after 24 h of incubation, the cell density of enteric bacteria increased by an average of 2.04 ± 0.19 log CFU/mL, which was lower than the growth of probiotics on all given prebiotics.



a-c means values (±standard deviation) labelled with different uppercase superscripts were significantly different.

Figure 1 Viability count of *Lactobacillus paracasei* (A) and *Escherichia coli* mixture (B) in different prebiotics at 0 hours and 24-hour incubation

This indicated that all commercial prebiotics and KGM's ability to support probiotics' growth was comparable with glucose. This property is one of the essential prerequisites for a substrate to have prebiotic potential [17]. Most bacteria can use glucose as a primary source, but not all bacteria can use prebiotics. The result reflected that these probiotics had a mechanism to use the test prebiotics as an energy source, as in previous studies [18-20]. For the prebiotic activity score, which reflects a substrate's specificity and ability to promote probiotics' growth, it is possible to perform rapid in vitro screening for the potential synbiotic combination [17]. Comparing prebiotic activity score, there were prebiotics that gave a significantly higher increase in several probiotic cell densities compared with medium added with glucose, namely GOS (0.1669^{a}±0.4), KGM (0.1437^{ab}±0.08) and water-soluble inulin (0.079^{b}±0.81), respectively. The results revealed that one probiotic strain preferred more than one type of prebiotic. *L. paracasei* was able to utilise various carbon sources; some enhanced the growth better than glucose [12-14]. The results of this experiment are consistent with previous research revealed that GOS containing glucose, galactose,

xylose, and fructose were found to have higher prebiotic activities than other types of prebiotics [20]. Additionally, it was noticed that KGM had prebiotic activity. This agrees with previous research that KGM had the potential to be a prebiotic, Moreover, KGM supports the viability of *L. paracasei* LYO-150 by providing nourishment, enhancing adherence, modulating gut microbiota, and producing beneficial metabolite [12,17,26]. Therefore, a combination of *L. paracasei* LYO 150 and GOS was chosen for developing symbiotic drinking jelly.

Konjac glucomannan was used to develop drinking jelly at different concentrations of 0.5, 0.75 and 1% w/v. The effects of alkaline solution were studied with potassium hydroxide (KOH) or sodium hydroxide (NaOH) at 0.14% v/v, while carboxy methyl cellulose (CMC) was used as a control as a commercial thickener. Characteristics of synbiotic drinking Jellies are shown in Table 1. The study found that increasing KGM concentration led to higher viscosity due to its thickening properties [2]. There were no significant differences in viscosity between drinking jelly with KOH or NaOH at 0.5% and 0.75% w/v concentrations. However, at 1% w/v KGM concentration, KOH-added drinking jelly showed higher viscosity than NaOH-added drinking jelly. Previous research indicates that KOH strengthens KGM gels more effectively than other alkali solutions [21]. KGM-based drinking jelly showed significantly higher viscosity than commercial thickeners like CMC. The optimal viscosity for drink jelly is between 1,104.4-1,032.4±57.6 mPa [22], similar to KGM 0.75% w/v with NaOH (1,091.0±69.29 mPa). Adding fruit juice to drinking jelly led to a notable decrease in pH levels within the acidic range (pH 3.7-3.8) compared to products without added fruit juice (pH 6.58-6.83). The colour parameter is a critical sensory attribute influencing consumer acceptance and purchase decisions. Analysis of colour parameters revealed no significant differences in the white index for KGM 0.5% and 0.75% w/v. However, the decrease in whiteness value and concurrent increase in colour intensity and colour difference (ΔE) was found in higher konjac glucomannan concentrations (KGM 1% w/v). This phenomenon is attributed to the dispersion of hydrocolloid polymers and water, reducing light scattering [23]. Meanwhile, analysis of the water-holding capacity revealed that no water layer separation occurred in all conditions. This indicates that drinking jelly effectively retains water, improving gel stability.

Table 1. Characteristics of drinking jellies with *Lactobacillus paracasei* LYO150 and GOS

Drinking Jelly Formula		Parameter				
		Viscosity	pH	ΔE	White Index	Color Intensity
KOH	0.5% KGM	850.05±59.75^{e}	3.76±0.01^{def}	83.300±1.12^{f}	96.034±0.006^{a}	40.210±0.01^{g}
	0.75% KGM	1717.50±74.24^{c}	3.77±0.00^{def}	90.875±0.10^{d}	94.890±0.003^{a}	41.540±0.05^{f}
	1% KGM	5984.00±15.18^{a}	3.77±0.007^{def}	93.240±0.01^{b}	73.985±0.764^{bc}	44.530±0.1^{c}
NaOH	0.5% KGM	860.25±11.10^{e}	3.75±0.007^{e}	89.390±0.04^{f}	94.4942±0.01^{a}	39.205±0.12^{h}
	0.75% KGM	1091.00±59.29^{c}	3.81±0.007^{f}	90.390±0.04^{d}	95.1757±0.036^{a}	46.325±0.09^{a}
	1% KGM	5327.50±4.96^{b}	3.77±0.007^{def}	94.150±0.11^{a}	75.9916±0.027^{bc}	46.245±0.035^{a}
CMC	0.5%	443.00±10.96^{f}	3.59±0.00^{bc}	90.470±0.05^{de}	96.5188±0.610^{a}	43.445±0.07^{d}
	0.75%	1523.50±57.98^{de}	3.58±0.01^{b}	90.105±0.03^{ef}	94.8901±0.011^{a}	44.390±0.04^{c}
	1%	3862.00±65.76^{b}	3.48±0.007^{a}	93.055±0.13^{b}	79.2414±0.781^{b}	45.405±0.09^{b}

*a-g mean values (±standard deviation) labelled with different uppercase superscripts were significantly different in column ($p<0.05$)

The sensory preference test shown in Table 2 shows the similarity between drinking jelly at KGM 0.5% w/v and 0.75% w/v g and commercial product in terms of viscosity and texture for KGM 0.75% w/v. This indicated that acceptability values were consistent with consumer preference

across the tested formulations. Therefore, KGM 0.75% w/v and NaOH were chosen to study probiotic survival in synbiotic drinking Jelly further.

Table 2. Sensory evaluation of drinking jelly from different concentrations of KGM

Parameter	Sensory score				
	Commercial Product (Jele' brand)	KGM 0.5% (NaOH)	KGM 0.75% (NaOH)	KGM 1% (NaOH)	CMC 0.75%
Appearance	$6.53±1.92^{ns}$	$6.33±1.54^{ns}$	$6.40±1.76^{ns}$	$6.20±1.69^{ns}$	$5.53±1.55^{ns}$
Flavours	$7.26±1.43^{a}$	$6.60±1.63^{b}$	$6.66±1.34^{b}$	$6.60±1.91^{b}$	$6.00±1.30^{bc}$
Viscosity	$6.80±1.61^{a}$	$5.33±1.64^{ab}$	$5.53±1.75^{ab}$	$4.46±2.19^{b}$	$4.33±2.09^{b}$
Texture	$7.13±1.55^{a}$	$6.40±1.45^{b}$	$6.93±1.62^{ab}$	$5.73±2.051^{b}$	$5.73±2.18^{b}$
Overall	$7.40±1.68^{a}$	$6.93±1.53^{b}$	$6.93±1.48^{b}$	$6.93±2.25^{bc}$	$6.46±1.84^{c}$

*a-c mean values (±standard deviation) labelled with different uppercase superscripts were significantly different in row ($p<0.05$)

Viability of probiotic bacteria in KGM drinking jellies after storage at 4 °c for 21 days during storage (Figure 2). The result found that KGM-GOS and KGM synbiotic drinking jelly can maintain the cell number growth from the first inoculum (8.56 log CFU/ml) with a slight decrease (0.5-0.25 log CFU/ml) during storage. KGM-GOS drinking jelly showed a higher survival rate of probiotics than KGM drinking jelly alone, especially on day 21. Compared with the control (without prebiotics), it resulted in the loss of probiotic cell viability in a 3.43 log cycle, and the viable cells dropped to lower than 6 log CFU/ml on day 14. The minimum viable probiotic cell density is required at a time of consumption to exert health effects and to ensure viable probiotic cells in final product regulations (> 6 log CFU/g) [24,25]. This indicated that drinking jelly without prebiotics had a relatively shorter shelf life than prebiotic-containing products. Taking both the prebiotic activity score and the survival rate into account, KGM could act as a synbiotic carrier, and the KGM drinking jelly with *L. paracasei* LYO150 and GOS was promising for future use.

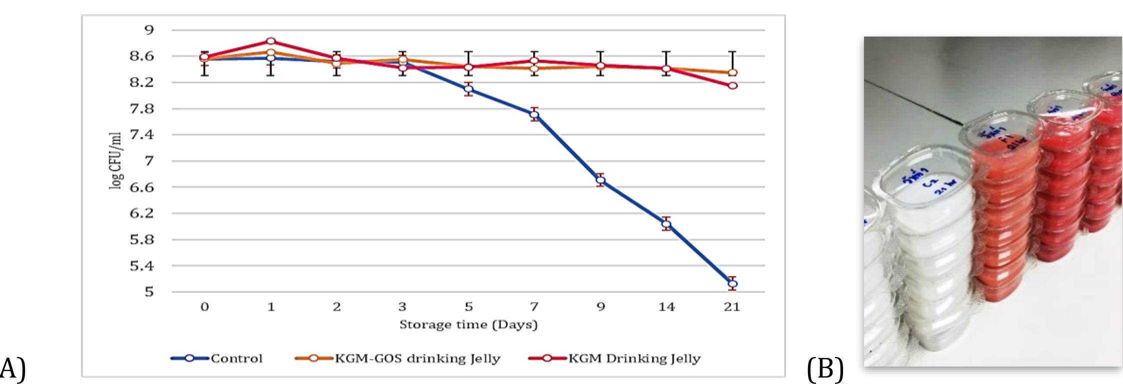


Figure 2. Viability of probiotic bacteria in drinking jelly after storage at 4 °c for 21 days (A) and KGM synbiotic drinking Jelly (B)

4. CONCLUSIONS

The study of synbiotic drinking konjac jelly presents prospects for functional food development. This study's findings reveal that the optimised formulation incorporates *Lactobacillus paracasei* LYO-150 and Galactooligosaccharide (GOS) into konjac drinking jelly. These include enhanced probiotic survival rates, favourable physical properties, and consumer acceptance.

Moreover, the study highlights the suitability of konjac glucomannan for protecting probiotics during processing and storage, further contributing to the product's stability and functionality. The synergistic effects of konjac glucomannan and prebiotics promote probiotic survival and enhance product attributes such as viscosity, texture, and sensory appeal. The synbiotic drink jelly offers both health-promoting benefits and consumer expectations. It contributes valuable insights into the potential applications of synbiotics in functional food formulations, paving the way for developing innovative and health-promoting products in the food industry.

5. ACKNOWLEDGEMENTS

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Comparative study of the mutagenic potential of conventionally and organically grown Chinese kale: a preliminary study in Nakhon Pathom province

DP-O024

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ABSTRACT

Pesticides have been extensively employed in the present to increase agricultural product quantities; however, the most of pesticides have been recorded for their detrimental effects on humans, such as induction of DNA mutations and eventually cancer. Agricultural products are typically produced in two ways: organic and conventional practices. Conventional practice mostly entails the use of pesticides and fertilizers, whereas organic practice is defined by pesticide-free management approach. Over several consecutive years, Chinese kale has been ranked as one of the ten vegetables most heavily contaminated with pesticides. However, there is no study on the comparison between the mutagenicity of Chinese kale planted in conventional and organic practices. Therefore, this study aimed to compare the mutagenic potential of conventionally and organically grown Chinese kale in Nakhon Pathom using Ames test. Nakhon Pathom was chosen because it has the largest Chinese kale growing area in Thailand. The results showed that conventionally grown Chinese kale contaminated with prothiophos, bifenthrin, cypermethrin and lambda-cyhalothrin. The concentrations of prothiophos, bifenthrin, and cypermethrin exceeded the maximum residue limits (MRLs) by 384, 9.4, and 4 times, respectively, while lambda-cyhalothrin was lower than the MRLs. Interestingly, both conventionally and organically produced Chinese kale revealed no mutagenicity potential as assessed by the Ames test using the standard guidelines (OECD guideline 471). Despite the fact that the genotoxicity of the two practices was comparable, consumers should be cautioned about conventionally grown Chinese kale due to the significant pesticide contamination and the fact that genotoxicity was not the only adverse effect observed of pesticides.

1. INTRODUCTION

Pesticides are commonly employed to increase productivity and ensure the quality of agricultural products [1]. Over the past decade, the agricultural industry has grown very competitive to maximize agricultural production, resulting in a rise in the usage of pesticides [2]. Several studies have indicated that chronic exposure to pesticides can produce detrimental health consequences, including cancer [3-5]. The International Agency for Research on Cancer (IARC) reported the carcinogenicity potential of five organophosphate pesticides, including glyphosate, malathion, and diazinon, which were classified as probably carcinogenic to humans, while tetrachlorvinphos and parathion were classified as possibly carcinogenic to humans [6]. In addition, the epidemiological data from Agricultural Health Study research, exposure to chlorpyrifos is related to an increased risk of breast, lung, and rectal cancer [7]. It has been established that pesticides induce cancer because of their capacity to attach to macromolecules, such as DNA and RNA, resulting in DNA mutations and genomic instability, which may eventually lead to cancer [8-10]. Many pesticides, such as chlorpyrifos and cypermethrin, have been shown to induce DNA mutations *in vitro* [11]. Moreover, 50 of the 228 pesticides were reported to be mutagenic when assayed with the bacterial reverse mutation assay (Ames test), which is one of the genotoxicity tests suggested by Organisation for Economic Co-operation and Development (OECD) [12].

Agricultural products are primarily cultured in two ways: organic practice and conventional practice. Organic approaches are known as management systems that do not utilize synthetic fertilizers or pesticides and instead focus on rotating crops, organic pest management, crop diversification, and soil enhancement with compost additions and animal and green manures [13], while the conventional approach is usually associated with high-input contemporary agriculture, which involves the use of synthetic chemical fertilizers, fungicides, herbicides, and pesticides [14]. As a result, conventional procedures may result in a greater presence of pesticide residues in the final product than organic practices.

According to the survey of the Thailand Pesticide Alert Network (Thai-PAN), Chinese kale was among the top ten vegetables contaminated with pesticide residues for a longer length of time, indicating the intensive usage of pesticides in this vegetable. Moreover, organophosphates and pyrethroids were commonly detected in Chinese kale [15-18]. Interestingly, Nakhon Pathom province has the largest Chinese kale growing area in Thailand (10,086.00 Rai) [19], which suggests the presence of a pesticide-mediated genomic instability risk factor. However, there is no study on mutagenicity in Chinese kale exposed to pesticides. Thus, this study aimed to compare the mutagenic potential of conventionally and organically grown Chinese kale in Nakhon Pathom province using Ames test. This study provides useful information in such a way as to obtain insight into the mutagenic potential of conventionally and organically grown Chinese kale, and it is important information for assessing risks and potential health effects on humans.

2. MATERIAL AND METHODS

2.1 Sample preparation

Conventionally and organically grown Chinese kale were collected from three planting areas in Nakhon Pathom province (Phutthamonthon, Nakhon Pathom, and Kamphaengsaen District). The organically grown Chinese kale were also collected from the same districts. Chinese kale from only three organic farming were collected in the present study since from total twenty-nine organic farms, three farms had Chinese kale at that time. All organic farming were approved by Organic Thailand, Ministry of Agriculture and Cooperatives. Chinese kale from conventionally or organically grown were pooled together. Unwashed parts of samples (500 g) were analyzed for pesticide residues. The

remaining fresh samples were cut into small pieces, freeze-dried for seven days, and ground into fine powder. The moisture contents of the dry samples were below 10%. The powdery samples were kept at -20°C until required for analysis.

2.2 Determination of pesticide residues

Conventionally and organically grown Chinese kale were analyzed for 75 pesticide residues, which were divided into four groups: (**group i**) twenty-five organochlorines (Aldrin, alpha-BHC, beta-BHC, delta-BHC, gamma-BHC, cis-Chlordane, trans-Chlordane, o,p'-DDT, p,p'-DDT, Dicofol, Dieldrin, Endosulfan, Endosulfan I, Endosulfan II, Endosulfan sulfate, Endrin, Heptachlor, Heptachlor-epoxide, Methoxychlor, o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, Mirex, Endrin ketone and Hexachlorobenzene), (**group ii**) twenty-eight organophosphates (Acephate, Azinphos-ethyl, Azinphos-methyl, Chlorpyrifos, Chlorpyrifos-methyl, Dichlorvos, Diazinon, Disulfoton, Dicrotophos, Dimethoate, EPN, Ethion, Fenitrothion, Malathion, Methamidophos, Methidathion, Mevinphos, Monocrotophos, Omethoate, Parathion-ethyl, Parathion-methyl, Phosalone, Pirimiphos-ethyl, Pirimiphos-methyl, Profenofos, Prothiophos, Triazophos and Phosphamidon), (**group iii**) eight pyrethroids (Bifenthrin, Cyfluthrin, Cypermethrin, Deltamethrin, Fenpropathrin, Fenvalerate, lambda-Cyhalothrin and Permethrin), and (**group iv**) fourteen carbamates (Aldicarb, Aldicarb sulfone, Aldicarb sulfoxide, Carbofuran, Carbofuran-3-hydroxy, Carbaryl, Methiocarb, Methomyl, Oxamyl, Propoxur, Fenobucarb, Promecarb, Isoprocarb and Meltolcarb).

The pesticide residues were determined based on the In-house method TPT-FS-229TM based on AOAC (2019), 2007.01, and the detection of carbamate was based on the In-house method TPT-FS-241TM based on AOAC (2019), 2007.01 [20]. The limit of detection (LOD) and limit of quantification (LOQ) for organochlorine, organophosphate, pyrethroids were 0.005 and 0.01 $mg \cdot kg^{-1}$, while LOD and LOQ for carbamates were 0.006 and 0.01 $mg \cdot kg^{-1}$.

2.3 Determination of mutagenic properties

The samples were extracted using 80% aqueous ethanol at 50°C while being stirred continuously for 1 hour. The supernatant was then collected by centrifugation at 3000*g* for 15 min. The remaining sample was re-extracted for other two times. The solvent was removed from supernatant by vacuum-rotary evaporation at 50 °C. The dried extract was dissolved in dimethyl sulfoxide (DMSO) and stored at −20°C until use.

Ames test or bacterial reverse mutation test was performed following the standard protocol of OECD guideline 471 [21]. In brief, the five concentrations of extract were incubated with *Salmonella Typhimurium*, including TA98, TA100, TA1535, TA1537, and TA102 in the presence and absence of metabolic activation system (S9 mix) from rat liver (Sigma-Aldrich, St. Louis, MO, USA). Incubation with S9 mix detects indirect-acting mutagens that require metabolic activation before becoming ultimate mutagens [22-24]. The number of revertant colonies were counted compared with the negative (DMSO) and positive control. Positive controls for the test without S9 activation were 4-nitroquinoline-1-oxide (4NQO, 0.2 µg/plate for TA98), sodium azide (NaN3, 2.5 µg/plate for TA100 and 0.5 µg/plate for TA1535), mitomycin C (MMC, 0.5 µg/plate for TA102), and 9-aminoacridine (9-AA, 50 µg/plate for TA1537), while, a positive control, 2-aminoanthracene (2-AA, 2 µg/plate), was used for the test with S9 activation.

2.4 Statistical determination

All data were expressed as the mean ± standard deviation (SD). The comparison of differences in pesticide residues between conventionally and organically grown Chinese kale was analyzed by Student's unpaired t-test. Significant differences were established at $p \leqslant 0.05$. The mutagenicity potential was performed using the analysis of variance (one-way ANOVA) followed by Dunnett's multiple comparison test. Significant differences will be established at $p \leqslant 0.05$. SPSS version 18 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA) was used in this study.

3. RESULTS AND DISCUSSION

3.1 Pesticide residues in conventionally and organically grown Chinese kale

In this study, four groups of pesticides (a total of seventy-five pesticides) were examined. The results presented in Table 1 indicate that the conventionally grown Chinese kale contained pesticides, particularly regarding prothiophos (3.84±0.31 $mg\cdot kg^{-1}$), bifenthrin (0.47±0.19 $mg\cdot kg^{-1}$), cypermethrin (0.08±0.06 $mg\cdot kg^{-1}$), and lambda-cyhalothrin (0.03±0.04 $mg\cdot kg^{-1}$). Prothiophos is classified as organophosphates, while bifenthrin, cypermethrin and lambda-cyhalothrin are classified as pyrethroids. In contrast, the organically grown Chinese kale did not contain any detectable pesticides. The concentrations of prothiophos, bifenthrin, and cypermethrin exceeded the maximum residue limits (MRLs) by 384, 9.4, and 4 times, respectively, while lambda-cyhalothrin was lower than the MRLs.

Table 1. Pesticide residues in conventionally and organically grown Chinese kale cultivated in Nakhon Pathom

Pesticides	Pesticide residues ($mg\cdot kg^{-1}$) in Chinese kale		MRLs ($mg\cdot kg^{-1}$)#
	Conventionally	Organically	
Prothiophos	3.84±0.31*	nd.	0.01
Bifenthrin	0.47±0.19*	nd.	0.05
Cypermethrin	0.08±0.06*	nd.	0.02
lambda-Cyhalothrin	0.03±0.04*	nd.	0.05

*Data were presented as the mean ± standard deviation (SD). Student's unpaired t-test was used to distinguish the difference between pesticide residues between conventionally and organically grown Chinese kale. The p value ⩽ 0.05 was indicated significant differences. nd.: not detected (less than LOD).

*Sources: Codex Alimentarius Commission (2022) and Food and Drug Administration, Thailand (Thai FDA) (2017).

The present data were consistent with a prior study demonstrating organically grown vegetables cultivated in Muang Nakhon Pathom District had much fewer pesticide residues than conventionally grown vegetables [25], implying A careful control of organic practices in this region. However, according to the Thailand Pesticide Alert Network's survey [17], it showed that 90% of non-organic certified Chinese kale collected from all part of Thailand were contaminated with pesticides. Moreover, the same survey also reported that 33.33% of organic certified fruits and vegetables were contaminated with pesticides. This suggests that pesticides are extensively used by agricultural farmers. Pesticides not only contaminated with plants, but also polluted with environment, including water and soil. It has been documented that soil from organic agriculture had low pesticide residues (70-90%) than conventional agriculture [26].

3.2 Mutagenicity of conventionally and organically grown Chinese kale

To examine the mutagenicity potential of conventionally and organically cultivated Chinese kale, the bacterial reverse mutation test (Ames test) was used. This assay is one of the reliable genotoxicity assays recommended by the OECD. Ames test employs five different *S. Typhimurium* in order to cover several types of DNA damages, such as point and frameshift mutations [21]. In addition, addition of S9 mix could also discriminate between direct and indirect-acting mutagens. Direct mutagens are agents that can attach to DNA and cause mutations, whereas indirect mutagens are agents that must be metabolized by hepatic enzymes before they become mutagens [27].

Mutagenicity of conventionally grown Chinese kale was shown in Table 2. In the absence of S9 mix, the negative control revealed some revertant colonies in all tested *S. Typhimurium* strains, which could be spontaneous mutations that naturally occur in bacteria [22], while positive controls revealed a significant increase in revertant colonies as compared to negative controls, demonstrating an effective testing system of this study. The extract of conventionally grown Chinese kale (10–5000 ug/plate) did not significantly raise the number of revertant colonies in comparison to the negative control, even at the highest dose, indicating the extract may not act as direct mutagens. Further study using S9 mix (Table 2), The positive control (2-aminoanthracene) was successfully activated by S9 mix leading to the high induction of revertant colonies in all tested bacterial strains. 2-aminoanthracene is a well-known indirect mutagen as it requires hepatic enzyme activation before becoming ultimate mutagens [28]. This suggests the efficiency of S9 activation in the current study. The data in Table 2 also shows that the extract of conventionally grown Chinese kale, up to 5000 ug/plate, did not induce DNA mutations, as no induction of revertant colonies was seen compared to the negative control. In conclusion, the extract of conventionally grown Chinese kale were devoid of genotoxicity.

Table 2. Mutagenicity of conventionally grown Chinese kale planted in Nakhon Pathom

Concentration (µg/plate)	Number of revertant colonies/plate				
	TA98	TA100	TA1535	TA1537	TA102
Absence of S9					
Negative control	51±4	52±3	8±2	6±1	103±14
10	56±3	55±5	9±2	7±1	100±38
100	57±6	56±4	11±4	7±1	122±28
1000	57±7	57±6	14±4	7±1	123±22
2500	70±7	64±4	16±4	9±1	133±31
5000	73±6	70±11	16±5	8±3	136±15
Positive control	1534±70*	916±62*	397±41*	678±51*	804±76*
Presence of S9					
Negative control	46±2	77±4	8±2	8±1	163±8
10	52±2	78±8	8±1	8±2	164±8
100	52±3	80±6	9±1	8±1	165±6
1000	53±5	76±9	10±2	8±1	165±6
2500	57±5	84±7	9±1	8±1	163±7
5000	60±5	87±9	10±3	9±1	168±6
Positive control	919±81*	1008±33*	227±10*	617±30*	1185±34*

*Data were reported as mean ± standard deviations (SD) from triplicate experiments. The one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test was used to distinguish the difference between the extracts (10-5000 µg/plate), positive control and negative control. The p value $\leqslant 0.05$ was indicated significant differences.

Mutagenicity of organically grown Chinese kale was reported in Table 3. Similar to conventionally grown Chinese kale, the extract of organically grown Chinese kale exhibited neither direct nor indirect mutagenic effects, thereby demonstrating the absence of genotoxicity.

It is evident from Table 3 that Chinese kale is devoid of any genotoxic properties; yet, consumer safety issues arise due to contamination with pesticides. In this study, we found that conventionally grown Chinese kale were contaminated with an organophosphate (prothiophos) and pyrethroids (bifenthrin, cypermethrin and lambda-cyhalothrin). Regrettably, there is a lack of conclusive evidence about the mutagenic potential of these pesticides, and there have been reports of discrepancies between the employed assays. Prothiophos (up to 5000 µg/plate) has been shown to lack of genotoxicity in Ames test [12]. A study reported that high dose of bifenthrin (3,750 to

15,000 μg/mL) induced DNA mutations in dose-dependent manner assessed by *Drosophila* wing spot test [29]. Bifenthrin also exhibited mutagenicity in peripheral blood erythrocytes of fish [30]. Cypermethrin induced genetic damage in male germ cells of *Drosophila melanogaster* [31], while cypermethrin (0.06 to 1.0 μg/mL) was not mutagens in SOS Chromotest in *Escherichia coli* [32]. In addition, lambda-cyhalothrin (0.12 to 1.0 μg/mL) exhibits mutagenicity in Ames test by induction of revertant colonies in *S. Typhimurium* TA98, TA100 and TA1535, while low concentration at 0.06 μg/mL did not induce mutations in the same assay [32]. On the other hands, lambda-cyhalothrin (0.06 to 1.0 μg/mL) were lack of genotoxicity tested by SOS Chromotest in *E. coli* [32]. Hence, one single genotoxicity may not powerful enough to conclude the mutagenicity of suspected compounds. It is worthwhile to continue additional study using a different genotoxicity assay based on a different basis.

Table 3. Mutagenicity of organically grown Chinese kale planted in Nakhon Pathom

Concentration (μg/plate)	Number of revertant colonies/plate				
	TA98	TA100	TA1535	TA1537	TA102
Absence of S9					
Negative control	51±3	53±3	8±2	7±1	102±8
10	51±6	55±4	8±1	8±1	107±14
100	56±4	56±2	8±3	8±2	115±15
1000	58±3	56±11	13±3	7±2	116±10
2500	59±4	62±11	13±2	8±2	118±7
5000	71±6	67±10	14±3	7±2	142±28
Positive control	1477±80*	956±71*	396±52*	695±13*	853±71*
Presence of S9					
Negative control	47±3	77±8	8±1	8±1	163±6
10	51±4	78±8	8±2	8±2	162±8
100	54±6	79±8	8±1	8±2	162±5
1000	54±3	72±9	8±1	8±2	166±7
2500	61±9	85±9	9±1	8±1	166±7
5000	61±4	87±6	10±1	8±2	168±6
Positive control	920±83*	1011±49*	226±12*	616±32*	1182±45*

*Data were reported as mean ± standard deviations (SD) from triplicate experiments. The one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test was used to distinguish the difference between the extracts (10-5000 μg/plate), positive control and negative control. The p value $\leqslant 0.05$ was indicated significant differences.

In this study, the concentration of prothiophos, bifenthrin, cypermethrin and lambda-cyhalothrin at 5000 μg/plate were 10.45, 1.27, 0.22 and 0.07 μg/mL, respectively. Compared with the previous mentioned information, it is not surprised that conventionally grown Chinese kale showed no mutagenicity potential, which might be due to the low doses of pesticides in the genotoxicity test. Further study by including these four pesticides as controls may provide more detail on this matter. Moreover, since plant extracts have been known for their rich in phytochemicals and these phytochemicals have been intensively studied for their health benefits, such as antioxidants, anti-mutagenesis and anti-cancer. Isidori M., et al. studied the extract of three vegetables contaminated with pesticides and found no induction of DNA mutations measured by the Ames test, while the same concentration of pure pesticides contaminated in those samples showed DNA mutations. The presence of natural products, possibly vitamins and phytochemicals, may enhance the overall antioxidant activities of food and may be responsible for the significant decrease in the activity of the pesticide-mediated mutagenicity [32].

4. CONCLUSIONS

The present study reveals that conventionally grown Chinese kale planted in Nakhon Pathom were contaminated with four pesticides and three of them were higher than the MRLs established by Codex and Thai FDA, while organically grown Chinese kale did not contain any detectable pesticides, suggesting a strong regulation for organic farming. In addition, both conventionally grown Chinese kale and organically grown Chinese kale were devoid of genotoxicity, as assessed by the Ames test. Although the genotoxicity was comparable between two practices, a safety advice to consumers should be given to conventionally grown Chinese kale since considerable pesticide contamination existed.

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Fatty acid composition and gene expression of carbohydrate-responsive element-binding protein in copepod *Apocyclops royi*-TH

DP-P086

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ABSTRACT

High-nutrient food is essential for aquatic animal larvae to support growth and improve survival rates more effectively. Copepod *Apocyclops royi* is categorized as a tiny crustacean that can synthesize long-chain polyunsaturated fatty acids (LC-PUFA) with high levels. The goal of the study is to examine the characteristics and role of carbohydrate-responsive element-binding protein (*Ar*CHREBP) gene in biosynthesis of fatty acids in the copepod *A. royi*-TH. The *Ar*CHREBP gene consists of an open reading frame of 2145 nucleotides and encodes a putative protein of 714 amino acids. *Ar*CHREBP shares high similarity to ChREBP2 gene from the copepod *Paracyclopina nana*. The results of the gene expression of *Ar*CHREBP on its vital role in fatty acid synthesis indicated that copepod *A. royi*-TH fed *Chlorella* sp. (CH) showed considerably greater levels of gene expression for *Ar*CHREBP and Delta 6 desaturase (*Ar*D6D) than copepod fed *Tetraselmis* sp. (TET). This increase was consistent with the discovery that the copepod *A. royi*-TH fed CH had a fatty acid composition that included higher levels of DHA than copepod fed TET. Copepod fed TET, on the other hand, exhibited a notably greater proportion of PUFAs compared to those fed CH. The findings suggested that the *Ar*CHREBP gene has a role in the fatty acid synthesis of the copepod *A. royi*-TH. The results have implications for the production of high-quality copepod food the aquaculture industry's larval aquatic animals.

1. INTRODUCTION

The copepod *Apocyclops royi*, a species of copepod native to Thailand (*A. royi*-TH), is a zooplankton in the crustacean group that is commonly used as live food for nursery aquatic animal larvae in the aquatic industry. The copepod *A. royi*-TH is capable of producing long-chain (C≥20) polyunsaturated

fatty acids (LC-PUFA) such as docosahexaenoic acid (DHA), eicosatetraenoic acid (EPA), and arachidonic acid (ARA) from α-linolenic acid (ALA) and linoleic acid (LA) through 2 steps. utilizing the desaturase enzyme to catalyze the addition of double bonds, and using the elongases enzyme to lengthen the fatty acid chain [1].

LC-PUFA is crucial for aquatic animals as it promotes growth, enhances immune response, and increases survival rates of larvae of aquatic organisms. Currently, research has found evidence of the beneficial impacts of using copepods as live food for several marine and aquatic animals, such as asian seabass (*Lates calcarifer*, Bloch) [2], yellowtail clownfish (*Amphiprion clarkii*) [3] and green mandarin fish (*Synchiropus splendidus*) [4].

Carbohydrate-responsive element-binding protein (ChREBP) is a transcription factor protein that plays an important role in the synthesis of fatty acids from acetyl-CoA to palmitic acid in the process of de novo lipogenesis and is responsible for regulating the expression of desaturase genes used to synthesize PUFAs [5]. The *Ar*ChREBP gene, a novel gene not previously identified in the copepod *A. royi*-TH, was found by examination of the *A.royi* copepod transcriptome data [1]. Nevertheless, the *Ar*ChREBP gene was shown to be related to the ChREBP2 gene of the copepod *Paracyclopina nana*.

It has been discovered in copepods that the dietary fat composition affects the production of fatty acids. According to a report by [6], research has shown that green algae *Chlorella* sp. may be a useful dietary source since it contains significant levels of essential substances (18:3n-3 and 18:2n-6), which are crucial building blocks for the synthesis of PUFAs. It is therefore thought to be a promising dietary source. Interestingly, copepod *Tigriopus japonicus* was shown to be capable of synthesizing LC-PUFA when it was fed *Chlorella* sp.

This study examined the fatty acid composition of the copepod *A. royi*-TH and also evaluated the gene expression of *Ar*ChREBP. Furthermore, the influence of the composition of dietary fatty acids on the synthesis of polyunsaturated fatty acids (PUFAs) in the copepod *A. royi*-TH was studied by analyzing the expression of the *Ar*ChREBP gene and the fatty acid content of the copepods. The obtained information will help in understanding the fatty acid synthesis process of the copepod *A. royi*-TH.

2. MATERIAL AND METHODS

2.1 *Culture of the copepod A. royi-TH*

The copepod *A. royi*-TH was grown under laboratory conditions. To study the influence of the fatty acid composition of the dietary, it was divided into 2 sets of experiments: Set 1, copepods were raised with the *Tetraselmis* sp. (control group), Set 2, copepods were raised with the *Chlorella* sp., a set of experiments was conducted in three replicates. The copepods were collected using a 33 µm filter cloth. The copepod samples for study were stored at −80°C and samples for total RNA extraction were stored in TRIzol™ Reagent solution (Invitrogen) and stored at -80°C.

2.2 *Analysis of fatty acids in the copepod A.royi-TH using GC/MS techniques.*

Lipids were extracted from *A. royi*-TH copepod samples fed with *Tetraselmis* sp. and *Chlorella* sp. using the method of [7], and fatty acids were analyzed using gas chromatography mass spectrometry (GC/MS).

2.3 *Characterization of the ArChREBP gene in copepod A.royi-TH*

The *Ar*ChREBP gene was identified from the transcriptome database of the copepod *A. royi*-TH. Nucleotide sequences and amino acid sequences are examined by using the ExPASy translation tool program (http://www.expasy.org/), the BLASTx program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the Clustal Omega program (https://www.ebi.ac.uk/Tools/msa/clustalo/). The SMART tool was utilized to assess significant protein domains (http://smart.embl-heidelberg.de/).

2.4 Total RNA extraction and first strand cDNA synthesis.

Total RNAs of copepod samples in each experiment were extracted using TRIzolTM Reagent according to the manufacturer's procedure (Thermo Fisher Scientific). The total RNA was treated with RNase-free DNase I (Promega) to remove any genomic DNA. Subsequently, the concentration and purity of the total RNA were determined using a NanoDropTM Spectrophotometer. Purified RNA was used to generate first-strand cDNA by using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). Then, the cDNA was employed to assess gene expression through the utilization of the semi-quantitative (sq)RT-PCR technique.

2.5 Examination of gene expression

The expression of the *Ar*ChREBP gene was analyzed in copepods that were fed two different diets with varying fatty acid compositions (*Tetraselmis* sp. and *Chlorella* sp.). The sqRT-PCR analysis of *Ar*ChREBP (Forward primer: 5' GATGACCAACGAACGCTTACAG 3'; Reverse primer: 5' CGATGGCAGTGAATACCCAGTA 3') and *Ar*D6D was carried out by conducting a PCR reaction according to Amparyup et al. 2022. The *Ar*18srRNA gene, which is a constitutively expressed gene, (the forward primer 5' CATATTGCGGACACTGGTTCT and the reverse primer 5' AAGAGTCTGGGCAAGGAAGAG) serves as a control gene. The amplification products were examined using 1.8% (w/v) agarose gel electrophoresis following staining with ethidium bromide. The experiments were performed in triplicate, with around 500 copepods used in each replication.

3. RESULTS AND DISCUSSION

3.1 Fatty acid composition in copepod A. royi-TH

The examinations of the fatty acid content of copepod *A. royi*-TH fed two different varieties of green algae, *Tetraselmis* sp. (TET) and *Chlorella* sp. (CH), are displayed in Table 1. Copepod *A. royi*-TH fed CH was found to have significantly (p<0.05) higher quantities of DHA and saturated fatty acids than copepod *A. royi*-TH fed TET. In contrast, copepod *A. royi*-TH fed TET was found to have significantly greater total PUFAs than copepod *A. royi*-TH fed CH (p<0.05).

Table 1. The fatty acid composition in copepod *Apocyclops royi*-TH fed two different diets

Fatty acids	*Ar*-Tetraselmis	*Ar*-Chlorella
C22:6 n-3 (DHA)	3.70 ± 0.06^{b}	7.37 ± 2.66^{a}
∑SFA	18.80 ± 0.07^{b}	39.74 ± 4.14^{a}
∑MUFA	8.28 ± 0.06^{a}	13.21 ± 4.22^{a}
∑SC-PUFA	37.20 ± 0.15^{a}	22.88 ± 1.87^{b}
∑LC-PUFA	8.21 ± 0.14^{a}	7.37 ± 2.66^{a}
∑n-3 PUFA	27.89 ± 0.08^{a}	16.24 ± 2.10^{b}
∑n-6 PUFA	17.52 ± 0.20^{a}	14.01 ± 1.38^{b}
∑PUFA	45.42 ± 0.28^{a}	30.25 ± 0.83^{b}

*a-b different letter within each column indicates statistically significant differences (p<0.05)

3.2 The characterization of ArCHREBP gene and gene expression analysis

The *Ar*ChREBP gene was discovered by analyzing the transcriptome data of the copepod *A.royi*-TH, as reported by [1]. This gene is a newly discovered transcription factor gene that has not been previously found in the copepod *A. royi*-TH. *Ar*ChREBP gene of copepod *A. royi*-TH has an open reading frame (ORF) of 2145 base pairs that codes for a protein with a polypeptide chain length of

714 amino acids, according to analysis of the gene. Using the BLASTx program, an analysis of the *Ar*ChREBP gene revealed 58% similarity to ChREBP2 gene of the copepod *Paracyclopina nana*. According to [8], it has been demonstrated that the ChREBP gene regulates both fatty acid desaturase activity and de novo lipogenesis.

To examine the gene expression in copepod *A. royi*-TH fed two types of algae with different fatty acid compositions, the *Ar*ChREBP gene-specific primer was used to analyze. The expression level of the *Ar*ChREBP gene was found to be significantly higher in copepod fed CH than in copepod fed TET (p<0.05)(Figure 1), which is similar to the notable rise in the expression of *Ar*D6D, a major desaturase enzyme in LC-PUFA synthesis in copepod *A. royi*-TH fed CH compared to those fed TET (Figure 2).

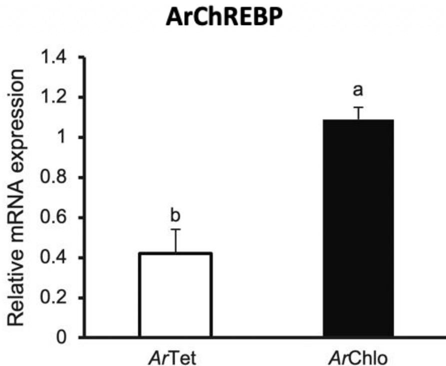

Figure 1. The mRNA expression of *Ar*ChREBP from the copepod *A. royi-TH* at different diets (*A.royi* that feeds on *Tetraselmis* sp.: *Ar*Tet and *A.royi* that feeds on *Chorella* sp.: *Ar*Chlo) The *Ar*18srRNA served as an internal reference gene. Data represents the mean±standard deviation (error bars) of triplicate samples. Means with different lowercase letter (above each bar) are significantly different (p<0.05; one-way ANOVA with Duncan's multiple range tests).

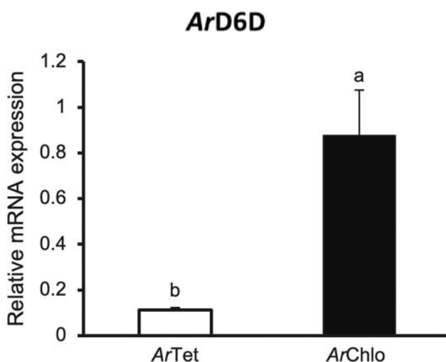

Figure 2. The mRNA expression of *Ar*D6D from the copepod *A. royi*-TH at different diets (*A.royi* that feeds on *Tetraselmis* sp.: *Ar*Tet and *A.royi* that feeds on *Chorella* sp.: *Ar*Chlo) The *Ar*18srRNA served as an internal reference gene. Data represents the mean±standard deviation (error bars) of triplicate samples. Means with different lowercase letter (above each bar) are significantly different (p<0.05; one-way ANOVA with Duncan's multiple range tests).

The results of *Ar*ChREBP and *Ar*D6D gene expression were consistent with the results of the fatty acid composition analysis of the copepod *A. royi-TH* fed CH. *Chlorella* sp. is an algae that has a lower amount of palmitic acid than *Tetraselmis* sp. [9]. It is possible that the *A. royi-TH* uses *Ar*ChREBP to stimulate the production of more palmitic acid because the role of ChREBP is to change Acetyl-CoA to palmitic acid in preparation for entering the DHA synthesis pathway. According to [5]

study, copepod ChREBP can regulate the expression of desaturation genes, which is consistent with the results of experiments in the copepod *A. royi*-TH that increasing *Ar*CREBP expression also increased the expression level of the *Ar*D6D gene.

Furthermore, the PUFA synthesis mechanism of the copepod *A. royi*-TH involves the *Ar*D6D enzyme, which catalyzes the desaturation step to convert ALA fatty acids into STA fatty acids. The *Ar*D6D enzyme is involved in the biosynthesis of DHA fatty acids by the process of introducing double bonds to fatty acids. The findings of the experiment indicate that copepods *A. royi*-TH, when fed with *Chlorella* sp., exhibited elevated levels of saturated fatty acids and DHA fatty acids.

4. CONCLUSIONS

The *Ar*ChREBP gene of the copepod *A. royi*-TH was successfully identified. The experiment aimed to assess the influence of two distinct diets (TET and CH) on the fatty acid content of copepods. The findings demonstrated that the copepod subjected to CH treatment displayed markedly elevated levels of docosahexaenoic acid (DHA). In addition, when comparing copepods fed TET to copepods fed CH, copepod *A. royi*-TH fed CH exhibited significantly elevated expression levels of the *Ar*ChREBP and *Ar*D6D genes. The study findings suggest that the fatty acid content of various food types influences the production of polyunsaturated fatty acids (PUFAs) in copepod *A. royi*-TH. Additionally, the *Ar*ChREBP gene may be involved in the fatty acid synthesis and DHA in copepod *A. royi*-TH. The findings of this study would be valuable in the production of high-quality copepods as food for feeding aquatic animal larvae for the aquatic industry.

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Stability of Entrapment of *vb_SalmoM-p*YM Phage by Using Modified Ca-alginate Beads with Soy Protein Isolate

DP-P096

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ABSTRACT

The *vb_SalmoM-p*YM are lytic phage that was isolated from pork and specific to *Salmonella* Typhimurium. Phage therapy for controlling *Salmonella* in chickens needs a feed administration route. The objective of this study was therefore to evaluate the stability of the entrapment of phages by using modified Ca-alginate beads with soy protein isolate (SPI). The entrapment efficiency of all treatment Ca-alginate beads was 97-98%, and there was no significant difference. Assessment of the lytic lysis of entrapped *Salmonella* for 6 h revealed no significant differences in bacterial survival across all treatments. The stability of the phage in the entrapment of Ca-alginate added to 0.15% (w/v) and 0.3% (w/v) SPI under various temperatures (4°C, 25ºC, 37ºC, 42ºC and 50ºC) for 24 h was determined, which showed that at 45 and 50 °C adding SPI to the Ca-alginate beads could protect *Salmonella* phages under high temperatures better than without SPI. Under acid condition of pH 2.5 for 2 h stability of phage in Ca-alginate beads added 0.3% (w/v) SPI was 84.9% higher than the control (73.51%). The survivability of phages under 0.1% (w/v) bile salt in Ca-alginate beads with 0.15% (w/v), 0.3% (w/v) SPI was 77.92% and 90.91%, respectively, which was better than without SPI. As a result, 0.3% (w/v) SPI-added Ca-alginate beads under 0.1% bile salt can retard the release of phage particles higher than 0.15% (w/v) of SPI-added Ca-alginate. It could be concluded that the soy protein isolate can support Ca-alginate to protect phages under high temperatures, acid and bile salt during the feed administration route to chickens for phage therapy.

1. INTRODUCTION

Bacteriophages (phages) are abundant in the ecosystem. Phages are an alternative biological agent to control bacteria pathogens because their capability of killing the bacterial host [1,2]. However, the conventional application of phage therapy in liquid form had limitations due to loss of viability during digestive tract. The previous study showed that biocontrol efficiency of bacteriophage was reduced under acidic conditions. Therefore, encapsulation was useful technique for protecting phages [3].

Ca-alginate beads have been generally used for encapsulation or entrapment of biological agent such as microorganism and enzymes [4,5]. Colom et al. [6] reported that the phage cocktail was encapsulated within alginate/$CaCO_3$ microcapsule resulting in phage survival under gastric juice in chickens, while all free phages were inactivated. The main problem with using Ca-alginate beads was their instability under to acidic conditions or high temperature for a long time [7]. Nevertheless, the incorporation of soy protein isolates and Ca-alginate into phage encapsulation has not been previously investigated. Babot et al. [8] reported that co-encapsulated between alginate and SPI were able to protect the probiotic bacteria under simulated gastric fluid (SGF) better than only alginate.

Therefore, this study was to evaluate the efficiency of soy protein isolate that can support Ca-alginate to protect phages under various temperatures, low pH and bile salts during the feed administration route to chickens for phage therapy.

2. MATERIAL AND METHODS

2.1 Bacteria and Bacteriophage

The *Salmonella* Typhimurium ATCC 13311 was bought from American Type Collection Culture (MicroBiologies, Inc, U.S.A.). Bacteriophage *vb_salmoM-p*YM. (Phage) was isolated from pork meat that bought from local market in Nakhon Ratchasima.

2.2 Salmonella cultivation

Salmonella Typhimurium (*S.* Typhimurium) was cultured on xylose lysine deoxycholate (XLD; HIMEDIA®, India) agar and incubated at 37 °C for 24 hours. Routine subculture of *S*. Typhimurium was performed in tryptic soy broth (TSB; HIMEDIA®, India). The broth was centrifuged to collect the bacterial cells. The cells were washed twice with salt magnesium sulphate buffer (SM buffer) and measured at OD 600 nm to determine the absorbance and cell concentration [9].

2.3 Bacteriophage propagation

Phages were mixed with a host suspension and then 500 ul of the mixture were added into 5 ml of molten TSB containing 0.6% agar and poured on the basal TSB containing 1.5% agar was incubated at 37 °C for 24 hr [10]. SM buffer was added on plate and placed on shaker incubator at 100 rpm, 4°C for 24 h. The phage suspension was filtered by using 0.2 µm syringe filter and stored at 4°C.

2.4 Enumeration of Salmonella

S. Typhimurium was determined by using spread plate technique. The ten-time serial dilution was performed and then cultured on XLD agar. The plates were incubated at 37 °C for 24 h. The colony-forming unit (CFU/ml) was calculated.

2.5 Enumeration of bacteriophage titer

Phage titer was determined using the agar overlay assay technique [11]. Briefly, phage suspension was diluted with ten-time serial dilution by using SM buffer. The 10 µl of diluted phage was dropped on the lawn as described on phage propagation without phage. The titer of phage was determined as the mean of three independent counts, as plaque forming units (PFU/ml) [10].

2.6 Bacteriophage entrapment in Ca-alginate beads

Phages were entrapped using sodium alginate (HIMEDIA®, Nashik, India) combined with or without soy protein isolate (Krungthepchemi, Bangkok, Thailand) according to Ma et al. [12]. Each

treatment was mixed by following the formula as described in Table 1. and adding phage suspension (10^8 PFU/ml). The beads were extruded by using a syringe 20 Gauge (G) needle and exposed in 4% (w/v) $CaCl_2$ that was stirrings at 100 rpm at room temperature. For enumeration of phage titres, 100 mg of beads were added into 5 ml of sterile 0.5M phosphate buffer saline (PBS; Na_2HPO_4 15.6 g and NaH_2PO_4 17.8 g per litter) pH 7.4 and shaking by using orbital shaker, 300 rpm at room temperature for 1-2 hr, or until dissolved [12]. The phage titre was examined.

Table 1. Compositions of modified bead used for phage entrapment

Treatment (T)	Sodium alginate (w/v) (%)	Soy protein isolate (w/v) (%)
T.1	3	0
T.2	3	0.15
T.3	3	0.3

2.7 Entrapment efficiency (EE)

Entrapment efficiency (EE) of phage in ca-alginate beads without/with SPI was determined according to Batalha et al. [10]. followed as the equation (1).

2.8 Lytic lysis assay of entrapped Salmonella phage (In vitro).

The Ca-alginate beads (100 mg) were placed into 5 ml SM buffer pH 7.4 and added *S.* Typhimurium cells at a concentration of 10^7 (PFU/ml) this was incubated at 41 °C with agitation 120 rpm for 6 hr. Five hundred microliters of sample was collected every 2 hr. The number of *Salmonella* bacteria were counted using the drop plate method [13] and incubated at 37 °C for 24 h. The colony-forming unit (CFU/ml) was calculated.

2.9 Effect of various temperatures and pH 2.5 on the stability of Salmonella phage.

The acidic stability (pH 2.5) of each entrapped *Salmonella* phage was evaluated. The 100 mg of each sample were placed into SM buffer pH 2.5 and then incubated at 37 °C for 2 hr. The number of phages was determined. For thermal stability, the 100 mg of alginate beads were placed into 1 ml SM buffer pH 7.4 and subsequently incubated at different temperatures 4°C, 25°C, 37°C, 42°C and 50°C for 24 hours [14]. The survival of phages in Ca-alginate bead were determined.

2.10 Effect of bile salt on the stability of Salmonella phage.

The 100 mg Ca-alginate beads were placed into 0.1% (w/v) bile salt, 0.85% NaCl pH to 6.8 and subsequently incubated at 41 °C with agitation 120 rpm for 1 hr. The titer of phage survival in the bile salt solution was determined by agar overlay assay.

2.11 Statistical analysis.

The experiments were conducted in triplicate, and the results are presented as means ± SE. Analysis of variance (ANOVA) was utilized for result comparison, followed by Tukey multiple range tests to compare means. Significance was determined at $p < 0.05$ using SPSS 26.0 for Windows (SPSS Inc., Chicago, USA).

3. RESULTS AND DISCUSSION

3.1 Entrapment efficiency (EE)

Salmonella phages were entrapped in Ca-alginate beads without or with soybean isolate protein (SPI). The entrapment efficiency (EE) of each encapsulation beads were evaluated (Table 1). The EE of each treatment was not significantly different around 97-98%. This result showed that addition of SPI in Ca-alginate beads did not affect on EE of Ca-alginate to entrapped *Salmonella* phages.

The diameter of the extruded beads in containing 0% SPI, 0.15% w/v SPI and 0.3% w/v SPI were 2.9 ± 0.25, 3.1 ± 0.20, and 3± 0.16 mm, respectively (Fig. 1).

Table 1. Entrapment efficiency for bacteriophage *vb_SalmoM-p*YM in Ca-alginate beads

Concentration of SPI (%w/v)	Encapsulation Efficiency
0	97.67 ± 0.58
0.15	98.34 ± 0.15
0.3	98.12 ± 0.19

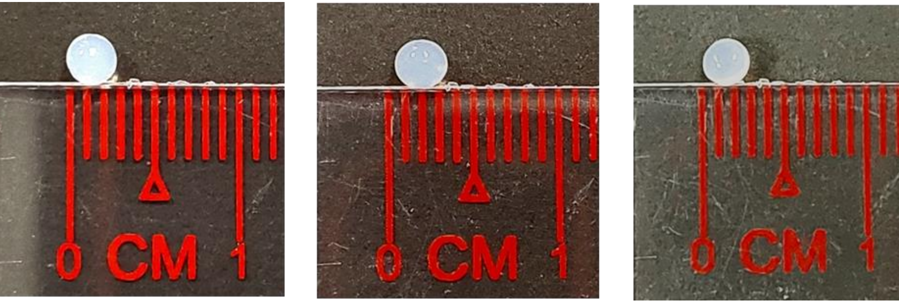

Figure 1. Illustration of ca-alginate beads. A: 0% SPI, B: 0.15% w/v SPI and C: 0.3% w/v SPI

3.2 Lytic lysis assay of entrapped Salmonella phage.

The lytic lysis of entrapped *Salmonella* was evaluated (Fig 2). The result showed that number of *Salmonella* Typhimurium in SM buffer was not significantly different during incubation each entrapped *Salmonella* phage (p>0.05). These results indicated that the Ca-alginate with or without SPI had mostly effective entrapment of *Salmonella* phage.

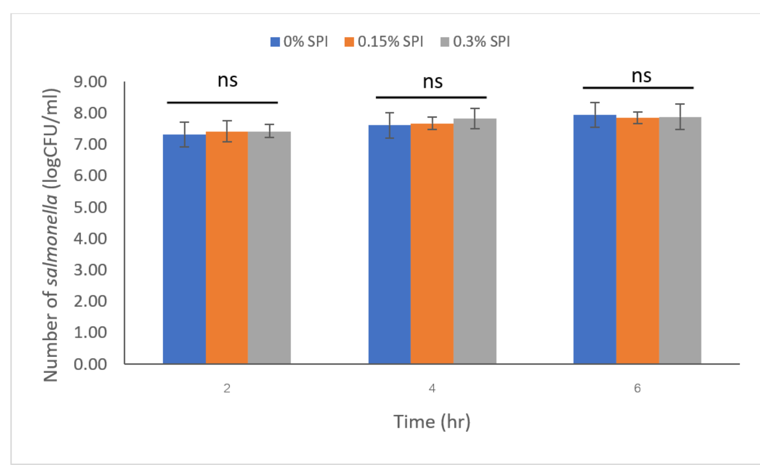

Figure 2. Lytic lysis of entrapped *Salmonella* phage on number of *Salmonella* Typhimurium

3.3 Stability of Salmonella bacteriophage under different temperatures

The Ca-alginate beads modified with/without SPI were evaluated under various temperature (Fig. 3). The number of *Salmonella* phage in each encapsulation was not significantly different at 4 and 37 °C. The titer of *Salmonella* phage in each treatment at 25, 42 and 50 °C was found significantly different. The addition of SPI in Ca-alginate bead could protect *Salmonella* phage under high temperature better than only Ca-alginate encapsulation. The free phage was decrease during stored at 42 and 50 °C. These results suggested that the adding of SPI in Ca-alginate could be increase efficiency of entrapment of *Salmonella* phage leading to protection phage under high temperature.

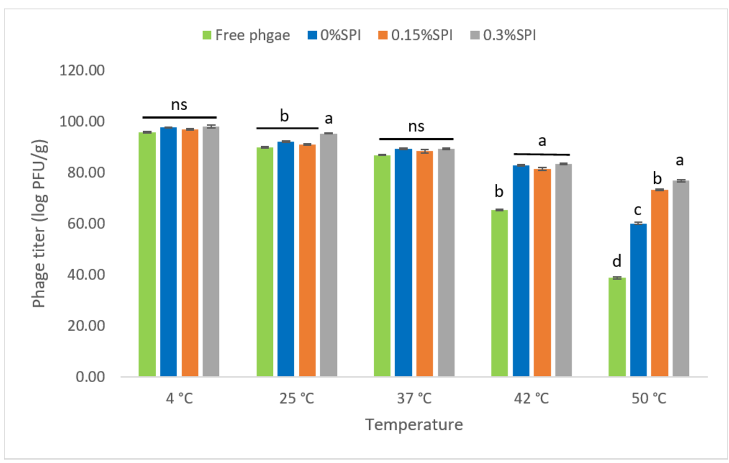

Figure 3. The survival of *Salmonella* bacteriophage in the modified bead at various temperatures; ns means not significantly different, a means significantly different compared with b, c and d within the same temperature

3.4 Stability of Salmonella bacteriophage in pH 2.5.

The stability of Ca-alginate beads-entrapped phages was incubated under pH 2.5 at 37 °C for 2 hr (Fig. 4). The viability of *Salmonella* phages entrapped in Ca-alginate beads without SPI was 73.15%. The survival of Ca-alginate beads with SPI 0.15% w/v and 0.3% w/v was 74.00% and 84.90%, respectively. These results showed that addition of SPI in Ca-alginate beads could be improved phage viability under low pH conditions. The Ca-alginate beads with SPI resulted in a reduction of porosity within the beads. At low pH, the carboxyl group has a positive charge, facilitating crosslinking between oppositely charged groups in the sodium alginate and SPI, which causes the beads to shrink, thereby reducing the leakage of *Salmonella* phage [8, 15]. Similarly, encapsulation of probiotic bacteria in alginate with SPI could be able to stable under GI for 1 hr [8].

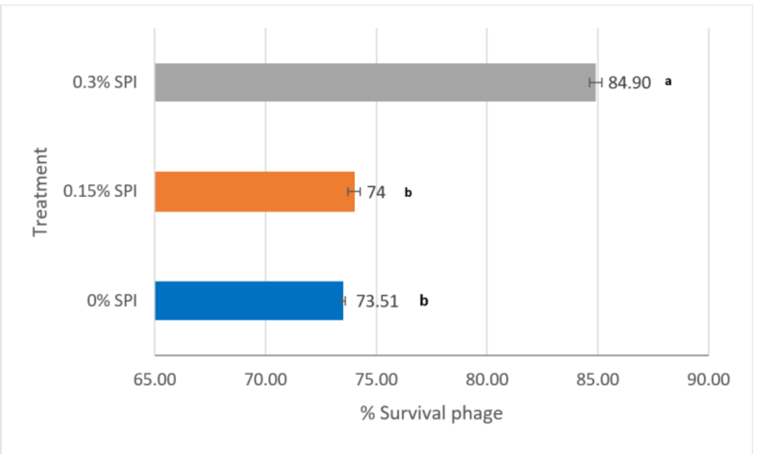

Figure 4. The stability of Salmonella bacteriophage in Ca-alginate bead under pH 2.5: a means significantly different compared with b.

3.5 Effect of bile salt on the stability of entrapped Salmonella phage

Previous research reported that free phages exposed to bile salts in the digestive tract resulted in a significant reduction [16]. Our study assessed the stability of phage in Ca-alginate beads were incubated in 0.1% bile salt solution (pH 6.8) at 41°C for 1 hr (Fig 5). The amount of phage remaining in Ca-alginate beads without SPI was 75%. The remaining phage of Ca-alginate beads with SPI 0.15% w/v and 0.3% w/v was 77.92% and 90.91%, respectively. The result showed that added SPI could be able to reduce phage leakage from the beads. The SPI was could be bound with alginate by electrostatic force between molecules to fulfilled the porous surface on Ca-alginate beads [17].

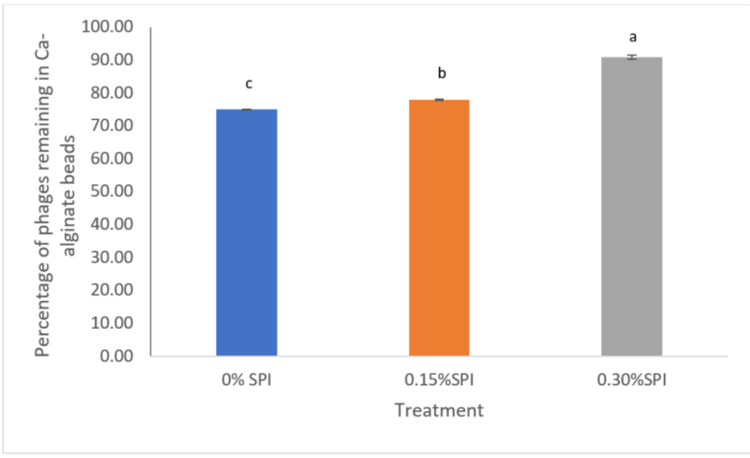

Figure 5. The number of phages remaining in Ca-alginate beads were incubated in bile salt: a means significantly different compared with b and c.

3.6 Mathematical Components

$$\%EE = \frac{Phage\ entrapped\ (PFU)}{Initial\ phage\ (PFU)} x100 \qquad (1)$$

4. CONCLUSIONS

This study demonstrated that the *vb_SalmoM-p*YM Phage was entrapped with ca-alginate beads has high efficiency entrapment. At high temperatures and under low pH conditions ca-alginate beads with SPI could improve the viability of phages. The ca-alginate beads were incubated in bile salt showed decreased phage leakage from the beads.

5. ACKNOWLEDGEMENTS

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Fatty Acid-Binding Protein of Copepod *Apocyclops royi*-TH: Expression and Its role in Polyunsaturated Fatty Acid Production.

DP-P111

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Keywords

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Hyposalinity
Fatty acid binding protein
Gene expression

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ABSTRACT

Copepod *Apocyclops royi* (*A. royi*-TH) is a crustacean zooplankton species found in Thailand, which serves as a vital primary food supply for aquaculture industry. *A. royi-TH* is a copepod that can synthesize long-chain polyunsaturated fatty acids (LC-PUFA). In this study, the role and expression of the fatty acid binding gene (FABP), a protein responsible for transporting fatty acids, was characterized. Analysis of the ArFABP gene in the copepod *A. royi*-TH revealed that it contains an open reading frame consisting of 465 base pairs, which codes for a protein composed of 154 amino acids. The ArFABP protein comprises the Lipocalin_7 domain region, which exhibits high similarity to the FABP protein found in the copepod *Caligus rogercresseyi*. The expression of the ArFABP gene was examined in *A. royi* copepod samples fed with *Tetraselmis sp*. (TET) compared with *Thalassiosira sp.* (THA) found that copepods in the THA group had significantly higher ArFABP gene expression levels than copepods in the TET group. This is consistent with the results of the fatty acid composition analysis. It was found that the copepods in the THA group had significantly higher proportions of LC-PUFA and arachidonic acid (ARA) than those in the TET group. Additionally, we found that the ARA content in the copepods raised at 15 ppt salinity (hyposalinity) was significantly higher than those in copepods raised at 25 ppt (control group). These results suggested that ArFABP may play a role related to fatty acid transport in LC-PUFA fatty acid synthesis in copepods. The obtained knowledge will result in the development of strategies to enhance the production of essential fatty acids in the copepod *A. royi-TH* which are important for the sustainability of aquaculture.

1. INTRODUCTION

Copepods are a type of the zooplankton that have a significant nutritional value. They can be utilized as a food source for raising aquatic larvae and have the potential to be economically beneficial for aquaculture.

Copepods can synthesize essential fatty acids, which serve as indications of nutritional value in aquatic animal feed. They have a crucial role in the development and survival rates, particularly in recently hatched fish and shrimp. *Apocyclops royi* is a species of copepod found in Thailand (*A. royi-TH*) that can be cultivated for large-scale production. It has the potential to be developed as a nutritious food source for aquatic larvae due to its high nutritional value. Copepod *A. royi-TH* has the ability to synthesize long-chain polyunsaturated fatty acids (LC-PUFAs), specifically Omega-3 fatty acids (DHA), Eicosapentaenoic acid (EPA), and Omega-6 fatty acids (ARA), in significant proportions [1].

Fatty acid binding proteins (FABPs) are a group of proteins that play a crucial role in the absorption and transportation of fatty acids. FABPs are small proteins, typically around 14-15 kDa, and belong to a group of proteins that have a specific affinity for binding to fatty acids inside cells. They play a crucial role in the regulation of lipid metabolism, including processes such as energy generation, fat storage, and fatty acid utilization [2,3]. Understanding the genes responsible for fatty acid production in copepods is crucial for developing efficient copepods for aquaculture biotechnology. Although there have been reports on the discovery and analysis of FABPs in crustaceans, there is currently no published report on the involvement of FABPs in the synthesis of fatty acids in copepods.

Salinity is a crucial determinant of the ecology and life cycle of aquatic habitats. Fluctuations in salinity can impact the traits of the population, the composition of the ecosystem, and the mechanism of lipid production in aquatic species [4-6]. The report on the effects of salinity on copepod *Paracyclopina nana* found that a salinity level of 35 ppt negatively affects their development and reproductive capacity [7]. On the other hand, it was observed that copepod *A. royi*-TH raised in a low salinity condition of 15 ppt had higher levels of arachidonic acid (ARA) and expression of the ArCb5D6D gene compared to copepods at a salinity of 25 ppt [8].

In this study, the characteristics of the ArFABP gene in copepod *A. royi*-TH were investigated. The role of ArFABP in the biosynthesis of fatty acids in copepod *A.royi*-TH was examined through gene expression analysis and investigation of the fatty acid composition in copepods fed with various diets. Furthermore, the impact of salinity on the production of fatty acids in copepods was studied by analyzing the expression of FABP and the composition of fatty acids in copepods. The acquired knowledge will lead to strategies for increasing the essential fatty acid production in the copepod *A. royi*-TH, which will in turn lead to opportunities for increasing the value of aquaculture products and the sustainability of aquaculture industry of Thailand.

2. MATERIAL AND METHODS

2.1 The cultivation and rearing of copepod A. royi-TH

Microalgae were utilized as a food source for the copepod *A. royi*-TH during a period of 15 days. The experiment was divided into two sets. The first set investigated the impact of salinity on the copepod *A. royi*-TH by feeding it with green microalgae *Tetraselmis* sp. (TET). The copepods were exposed to two different salinity levels: 15 ppt (low salinity group) and 25 ppt (control group). The second group of copepod *A. royi*-TH, was provided with diatom *Thalassiosira* sp. (THA) at a salinity of 25 ppt. This was done to assess its performance in comparison to the TET group, which was also fed under the same salinity conditions. The collection of *A. royi*-TH copepod samples for the investigation of fatty acid components was conducted using a 33 µm filter cloth and stored in a frozen state at a temperature of -80 °C. The samples for the extraction of total RNA are stored in TRIzol™ Reagent solution and kept frozen at -80 °C

2.2 The fatty acid composition in copepod A. royi-TH using the GC/MS method

The extraction of fat from samples of copepods reared on TET under salinity conditions of 15 ppt and 25 ppt, as well as copepods reared on THA and TET under salinity conditions of 25 ppt, was performed using a modified version of the method developed by Lepage and Roy [9]. Afterwards, the components of the fatty acids were analyzed using gas chromatography-mass spectrometry (GC/MS). The analysis was performed by conducting three replicates of experimental trials. The obtained data were then subjected to analysis of variance (ANOVA) to assess the variability, and the mean differences were compared using Duncan's multiple range test at a confidence level of 95% using the SPSS software.

2.3 Characterization of the ArFABP gene in the copepod A.royi-TH

The nucleotide sequence and amino acid sequence of the ArFABP gene were examined using the ExPASy translated tool, available at http://web.expasy.org/translate/. The sequence similarity of ArFABP gene was assessed by comparing it to the gene sequences already documented in the GenBank database using the BLASTx tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Predict protein domains was analyzed using the SMART program (http://smart.embl-heidelberg.de/). Sequence alignment of ArFABP amino acid sequence is determined by performing sequence alignment with genes of other related species using the Clustal Omega tool (https://www.ebi.ac.uk/Tools/msa/clustalo/).

2.4 The total RNA extraction and first strand cDNA synthesis

RNA extraction was performed on each set of experimental samples (each set consisting of 3 replicates) using the TRIzol™ Reagent extraction kit. The extracted RNA was then treated with RNase-free DNase I enzyme to remove any contaminating DNA (Promega). The RNA concentration was determined, and the quality of the RNA samples was evaluated using the Nano Drop 2000 instrument from Thermo Scientific. The first strand cDNA was synthesized using the ImProm-II™ Reverse Transcription System Kit (Promega) for gene expression analysis by the RT-PCR technique.

2.5 Gene expression analysis of the ArFABP gene in the copepod A.royi-TH

The gene expression of ArFABP was determined by the following primers: Forward primer: 5' GGCAACGACCAGTTTTCAAT 3'; Reverse primer: 5' GCATTCACTCCGTAGCCACT 3' using copepod samples reared in different salinities (15 ppt and 25 ppt) and samples reared with different types of algae (TET and THA) by semi-quantitative RT-PCR analysis, as described in [1]. Subsequently, the ArFABP gene's expression level was determined using 1.8% agarose gel electrophoresis and analyzed using Gel documentation with the GeneSys program. The mRNA expression levels were measured using the Gel Pro 3.1 simple analysis software, and the relative mRNA expression of the target gene was calculated using the Ar18srRNA gene as a control. The Ar18srRNA gene was amplified using the forward primer 5' CATATTGCGGACACTGGTTCT 3' and the reverse primer 5' AAGAGTCTGGGCAAGGAAGAG 3'. Three replicates of the experiment were performed to compare the gene expression in each set of experiments in the copepod.

3. RESULTS AND DISCUSSION

3.1 Identification and characterization of the ArFABP gene in copepod A. royi-TH

The characteristic analysis of the ArFABP gene in *A. royi*-TH revealed that it consists of an open reading frame (ORF) with a size of 465 base pairs, which encodes a protein consisting of 154 amino acids. An analysis of the important domain using the SMART program revealed that the protein ArFABP consists of the Lipocalin_7

domain. Using the BLASTP software, the ArFABP was compared to genes in the GenBank database, and it was found that the ArFABP had a 55% similarity to the FABP protein in copepod *Caligus rogercresseyi.*

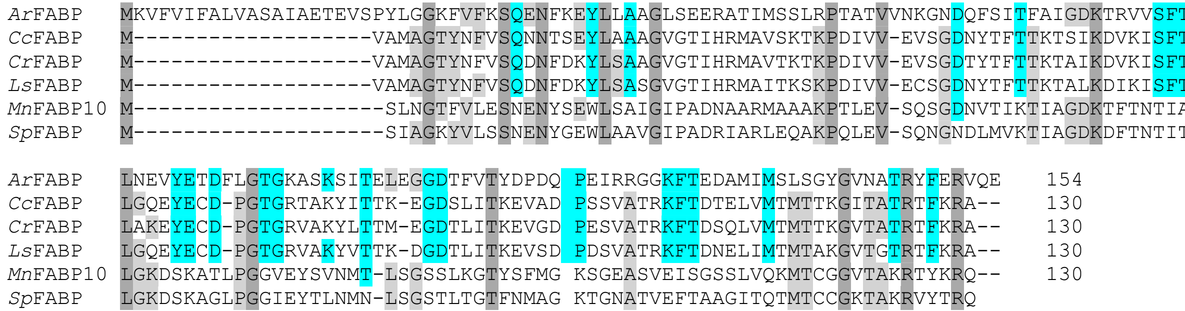

Figure 10. Multiple alignment of the deduced amino acid sequence of ArFABP from *A. royi*-TH with the other FABP proteins including, copepod *Caligus clemensi* (ACO10912.1), copepod *Caligus rogercresseyi* (ACO10912.1), copepod *Lepeophtheirus salm*onis (XP_040579648.1), prawn *Macrobrachium nipponense* (AFD29289.1)

3.2 Determination of fatty acid composition of copepod A. royi-TH

The analysis of the fatty acid composition in copepod *A. royi-TH*, which were fed with TET and THA, was conducted using GC/MS. The results showed that the LC-PUFA content was 8.62% and 26.30% in copepod *A. royi*-TH fed with TET and THA, respectively. The analysis revealed that the concentrations of omega-3 fatty acids in copepods were 26.26% and 28.32% respectively. In addition, copepods that were fed with TET had a DHA (C22:6 n-3) content of 3.61%, whereas THA had a substantially greater content of 16.62%. On the other hand, the levels of omega-6 fatty acids in both states of copepods were 14.74% and 6.14% respectively. ARA (C20:4 n-6) fatty acid was found in copepods fed with TET at a level of 1.07%, while THA had a level of 2.23%. This information is summarized in **Table 1.**

Table 8. Fatty acid analysis in the two different feeding conditions (TET and THA) of the cyclopoid *A. royi*-TH

Fatty acid	ArTET	ArTHA
DHA	3.61±0.55 a	16.62±1.37 b
EPA	2.61±0.17 a	6.89±0.29 b
ARA	1.07±0.12 a	2.23±0.03 b
∑n-3 PUFA	26.26±2.19	28.32±1.49
∑n-6 PUFA	14.74±1.06 a	6.14±0.52 b
∑SC-PUFA	32.39±3.18 a	8.44±0.32 b
∑LC-PUFA	8.62±0.95 a	26.30±1.68 b
∑PUFA	41.00±3.17	34.46±0.97

*Data represents the mean±standard deviation of triplicate samples. Means with different lowercase letter (shown above) are significantly different ($p < 0.05$; one-way ANOVA with Duncan's multiple range tests).

The fatty acid composition of copepod *A.royi*-TH was studied using the GC/MS method under low salinity conditions (15 ppt) and under controlled salinity conditions (25 ppt) in **Table 2**, using

TET as a food source The analysis reveals that the PUFA content of copepod *A. royi*-TH is determined to be 45.42% and 41.00%. The analysis of the omega-3 fatty acid content in copepod *A. royi*-TH, which were raised at salinities of 15 ppt and 25 ppt, revealed values of 27.89% and 26.26% respectively. In addition, DHA is also found under low-salt conditions in the copepod, with a value of 3.70%, and in controlled salinity conditions with a value of 3.61%. The levels of omega-6 fatty acids in both conditions were 17.52% and 14.74% respectively. The copepods cultivated in a salinity of 15 ppt exhibited an ARA concentration of 1.64%, while those cultured in a salinity of 25 ppt displayed an ARA concentration of 1.07%. The results of this investigation, consistent with the findings of a previous study [10], showed a significant increase in ARA synthesis in shrimp *L. vannamei* when they were reared in low salinity environments.

Table 9. The fatty acid composition in copepod *A. royi-TH* exposed to different salinities (15 ppt and 25 ppt)

Fatty acid	ArTET15 (hyposalinity)	ArTET25 (control)
C20:5 n-3 (EPA)	2.87 ± 0.05[a]	2.61 ± 0.17
C22:6 n-3 (DHA)	3.70 ± 0.06[a]	3.61 ± 0.55[a]
C20:4 n-6 (ARA)	1.64 ± 0.03[a]	1.07 ± 0.12[b]
∑SFA	18.80 ± 0.07[b]	20.86 ± 1.18[a]
∑MUFA	8.28 ± 0.06[a]	8.70 ± 1.96[a]
∑SC-PUFA	37.20 ± 0.15[a]	32.39 ± 3.18[a]
∑LC-PUFA	8.21 ± 0.14[a]	8.62 ± 0.95[a]
∑n-3 PUFA	27.89 ± 0.08[a]	26.26 ± 2.19[a]
∑n-6 PUFA	17.52 ± 0.20[a]	14.74 ± 1.06[b]
∑PUFA	45.42 ± 0.28[a]	41.00 ± 3.17[a]

* Data represents the mean±standard deviation of triplicate samples. Means with different lowercase letter (shown above) are significantly different ($p < 0.05$; one-way ANOVA with Duncan's multiple range tests).

3.3 Gene expression analysis in copepod A. royi-TH

The expression of the ArFABP gene in copepod *A. royi*-TH reared with different types of algae was significantly higher ($p < 0.05$) in copepods reared with THA compared to those reared with TET. This was consistent with the findings regarding the levels of fatty acids, specifically LC-PUFA and ARA, which were higher in the THA group compared to the TET group. Additionally, the analysis of gene expression revealed that the ArFABP gene showed a substantial increase in expression in copepod *A. royi*-TH that were raised in a salinity of 15 ppt, compared to those raised in a salinity of 25 ppt ($p < 0.05$).

The expression of the ArFABP gene in both the algae-fed copepod samples and the copepod samples under different salinity conditions had the same effect as the expression of the ArD6D gene, which is a desaturase gene responsible for the production of complex unsaturated fatty acids, as reported in the copepod *A. royi* [1]. This research is similar to the study on FABP in the copepod *Calanus finmarchicus*. It was found that FABP may have a role in storing wax esters in the oil sac, and its expression may decrease as the oil sac becomes full [11]. Furthermore, a study conducted on the swimming crab *Portunus trituberculatus* examined a FABP and its impact on the fatty acid composition in various tissues. The results showed that the fatty acid profile aligned with the expression of FABP, indicating that FABP plays a role in the transportation and storage of unsaturated

fatty acids in P. trituberculatus [12]. Together, these findings indicate that that the ArFABP gene may be involved in the transport of fatty acids in the synthesis of LC-PUFA fatty acids in copepod *A. royi*-TH.

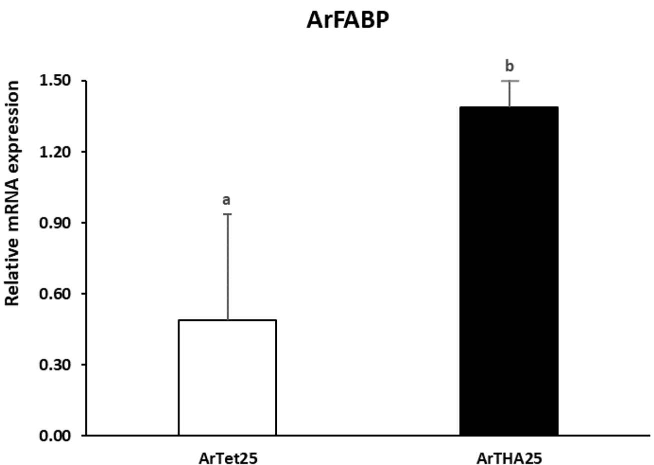

Figure 2. The mRNA expression of ArFABP from the copepod *A. royi*-TH at different feed (*Tetraselmis sp*;TET and *Thalassiosira sp.*;THA). The Ar18srRNA served as an internal reference gene. Data represents the mean ± standard deviation (error bars) of triplicate samples. Means with different lowercase letter (above each bar) are significantly different.

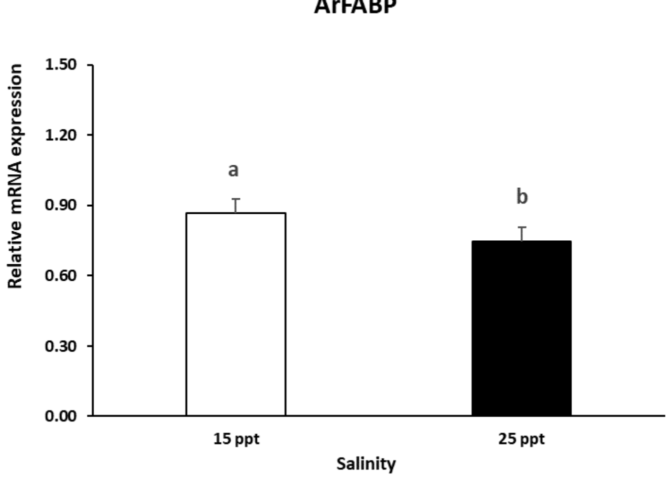

Figure 3. The mRNA expression of ArFABP from the copepod *A. royi*-TH at different salinity (15 ppt and 25 ppt). The Ar18srRNA served as an internal reference gene. Data represents the mean ± standard deviation (error bars) of triplicate samples. Means with different lowercase letter (above each bar) are significantly different.

4. CONCLUSIONS

This study has successfully identified the ArFABP gene, which is associated with the production of LC-PUFA in copepod *A. royi*-TH. The results of this work suggest that the ArFABP gene may have a function in transporting fatty acids for the production of LC-PUFA fatty acids in copepod

A. royi-TH. The insights obtained from this research report could be advantageous for the future advancement of copepod as a functional feed in the field of food biotechnology.

5. ACKNOWLEDGEMENTS

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Inhibitory Properties of Duckweed Extracts on Digestive Enzymes: an Emerging Role of Duckweeds as Functional Food to Fight Against Diabetes and Obesity

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Keywords

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Diabetes
Obesity
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ABSTRACT

Duckweeds are small floating aquatic plants that thrive on freshwater. Due to their high protein and carbohydrate content, duckweeds are commonly used as animal feed. Recently, they are considered as nutritious human food though the adverse effect of duckweeds on digestive systems has not been clearly elucidated. Therefore, inhibition of digestive enzyme activity by duckweed extracts were evaluated. Duckweeds, including giant duckweed (*Spirodela polyrhiza*), lesser duckweed (*Lemna aequinoctialis*), and water meal (*Wolffia globosa*), were axenically cultured in the laboratory and co-extracted using ethanol and hexane. Extracts were tested inhibitory effect on alpha-amylase, lipase and trypsin. Acarbose, Orlistat and phenylmethylsulfonyl fluoride (PMSF) were used as positive controls for assaying alpha-amylase, lipase and trypsin, respectively. Results revealed that hexane extract of *W. globosa* strongly inhibited activity of alpha-amylase (IC_{50} = 90.82 µg/ml) whereas its ethanolic extract slightly inhibited activity of lipase (IC_{50} > 300 µg/ml). Meanwhile, hexane extract of *S. polyrhiza* partially inhibited activity of both alpha-amylase and lipase (IC_{50} > 300 µg/ml). As expected, none of duckweed extracts inhibited activity of trypsin, suggesting that they were safe for consumption, ensuring normal protein digestion and amino acid uptake, respectively. Intriguingly, both ethanol and hexane extracts of *L. aequinoctialis* had relatively high phenolic content, 43.65 ±1.26 $mgGAE.g^{-1}$ and 50.82±1.26 $mgGAE.g^{-1}$, respectively, though ethanolic extract of *W. globosa* yielded the highest phenolic content at 62.33±1.91 $mgGAE.g^{-1}$. Taken together, results suggest that water meal and giant duckweed are promising to be used as novel functional food, particularly, for those who are affected by diabetes mellitus and obesity.

1. INTRODUCTION

Duckweeds are the smallest flowering plants that grow in freshwater. They belong to *Lemnaceae* family, comprising 5 genera, including *Spirodela*, *Landoltia*, *Lemna*, *Wolffia* and *Wolffiella* [1] though the common species found in Thailand are *Spirodela polyrhiza*, *Landoltia punctata, Lemna* spp. and *Wolffia globos*a.

Duckweeds grow relatively fast, their doubling time is in the range of 1.34-4.54 days under standardized cultivation conditions, surpassing other agricultural crops [2]. For this reason, duckweeds have drawn a lot of attention from scientists and investors worldwide to explore and utilize their advantages. Recently, biotechnologists have identified water meal (*W. globosa*) as a candidate for an alternative food supply, known as the superfood “Mankai” [3]. Not only have numerous duckweed species been used for nutritious human food [4] and animal feed [5], but their pharmaceutical benefits have also been extensively studied [6].

Several works on nutraceutical and pharmaceutical effects of duckweeds have mainly focused on nutrient (protein, carbohydrate, vitamin), antioxidant activity, anticancer activity and antibiotic activity [4-6], but there are couple studies emphasizing on antiglycemic- and antiadipogenic effects of duckweeds [7,8]. These two properties are crucial for patients who suffer from diabetes mellitus and obesity leading to cardiovascular complications. Even though the latter is rather the actual major public health problems, interventions by manipulating food environment are pivotal as obesity and diabetes mellitus more likely double the onset of cardiovascular disease [9].

Thus, using the adverse effect of phytochemicals existing in duckweeds on the activity of digestive enzymes, especially amylase (starch hydrolysis) and lipase (fat hydrolysis), could simply control sugar and lipid level in bloodstream. This mechanism basically supports the concept of functional food which promotes health and prevents disease at safe and sufficient concentration [10]. By preventing hydrolysis of starch and fat, sugar and fatty acid absorption is minimized thereby allowing the control of these nutrient uptakes. Even though there are several medicinal plants exhibit antidiabetic- and anti-obesity properties [11,12], this information is insufficient in duckweeds. Therefore, this work will shed light on the role of duckweeds as a novel functional food for protecting against diabetes and obesity.

2. MATERIAL AND METHODS

2.1 Duckweed cultivation and extraction

Three different species of duckweeds, including giant duckweed (Spirodela polyrhiza), lesser duckweed (Lemna aequinoctialis), and water meal (Wolffia globosa) were collected from natural reservoirs in Burapha University, Chonburi, Thailand (GPS coordinates: 13.278699, 100.923863). All duckweeds were washed with tap water three times, respectively. L. aequinoctialis and S. polyrhiza were subject to trimming and surface sterilization using 0.3% NaClO for 1 and 3 minutes, respectively. Whereas W. globosa was wrapped with straining cloth prior to surface-sterilization by submerging in 2.0% Povidone-iodine (PVP-I) for 30 seconds. All surface sterilized duckweeds were then rinsed with sterile distilled water at least three times and cultured on solid MS medium [13] to allow frond regeneration. After two weeks, duckweeds were subsequently transferred to liquid Hoagland’s medium [14] and axenically cultivated for use in further experiment. The growth condition was 16-hour light: 8-hour dark photoperiod with an irradiance of 55 $\mu mol.m^{-2}.s^{-1}$ from fluorescent light bulbs at 25±2 °C.

Duckweeds were ground into fine powders using liquid nitrogen and kept at -20 °C. Ground samples (6 g) were extracted with 60 ml of co-solvent solution comprising hexane and 95% ethanol at a ratio of 2:1 by volume. The extract procedures were carried out at ambient temperature (25 °C) for 20 minutes. The extracts were centrifuged at 2,700xg for 10 minutes and filtered through glass filters. The immiscible filtrates were separated using the separatory funnel. Each phase was dried to remove organic solvent (hexane or ethanol) in the vacuum chamber, respectively. The crude extracts were dissolved in dimethyl sulfoxide (DMSO) and stored at -20 °C for further analysis.

2.2 Enzyme assays

Pancreatic lipase inhibition assay Baseline lipase activity was measured using 50 U of porcine pancreatic lipase (L3126, Sigma-Aldrich, St. Louis, Mo., U.S.A) in a 990 µl reaction buffer (50 mM Tris-hydrochloride, 4 mM sodium deoxycholate, 0.12 mM $CaCl_2$, 0.4 mM p-nitrophenyl palmitate (p-NPP), pH 7.8) and 10 µl DMSO [15]. The reaction was conducted at 37 °C. DMSO was substituted duckweed extracts and the positive control, Orlistat (Xenical®, Switzerland) dissolved in DMSO at varying concentration to monitor the inhibition of lipase activity. Absorbance changes were recorded at 410 nm every 30 seconds to determine the reaction velocity. The rate of each reaction was compared with the baseline activity (100%) to generate relative activity.

Pancreatic alpha-amylase inhibition assay Baseline alpha-amylase activity was measured using 0.4 U of porcine pancreatic alpha-amylase (A3176, Sigma-Aldrich, St. Louis, Mo., U.S.A) in a 990 µl reaction buffer (10 mM sodium phosphate and 0.015% starch, pH 6.9) and 10 µl DMSO [15]. Alpha-amylase assay was conducted at 37 °C for 15 minutes. To stop the reaction and measure its activity, 1 ml of 5 mM iodine solution was added. Absorbance at 620 nm was measured at 1, 5, 10, and 15 minutes after the reaction was stopped. The inhibitory effects of duckweed extracts and the positive control, Acarbose (Glucobay®, Indonesia), were measured using DMSO replacement and comparison approach as described above.

Pancreatic trypsin inhibition assay Baseline trypsin activity was measured using 20 U of porcine pancreatic trypsin (T7168, Sigma-Aldrich, St. Louis, Mo., U.S.A) in a 990 µl reaction buffer (25 mM Tris-hydrochloride, 10 mM $CaCl_2$, and 1 mM benzoyl-DL-arginine p-nitroanilide hydrochloride (BAPNA), pH 7.6) and 10 µl DMSO [15]. The reaction was carried out at 37 °C. The inhibitory effects of duckweed extracts and the positive control, phenylmethanesulfonyl fluoride (PMSF), were measured using DMSO replacement and comparison approach as described above at 410 nm every 1 minute.

2.3 Total phenolic content determination

The total phenolic content of duckweed extracts was analyzed following the method described previously [16] with slight modifications. In summary, 0.5 ml of crude duckweed extracts was mixed with 0.5 ml of 0.5 N Folin-Ciocalteu reagent and incubated for 6 minutes. Subsequently, 2 ml of 5% sodium carbonate was added, and the mixture was kept in the dark for 30 minutes. The absorbance was then measured at 760 nm. Gallic acid served as the standard, and the results were quantified and expressed as gallic acid equivalents.

2.4 Statistical analysis and calculation

Results were statistically analyzed using one-way ANOVA. Significance of difference from the negative control (DMSO) and positive control (Orlistat, Acarbose, and PMSF) was determined by Tukey's test at 95% (p <0.05) confidential level using the Minitab software version 18.

Enzyme activity was estimated from reaction velocity or the slope of the plot between reaction time and changes of absorbance at designated wavelength. The full enzyme activity, $V_{control}$, was set as 100%, and the enzyme activity corresponding to the reaction with duckweed extracts, V_{sample}, was used to calculate remaining activity percentage by the following equation:

$$Activity = 100\ x\ \frac{Vcontrol - Vsample}{Vcontrol}$$

IC_{50} (the half maximal inhibitory concentration) values were estimated using Microsoft Excel and ED50plus v1.0.

3. RESULTS AND DISCUSSION

3.1 Axenic culture of duckweeds

Naturally, duckweeds float on the surface of freshwater. Beneath their fronds (stem and leaf), algae often grow alongside them (See Figure 1A). This cohabitation can potentially lead to misinterpretation of results, even when cultivated in a closed system. Therefore, it is advisable to surface sterilize the duckweeds and maintain them in aseptic conditions (axenic culture). This practice enables a clear examination of duckweed morphology (See Figure 1). Supported by DNA sequencing results (data not shown), it is confirmed that the three duckweed species utilized in the experiment were giant duckweed (*S. polyrhiza*), lesser duckweed (*L. aequinoctialis*), and water meal (*W. globosa*).

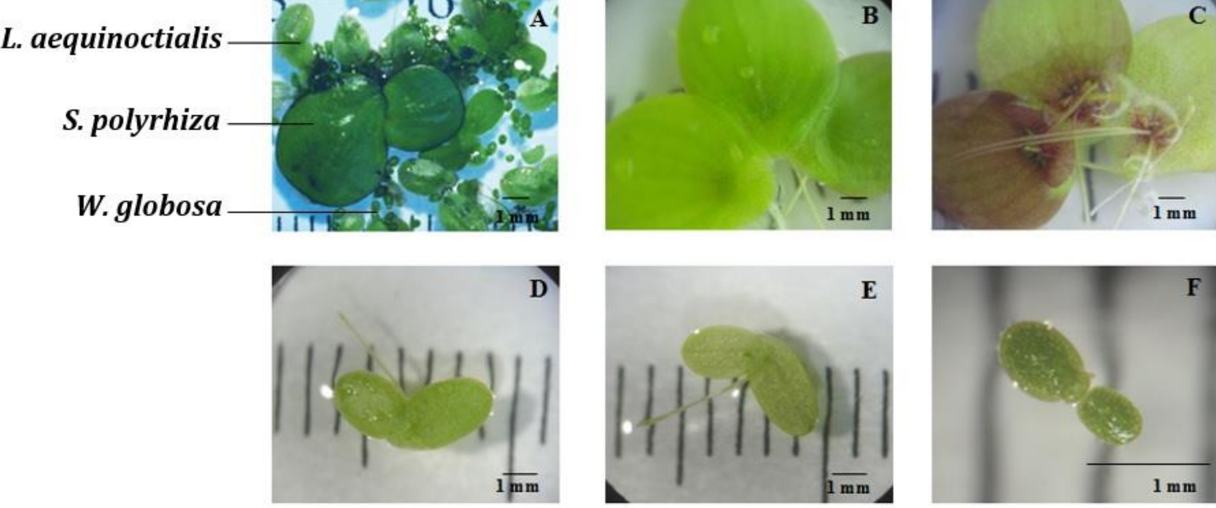

Figure 11. Comparison of duckweeds collected from natural reservoirs (A) and axenically cultivated in the laboratory (B-F). Morphology and size of *S. polyrhiza* (B-C), *L. aequinoctialis* (D-E) and *W. globosa* (F) are different. (scale bar = 1 mm)

3.2 Inhibition of lipase activity

Lipase is a key enzyme responsible for hydrolyzing dietary fat in the digestive system. Orlistat, an anti-obesity medication, effectively inhibits pancreatic lipase activity, thereby reducing fat absorption in the intestine. In this study, we used Orlistat as a positive control for assaying the inhibition of porcine pancreatic lipase in vitro. Orlistat exhibited a strong inhibitory effect on pancreatic lipase, with an IC_{50} of 0.27 µg/ml (See Figure 2 and Table 1), which closely matched the

FDA-approved datasheet (IC_{50} = 0.12 µg/ml) [17]. This finding suggests that our control experiment was comparable and reproducible.

Interestingly, only hexane extract of *S. polyrhiza* and ethanol extract of *W. globos*a showed partial inhibition of lipase activity (IC_{50} > 300 µg/ml) (See Figure 2 and Table 1). This indicates that the bioactive compounds exhibiting lipase inhibitory properties in both duckweeds differed in polarity. Furthermore, the phenolic compounds found in these duckweeds might contribute to lipase inhibition, as both extracts had relatively higher total phenolic content compared to the other extract fractions in each species (See Table 1). A previous study also suggested a potential relationship between the total phenolic content obtained from fermented and non-fermented oats and the inhibition of lipase. Among several phenolic compounds, caffeic acid, ferulic acid, and p-coumaric acid were found to synergistically inhibit lipase activity [18].

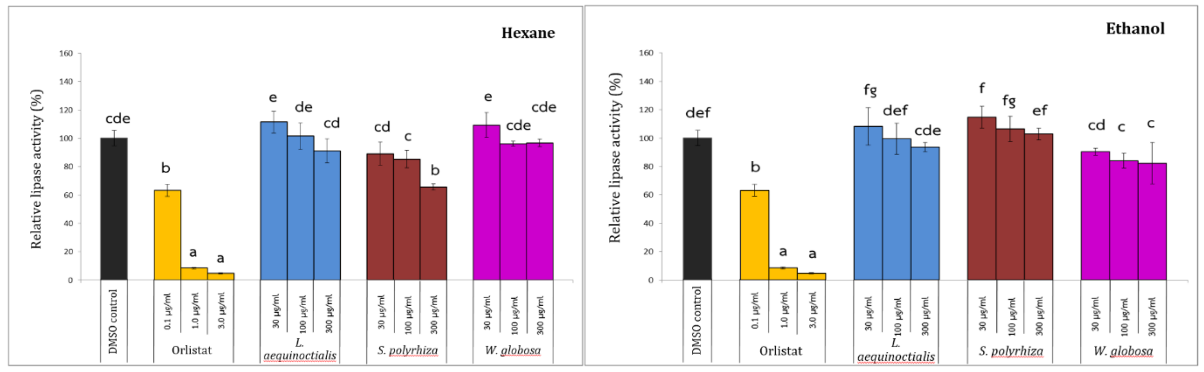

Figure 2. Inhibition of porcine pancreatic lipase by duckweed extracts obtained from hexane fraction (left) and ethanol fraction (right) at different concentration. Relative lipase activity was compared with DMSO control and Orlistat. Different letters indicate statistically significant differences (p<0.05). (Bars = S.D.)

3.3 Inhibition of alpha-amylase activity

Alpha-amylase is one of the key hydrolytic enzymes involved in carbohydrate metabolism, responsible for breaking down starch and glycogen into glucose and maltose in the digestive system. Acarbose, an anti-diabetic medication, effectively inhibits pancreatic alpha-amylase, with an IC_{50} ranging from 1.24 - 812.83 µg/ml depending on experimental conditions [19]. In this study, we utilized Acarbose as a positive control to assess the inhibition of porcine pancreatic alpha-amylase in vitro. Acarbose demonstrated a strong inhibitory effect on pancreatic alpha-amylase, with an IC_{50} of 140.96 µg/ml (See Figure 3 and Table 2), aligning with previous reports , indicating the reliability of our control experiment.

It was evident that only duckweed extracts derived from the hexane fraction exhibited inhibition of porcine pancreatic alpha-amylase. The hexane extract of *Wolffia globosa* demonstrated the most potent alpha-amylase inhibitory property, with an IC_{50} of 90.82 µg/ml, slightly outperforming Acarbose (See Figure 3 and Table 1). Additionally, the hexane extract of *Spirodela polyrhiza* exhibited subtle inhibition, while the hexane extract of *Lemna aequinoctialis* unexpectedly enhanced alpha-amylase activity (See Figure 3). This unexpected pattern was attributed to bioactive compounds, potentially phenolic compounds, in the extract interfering with iodine in the assay, resulting in a reverse effect (data not shown). Various classes of phytochemicals, such as flavonoids, terpenoids, polysaccharides, phenolic acids, and tannins, have been reported to inhibit alpha-amylase, suggesting that phenolic compounds in duckweeds may not play a significant role in alpha-amylase inhibition.

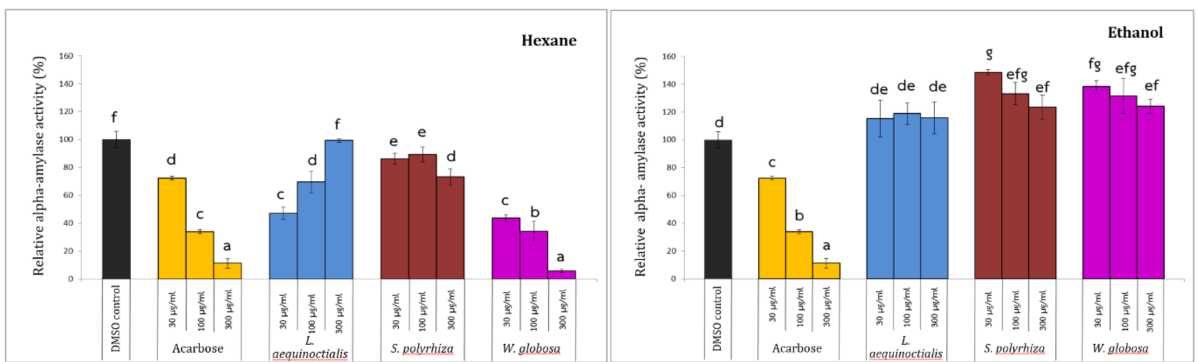

Figure 3. Inhibition of porcine pancreatic alpha-amylase by duckweed extracts obtained from hexane fraction (left) and ethanol fraction (right) at different concentration. Relative alpha-amylase activity was compared with DMSO control and Acarbose. Different letters indicate statistically significant differences (p<0.05). (Bars = S.D.)

3.4 Inhibition of trypsin activity

Pancreatic trypsin was utilized as a control experiment to determine the specificity of enzyme inhibition in the digestive system. Plants in the Leguminosae family, such as soybeans, are the primary source of trypsin inhibitors [20]. Phenylmethylsulfonyl fluoride (PMSF) acts as an irreversible inhibitor of serine proteases, binding specifically and forming a covalent bond with the serine residue at the active site of trypsin, rendering the enzyme inactive [21]. In this study, PMSF was employed as a positive control to assess the inhibition of porcine pancreatic trypsin *in vitro*, demonstrating a significant inhibitory effect on pancreatic trypsin with an IC_{50} of 40.51 µg/ml (See Figure 4 and Table 1).

As anticipated, none of the duckweed extracts exhibited inhibition of porcine pancreatic trypsin (See Figure 4 and Table 1). This outcome underscores the potential of duckweeds as a novel protein source, as their assimilation was not hindered during the digestive process. Several studies have highlighted duckweeds as a superfood for humans, particularly *Wolffia globosa* [3,4,6]. Not only does it boast a high protein content, but its protein is also readily absorbed into the bloodstream [22], partly due to its complete digestion without significant inhibition. Furthermore, previous research has indicated that the digestible indispensable amino acid score (DIAAS) of *Wolffia arrhiza* is 0.75, indicating that duckweed protein is highly suitable for human consumption [23].

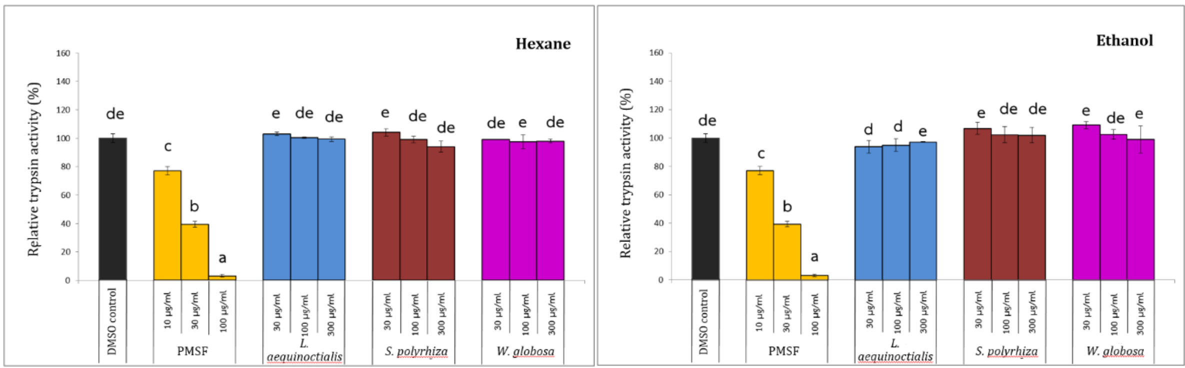

Figure 4. Inhibition of porcine pancreatic trypsin by duckweed extracts obtained from hexane fraction (left) and ethanol fraction (right) at different concentration. Relative trypsin activity was compared with DMSO control and PMSF. Different letters indicate statistically significant differences (p<0.05). (Bars = S.D.)

3.5 *Total phenolic content in duckweed extracts*

Phenolic compounds are major plant metabolites that exhibit various biological activities, including antioxidant, anti-inflammatory, and antibiotic properties [24]. We analyzed the total phenolic content to gain insight into the phenolic compound composition of duckweed extracts. It was surprising to find that most duckweed extracts yielded higher phenolic content in the hexane fraction, except for the extract of *Wolffia globosa*, which showed a higher phenolic content in the ethanol fraction at 62.33 ± 1.91 mgGAE.g^{-1} (See Table 1 Interestingly, a recent report indicated that *W. globosa* contains 40.83 ± 4.99 mgGAE.g^{-1} when extracted with ethanol [25] which is one-third lower than our findings. This suggests that co-solvent extraction with hexane and ethanol resulted in a higher phenolic content compared to extraction solely with ethanol.

Surprisingly, among all duckweed extracts, the extract of *Spirodela polyrhiza* exhibited the lowest phenolic content at 12.73 ± 1.49 mgGAE.g^{-1} in the ethanol fraction and 27.08 ± 4.71 mgGAE.g^{-1} in the hexane fraction. Despite the fact that the extract of *L. aequinoctialis* showed no significant inhibitory effect on digestive enzymes, it displayed a relatively high overall phenolic content in both fractions, with values of 43.65 ± 0.88 and 50.82 ± 1.26 mgGAE.g^{-1}, respectively (See Table 1). This suggests that *L. aequinoctialis* might be suitable as animal feed or a novel food for patients with digestive anomalies, as it also contains high levels of protein and carbohydrates [6] while demonstrating a low adverse effect on the digestive system, as reported in this study.

While our experiment has not definitively shown whether phenolic compounds are the major factors contributing to enzyme inhibition, in general, phenolic compounds can inhibit enzyme activity by interacting with enzymes through various mechanisms, primarily due to their structural properties, notably the presence of multiple hydroxyl groups. These compounds are recognized for their ability to form covalent or hydrogen bonds with enzymes, thereby modifying their active sites and impeding their ability to effectively catalyze substrates. Moreover, phenolic compounds can also form complexes with substrates, hindering enzyme access to the substrates [26].

Table 1. Summary of inhibitory properties of duckweed extracts on digestive enzymes and relationship with the total phenolic content

Source of extraction	Solvent fraction	Lipase inhibition	Amylase inhibition	Trypsin inhibition	Phenolic content (mgGAE.g^{-1})
L. aequinoctialis	Hexane	NS	ND	NS	50.82 ± 1.26^{d}
	Ethanol	NS	NS	NS	43.65 ± 0.88^{c}
S. polyrhiza	Hexane	+++	++	NS	27.08 ± 4.71^{b}
	Ethanol	NS	ND	NS	12.73 ± 1.49^{a}
W. globosa	Hexane	NS	IC_{50} = 90.82 µg/ml	NS	29.81 ± 1.63^{b}
	Ethanol	+	ND	NS	62.33 ± 1.91^{e}
Orlistat		IC_{50} = 0.27 µg/ml	-	-	-
Acarbose		-	IC_{50} = 140.96 µg/ml	-	-
PMSF		-	-	IC_{50} = 40.51 µg/ml	-

* Abbreviation: NS = not significant, ND = not determined, IC_{50} = half maximal inhibitory concentration
** Symbol: + = 10-20 % inhibition, ++ = 20-30% inhibition, +++ = 30-40% inhibition
*** Different letters indicate statistically significant differences (p<0.05)

4. CONCLUSIONS

Duckweeds are small aquatic plants that were once considered as weeds or simply animal feed. Due to their unique properties, such as fast growth and high nutritional value, they have recently

garnered significant attention in response to climate change and health concerns. This research has unveiled a new dimension of duckweeds in food innovation by harnessing their ability to inhibit digestive enzymes, potentially transforming them into functional foods to address issues related to diabetes and obesity. Notably, the current market trend is shifting towards the utilization of watermeal (*Wolffia globosa*) as a superfood, a direction supported by our findings. These findings not only highlight the numerous nutritional benefits of duckweeds but also suggest that specific varieties like watermeal and giant duckweed (*Spirodela polyrhiza*) could be developed into anti-diabetic and anti-obesity products in the future.

5. NOMENCLATURE

U	enzyme unit	$\mu mol.min^{-1}$
IC_{50}	half maximal inhibitory concentration	μg/ml

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Evaluation of Prebiotic Activity Score of Konjac Glucomannan Hydrolysate and Commercial Prebiotic

DP-O202

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Keywords

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Prebiotic activity score
Probiotic
Dietary fiber

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ABSTRACT

Konjac glucomannan hydrolysate (KGMH) is a non-calorie food and a source of indigestible dietary fiber with potential health benefits. This study seeks to evaluate the prebiotic activity score (PAS) of KGMH in comparison to commercial prebiotics including inulin and fructooligosaccharides (FOS). The PAS measures prebiotic efficacy based on its ability to promote probiotic growth and inhibit pathogen growth. Probiotic strains (*Lactobacillus acidophilus* DDS-1®, *L. rhamnosus* LGG®, *L. paracasei* 431®, *L. salivarius* B37, *Bifidobacterium breve*, and *B. longum*) and the pathogen *Escherichia coli* ATCC 25922 were inoculated at 1% (v/v) into their respective culture media supplemented with 1% (w/v) of either commercial prebiotic or KGMH: Tryptic Soy Broth (TSB) for enteric culture and de Man, Rogosa and Sharpe (MRS) broth for probiotics. Cultures were incubated at 37 °C for 72 hours. Bacterial cell counts were determined at 0, 12, 24, 48, and 72 hours, and PAS was calculated. *L. acidophilus* with KGMH exhibited the highest PAS at 48 hours, while FOS demonstrated the highest score at 24 and 72 hours. Similarly, *L. paracasei* with KGMH showed the highest PAS at 24 hours, with inulin exhibiting the highest score at 48 and 72 hours. KGMH displayed the highest for *L. salivarius* at 24 hours. *Bifidobacterium* strains showed non-significant differences in prebiotic activity scores between KGMH and other prebiotics. Overall, KGMH demonstrated the highest PAS alongside all strains at 24 hours. These findings underscore KGMH's potential health benefits and provide a basis for optimizing synbiotic combinations of probiotics and prebiotics.

1. INTRODUCTION

Konjac glucomannan hydrolysate (KGMH) is a hydrolyzed form of konjac glucomannan. It contains oligosaccharides and can be used for encapsulation (1). KGMH are natural polysaccharides with numerous applications in both the food and non-food sectors, including the chemical and health industries, owing to their non-hazardous, non-toxic, and biocompatible properties, as well as their good solubility.

It is also known as a non-calorie food and a source of indigestible dietary fiber, which is resistant to hydrolysis by digestive enzymes in the human gut (2). A previous study suggested that the distinctive characteristics of KGMH make it universally advantageous as a prebiotic, suitable for incorporation into a diverse array of food, feed, and healthcare/pharmaceutical products (3). KGMH is a prebiotic source that provides an ability as an edible film properties (4). It can coat any food materials to protect bioactive compounds in spray drying method (5). This incorporation offers consumers an avenue to enhance their gut health through the consumption of a prebiotically beneficial component. Nevertheless, KGMH has never been studied for its prebiotic capacity in some strains of probiotics.

Prebiotics consist of indigestible components that optimize the growth of probiotics, within the digestive systems, serving as nourishment for probiotics. Prebiotics are often carbohydrate-based, such as fructooligosaccharides (FOS) and inulin. The acquisition of an appropriate combination of probiotics and prebiotics holds the potential to optimize the effectiveness of food products, thereby offering maximal health benefits. (6) Ideal prebiotics should fulfill specific criteria, including resistance to stomach acids, bile salts, and intestinal hydrolyzing enzymes, lack of absorption within the upper gastrointestinal tract, and ease of fermentation by the beneficial intestinal microflora (7).

Probiotics are live microorganisms that confer health benefits to the host when consumed in adequate amounts. They are identified by their specific strain, which includes the genus, the species, the subspecies, and an alphanumeric strain designation (8). The popularly used probiotic microorganisms are *L. rhamnosus*, *L. reuteri*, *Bifidobacteria,* and certain strains of *L. casei* (9). *Lactobacillus*, a gram-positive bacterium with the capability to convert sugars into lactic acid, has been explored as a probiotic in numerous studies (10). *Bifidobacterium*, a gram-positive, nonmotile, anaerobic bacteria, is among the gut microbiota believed to provide positive health benefits to their host (11). However, an investigation into KGMH's prebiotic activity score particularly concerning *Lactobacillus* and *Bifidobacterium* strains, has yet to be conducted. This present study aims to evaluate the prebiotic activity score (PAS) of KGMH across four strains of *Lactobacillus* and two strains of *Bifidobacterium* which are *L. acidophilus* DDS-1®, *L. rhamnosus* LGG®, *L. paracasei* 431®, *L. salivarius* B37, *B. breve*, and *B. longum*.

2. MATERIAL AND METHODS

The pairing method was used to compare the growth of two groups of microorganisms, probiotic and pathogenic, over a period of 72 hours in the presence and absence of the substance of interest. PAS is a score indicating that the substance of interest is promoting probiotic growth and/or inhibiting pathogens. Low scores mean low promotion or no promotion at all (12). For this study, we compared the PAS of KGMH and other commercial prebiotic products including inulin and FOS.

2.1 Material

- Probiotic strain (*L. acidophilus* DDS-1®, *L. rhamnosus* LGG®, *L. paracasei* 431®, *L. salivarius* B37, *B. breve*, and *B. longum*)
- Pathogen (Enteric culture: *Escherichia coli* ATCC 25922)
- Culture media (MRS: deMan Rogosa Sharpe and TSA: Tryptic Soy Agar from Himedia™-India)
- Prebiotic (Inulin, Fructooligosaccharide, and KGMH from Chulalongkorn University-Thailand).

2.2 Methods

PAS was performed according to Huebner et al. (2007) (13). In brief, two groups of microorganisms (probiotic and pathogenic) were cultured separately in liquid media under 37°C for 72 hours (MRS media for probiotics and TSA media for enteric cultures). Subsequently, they were diluted to a concentration of 10 log CFU/mL. Next, 1% (v/v) of both the probiotic and enteric cultures

were inoculated into 10 mL of MRS or TSA media containing 0.1 g of prebiotics. After an additional 12, 24, 48 and 72 hours of incubation, bacterial cell counts were determined using the pour plate technique. The probiotic culture was poured onto MRS media, while the enteric cultures was poured onto the TSA media plates. The effect of the prebiotic was determined using the PAS equation:

$$\mathrm{PAS} = \left(\frac{\mathrm{logPx24} - \mathrm{logPx0}}{\mathrm{logPg24} - \mathrm{logPg0}}\right) - \left(\frac{\mathrm{logEx24} - \mathrm{logEx0}}{\mathrm{logEg24} - \mathrm{logEg0}}\right)$$

Where:
Px is the number of colonies of probiotics that grow in media containing prebiotics.
Pg is the number of colonies of probiotics that grow in media containing glucose.
Ex is the number of colonies of the enteric mixture that grow in media containing prebiotics.
Eg is the number of colonies of the enteric mixture that grow in media containing glucose.

2.3 Statistical analysis

Statistical analysis will be performed using Factorial ANOVA (SPSS Statistics Data Editor, Edition 28) to determine whether there were any significant differences in the effect of the substrates, differences at each time, with Duncan's post-test. All significant differences between means will be assessed at the significant level of $\alpha = 0.05$ (95% confidence level). P value < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Growth of lactobacilli, bifidobacteria, and enteric mixture on KGMH and commercial prebiotic

The investigation of the growth of probiotic and enteric mixtures on various prebiotics aimed to elucidate the comparative growth patterns of these microbial strains on different substrates. Viable cell counts of all microorganisms used in this experiment are detailed in Table 1. Significant differences in viable cell counts were observed among bacterial cultures and carbon sources at various time points. Figure 1 depicts the trajectory of microbial proliferation. All probiotic strains attained the stationary phase after 48 hours, albeit certain strains, such as *L. acidophilus*, *L. salivarius*, and *L. paracasei*, exhibited diminished viability leading to a decline after 24 hours. Conversely, the growth pattern of the enteric mixture featured modest proliferation within the initial 12 hours, culminating in the stationary phase at the 24-hour mark. At 12 hours, *L. acidophilus*, *L. rhamnosus*, and *L. salivarius* showed higher viable cell counts when grown on FOS compared to glucose, while *B. breve* exhibited similar trends with inulin. At 24 hours, significant differences were observed among various carbon sources for all bacterial cultures, with *L. acidophilus*, *L. rhamnosus*, and *L. paracasei* showing higher viable cell counts on FOS compared to glucose. Moreover, at 48 and 72 hours, consistent trends were observed, with certain carbon sources consistently supporting higher viable cell counts across different bacterial cultures. For instance, at 48 hours, *L. acidophilus*, *L. rhamnosus*, and *B. breve* exhibited higher viable cell counts on FOS compared to glucose, while at 72 hours, *L. acidophilus* and *L. paracasei* showed similar trends. However, compared to growth on the common carbon source, glucose (2.34 cfu mL^{-1}), some prebiotics exhibited lower growth at 12 hours, such as FOS (1.04 cfu mL^{-1}) and KGMH (0.91 cfu mL^{-1}). These findings may indicate potential inhibition properties of these prebiotics during the early log phase, suggesting complex interactions between the enteric mixture and different prebiotic substrates.

Table 1. Viable cells between time 0 and time 72 h, reported as log10(cfu mL^{-1})±standard deviation, for bacterial cultures grown with various carbohydrates

Bacterial Culture	time	Glucose	Inulin	FOS	KGMH	*p* value
L.acidophilus	12 h	1.80±0.07^{bD}	1.86±0.10^{bB}	2.20±0.09^{aD}	1.64±0.05^{cD}	<0.001
	24 h	5.37±0.21^{aA}	4.04±0.13^{dA}	4.39±0.07^{cC}	4.89±0.05^{bA}	<0.001
	48 h	3.64±0.10^{cC}	3.93±0.11^{bA}	4.92±0.08^{aA}	3.30±0.07^{dC}	<0.001
	72 h	4.32±0.06^{bB}	4.04±0.06^{cA}	4.64±0.06^{aB}	3.67±0.07^{dB}	<0.001
	p value	<0.001	<0.001	<0.001	<0.001	
L.rhamnosus	12 h	2.54±0.07^{aD}	2.26±0.10^{bC}	2.30±0.04^{bD}	2.22±0.05^{bD}	<0.001
	24 h	4.08±0.12^{cC}	4.42±0.03^{bA}	4.14±0.04^{cB}	4.60±0.04^{aA}	<0.001
	48 h	5.30±0.05^{aA}	3.40±0.07^{cB}	4.76±0.04^{bA}	2.70±0.04^{dC}	<0.001
	72 h	4.95±0.11^{aB}	3.31±0.05^{cB}	3.46±0.12^{bC}	3.58±0.04^{bB}	<0.001
	p value	<0.001	<0.001	<0.001	<0.001	
L.salivarius	12 h	1.72±0.04^{dC}	2.09±0.07^{bB}	2.35±0.03^{aD}	1.97±0.03^{cC}	<0.001
	24 h	4.59±0.05^{aA}	2.92±0.02^{cA}	2.96±0.02^{cB}	3.97±0.05^{bA}	<0.001
	48 h	1.80±0.04^{cC}	1.94±0.05^{bD}	2.87±0.04^{aC}	1.85±0.04^{bcC}	<0.001
	72 h	2.97±0.04^{bB}	2.85±0.02^{bcA}	3.66±0.06^{aA}	2.77±0.42^{bcB}	<0.001
	p value	<0.001	<0.001	<0.001	<0.001	
L.paracasei	12 h	1.49±0.05^{cD}	1.90±0.03^{aD}	1.75±0.01^{bD}	0.91±0.05^{dD}	<0.001
	24 h	6.69±0.02^{aA}	6.98±0.02^{aA}	5.85±0.11^{cA}	6.87±0.02^{bA}	<0.001
	48 h	6.24±0.10^{aB}	4.95±0.06^{bB}	3.99±0.06^{cB}	3.95±0.05^{cC}	<0.001
	72 h	4.33±0.03^{cC}	4.60±0.01^{aC}	3.58±0.06^{dC}	4.43±0.04^{bB}	<0.001
	p value	<0.001	<0.001	<0.001	<0.001	
B.longum	12 h	3.96±0.24^{cD}	4.26±0.03^{bC}	4.91±0.02^{aB}	4.84±0.02^{aC}	<0.001
	24 h	6.19±0.20^{bA}	6.91±0.25^{aA}	5.86±0.04^{bA}	6.36±0.42^{bA}	0.004
	48 h	5.28±0.15^{cB}	4.76±0.10^{dB}	6.13±0.13^{aA}	5.67±0.15^{bB}	<0.001
	72 h	4.52±0.20^{aC}	4.42±0.24^{abC}	4.37±0.30^{abC}	3.84±0.28^{cD}	0.029
	p value	<0.001	<0.001	<0.001	<0.001	
B.breve	12 h	2.04±0.02^{abD}	1.96±0.15^{bC}	1.91±0.03^{bC}	2.12±0.03^{aC}	0.041
	24 h	5.22±0.13^{aA}	4.85±0.15b^{cA}	4.89±0.04^{bA}	4.86±0.01^{bA}	0.002
	48 h	4.36±0.09^{cB}	4.70±0.11^{abA}	4.83±0.02^{aA}	4.60±0.04^{bA}	<0.001
	72 h	4.03±0.11^{abC}	3.89±0.12^{abcB}	4.12±0.14^{aB}	3.75±0.12^{cB}	0.036
	p value	<0.001	<0.001	<0.001	<0.001	
Enteric mixture	12 h	2.34±0.02^{bC}	3.06±0.02^{aD}	1.04±0.48^{cB}	0.91±0.04^{cD}	<0.001
	24 h	3.27±0.22^{cB}	5.24±0.18^{aC}	4.43±0.08^{bA}	4.60±0.06^{bC}	<0.001
	48 h	3.09±0.07^{dB}	5.44±0.03^{aB}	4.52±0.04^{cA}	5.26±0.01^{bA}	<0.001
	72 h	5.17±0.09^{bA}	6.24±0.01^{aA}	4.11±0.04^{cA}	5.11±0.08^{bB}	<0.001
	p-value	<0.001	<0.001	<0.001	<0.001	

* Results are given as mean ± SD (n=3). Mean values with different superscript letters in the same column (lowercase letter) or row (capital letter) mean that the values are significantly different ($p<0.05$). FOS, fructooligosaccharides; KGMH, konjac glucomannan hydrolysate.

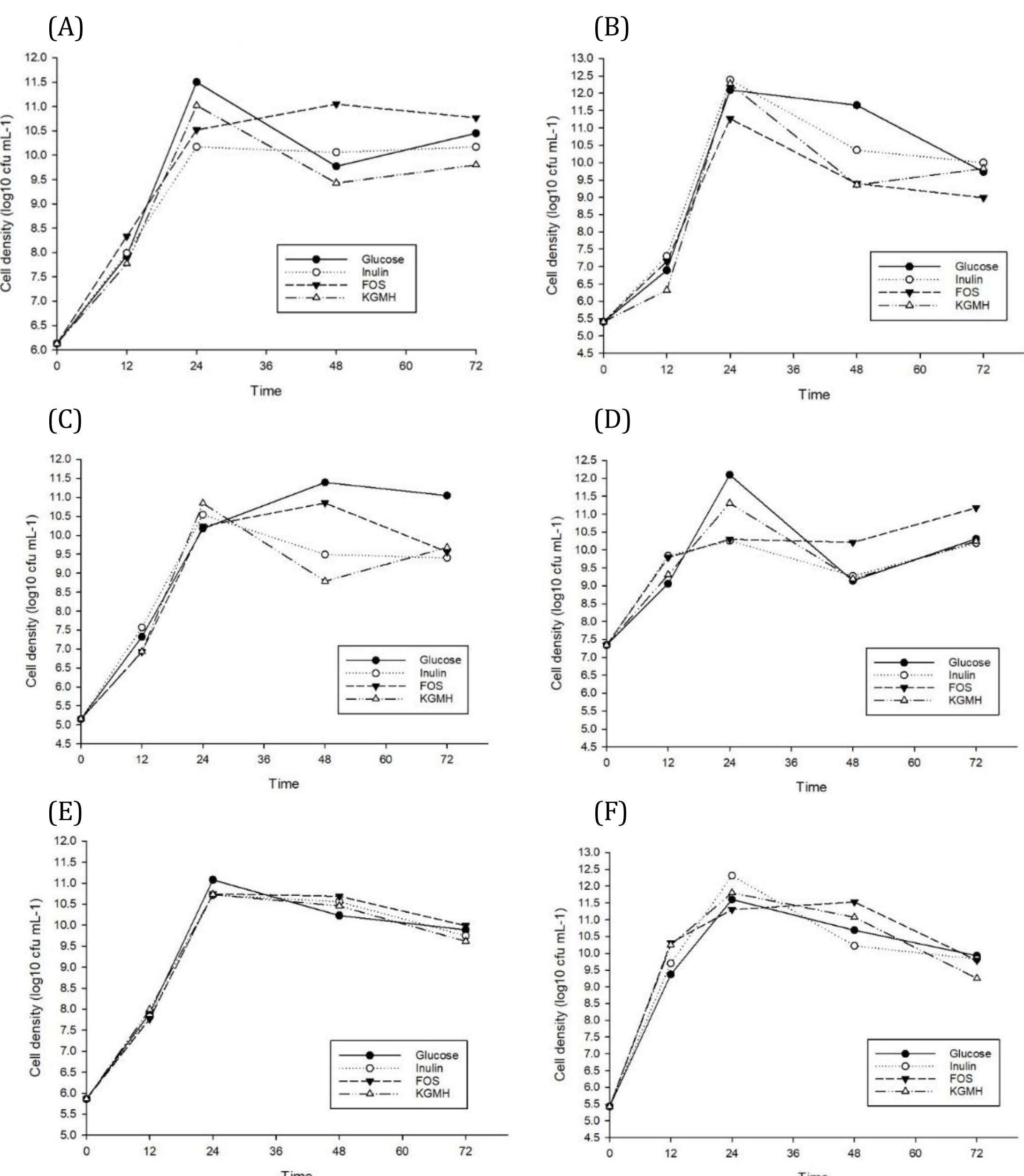

Figure 1. Growth of probiotics on KGMH and commercial prebiotic, FOS and inulin. (A) *L. acidophilus*, (B) *L. paracasei*, (C) *L. rhamnosus*, (D) *L. salivarius*, (E) *B. breve* and (F) *B. longum*

3.2 Prebiotic activity score

The Prebiotic Activity Score (PAS) experiment was conducted to investigate the prebiotic properties by comparing the growth support of each prebiotic on probiotics relative to an enteric mixture. Growth was measured by the growth rate of microorganisms increasing from the general carbon source, glucose. Table 2 presents the PAS of inulin, FOS, and KGMH by comparing them with various probiotic strains, including *L. acidophilus*, *L. rhamnosus*, *L. salivarius*, *L. paracasei*, *B. longum*, and *B. breve* at 24, 48, and 72 hours of incubation. A positive value of PAS indicates that the prebiotics can support the growth of specific strains of probiotics better than the enteric mixture. Conversely, a negative value of PAS may indicate that the prebiotics did not support the growth of probiotics or inhibit the growth of the enteric mixture better than glucose. Differences between PAS during the

periods indicate the suitable time for probiotic growth support from these prebiotics. Results show that, at 24 hours, *L. acidophilus* showed negative results on PAS for all prebiotics, suggesting that the enteric mixture could grow better than *L. acidophilus* in media containing prebiotics. However, at 48 hours, the PAS of *L. acidophilus* was positive for all prebiotics, with particularly high scores for inulin (3.73±0.08), FOS (2.55±0.01), and KGMH (4.34±0.03). This PAS score of KGMH and *L. acidophilus* was also higher than that of all other prebiotics and probiotics. Additionally, KGMH also showed the highest score for *L. salivarius* (0.95±0.04) and *L. paracasei* (1.01±0.01) at 24 hours. Inulin exhibited the highest score for *L. paracasei* at 48 hours (0.89±0.01). Inulin showed a high PAS score which was not significantly different from KGMH with *L. paracasei* at 72 h. FOS showed the highest score for *L. acidophilus* at 72 hours (1.23±0.03), *L. rhamnosus* at 48 hours (0.95±0.01), *L. salivarius* at 72 hours (1.07±0.06), *B. longum* at 48 hours (1.08±0.01) and *B. breve* at 48 hours (1.05±0.01). FOS and KGMH showed no significant differences in PAS score at 24 hours of *B. breve*. While the PAS score of B. breve at 72 hours showed no significant difference among different prebiotics.

Table 2. Prebiotic Activity Score of various probiotic strains

Strains	time	Inulin	FOS	KGMH	*p* value
L.acidophilus	24 h	-1.20±0.04^{b}	-0.44±0.06^{a}	-0.45±0.07^{a}	<0.001
	48 h	3.73±0.08^{b}	2.55±0.01^{c}	4.34±0.03^{a}	<0.001
	72 h	0.59±0.06^{c}	1.23±0.03^{a}	1.01±0.08^{b}	<0.001
L.rhamnosus	24 h	0.49±0.03^{b}	1.01±0.01^{a}	1.05±0.05^{a}	<0.001
	48 h	0.83±0.01^{b}	0.95±0.01^{a}	0.77±0.01^{c}	<0.001
	72 h	0.85±0.01^{b}	0.87±0.01^{ab}	0.88±0.01^{a}	<0.001
L.salivarius	24 h	0.36±0.04^{c}	0.86±0.04^{b}	0.95±0.04^{a}	<0.001
	48 h	1.01±0.01^{b}	1.12±0.01^{a}	1.01±0.01^{b}	<0.001
	72 h	0.99±0.01^{b}	1.07±0.06^{a}	0.98±0.04^{b}	0.023
L.paracasei	24 h	0.53±0.01^{c}	0.93±0.01^{b}	1.01±0.01^{a}	<0.001
	48 h	0.89±0.01^{a}	0.81±0.01^{b}	0.80±0.01^{b}	<0.001
	72 h	1.03±0.01^{a}	0.92±0.01^{b}	1.01±0.01^{a}	0.010
B.longum	24 h	0.55±0.03^{b}	0.97±0.03^{a}	1.01±0.03^{a}	<0.001
	48 h	0.95±0.02^{c}	1.08±0.01^{a}	1.04±0.01^{b}	<0.001
	72 h	0.99±0.01^{a}	0.98±0.01^{a}	0.93±0.01^{b}	<0.001
B.breve	24 h	0.47±0.09^{b}	0.97±0.01^{a}	0.97±0.01^{a}	<0.001
	48 h	1.03±0.01^{b}	1.05±0.01^{a}	1.02±0.01^{b}	0.002
	72 h	0.99±0.01^{ns}	1.01±0.03^{ns}	0.97±0.01^{ns}	0.079

* Results are given as mean scores ± SD (n=3). Mean values with different superscript letters in the same column denote that the values are significantly different (p<0.05).
ns, not significant; FOS, fructooligosaccharides; KGMH, konjac glucomannan hydrolysate.

3.3 Discussion

In this study, PAS of KGMH exhibits the highest value with *L. acidophilus*, *L. salivarius*, *B. longum* and *B. breve* at 24 hours. Inulin exhibits the highest value in *L. paracasei* at 72 hours with no significantly different from KGMH. FOS exhibits the highest value in *L. acidophilus* at 72 hours, *L. rhamnosus* at 48 hours, and *L. salivarius* at 72 hours. A different PAS value for certain strains may be attributed to the characteristics of prebiotics suitable for each strain. Research conducted by Huebener et al. (13) illustrated that different microorganisms exhibit diverse metabolic capacities, resulting in varying abilities to metabolize prebiotics. This diversity contributes to discrepancies in their responses to prebiotic substances. Certain bacteria, such as lactic acid bacteria, require

specialized systems for the degradation and utilization of prebiotics. KGMH shows its potential as a prebiotic with positive effects on *Lactobacillus* strains which are *L. acidophilus* DDS-1®, *L. rhamnosus* LGG®, *L. paracasei* 431® and *L. salivarius* B37. It also shows positive effects on *Bifidobacterium* strain which are *B. longum* and *B. breve*. The presence or absence of these metabolic systems, encoded in their genetic makeup, significantly shapes their interaction with prebiotics. These results are in line with earlier findings by Rubel et al. (14), which suggested a positive correlation between increased prebiotic activity and the proliferation of beneficial microorganisms alongside a reduction in pathogenic ones. It suggests that beneficial microorganisms, including those targeted by KGMH, exhibit a preference for prebiotics such as resistant starch over glucose. As a result, these microorganisms efficiently utilize prebiotics, restraining the proliferation of glucose-favoring harmful microorganisms. This underscores the varied responses of different microorganisms to prebiotics, particularly KGMH, which influence their growth dynamics and interactions with glucose (1). This potential mechanism could validate the prebiotic efficacy of KGMH, as evidenced by the PAS values of probiotics, wherein KGMH displayed the highest PAS value after 48 hours across most strains.

In contrast, all prebiotics in this study, inulin, FOS, and KGMH showed negative PAS values with *L. acidophilus* at 24 hours. This variation may be ascribed to *Lactobacillus* strains exhibiting reduced affinity for growth in a prebiotic medium lacking carbon sources beyond glucose. This observation is consistent with the notion that enteric strains experience less limitation in carbon source availability within the medium compared to probiotic strains, as indicated by Rubel et al. (14). The PAS values underscore the selective fermentation demonstrated by *Lactobacillus* strains towards various prebiotic carbohydrates. Another potential mechanism contributing to the negative PAS value of this prebiotic involves the proliferation of E. coli (7). This investigation reveals the highest proliferation of E. coli with glucose, followed by inulin, FOS, and KGMH. This can be elucidated by a prior study by Tsuda et al. (15) which found that E. coli initially favors the utilization of monosaccharides and lactose in the initial hours of fermentation, preceding the utilization of prebiotics.

The limitation of this study is that it does not determine the by-product from probiotic metabolism to confirm the support of prebiotics to the growth of certain strains, so further study needs to investigate the pH, short-chain fatty acids, and by-product after fermentation to clarify the compatibility of the synbiotic.

4. CONCLUSIONS

KGMH exhibited a positive PAS value for all strains of probiotics except L. acidophilus at 24 hours, but it showed the lowest negative PAS value for *L. acidophilus* at 24 hours. It showed a higher number of increased cells in all probiotic cultures compared to glucose. Thus, KGMH demonstrates high potential as a novel prebiotic. This study highlights KGMH's ability to support the growth of *L. acidophilus* DDS-1®, *L. rhamnosus* LGG®, *L. paracasei* 431®, *L. salivarius* B37, *B. breve*, and *B. longum*, comparable to inulin and FOS. Further research is needed to warrant its mechanisms and effects on other strains, aiming to unravel the full spectrum of health benefits of KGMH. It provides benefits as a non-calorie dietary fiber and prebiotics. The benefit of KGMH on health will be studied to warrant the gut health-benefit.

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Probiotic and synbiotic mulberry jelly drinks: Quality and preliminary shelf-life evaluations

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ABSTRACT

The objective of this study was to evaluate the effect of the addition of *Lactobacillus casei* and/or prebiotic (resistant dextrin or polydextrose) on the quality parameters of mulberry jelly drink (MJ) mixed with fruit juices during refrigerated storage for 4 weeks. There were four treatments set in the current research such as control MJ (CMJ), MJ added with *L. casei* (PRO-MJ), PRO-MJ fortified with resistant dextrin (SYN-MJ-RD) and fortified with polydextrose (SYN-MJ-PD). The viability of *L. casei*, pH, total soluble solid (TSS), total anthocyanin content, and antioxidant activities were evaluated for 4 weeks at 1 week interval. The addition of the probiotic culture improved the nutritional values during storage. It was found that slight decreases in pH and TSS were found during storage while the anthocyanin content as well as antioxidant activities gradually increased. The synbiotic products had more bioactive components in addition to the enhanced qualities derived from the probiotic culture. The probiotic counts in all synbiotic jelly drinks and PRO-MJ exceeded 106 CFU/g. This work shows that the addition of commercial prebiotics and lyophilized L. casei can result in synbiotic MJ products with enhanced nutritional qualities. This result showed that the synbiotic mulberry jelly beverages maintain a minimum required standard count of >10^6 CFU/g as set by the Thai FDA.

1. INTRODUCTION

Recently, there has been an interest in the development of fruit and vegetable juices as functional beverages with probiotics. Probiotics beverages are consumed universally by a wide range of people, and the reason lies behind probiotics' inherently healthy characteristics and being free from incompatible components such as lactose and casein. Mulberry (Morus australis Poir), which belongs to the genus Morus and the family Moraceae, is broadly cultivated in different climatic zones. Mulberry juice is proven to be a very concentrated source of polyphenols and anthocyanins (1). Fruits and vegetables with high phenolic content have been studied extensively for its cancer preventing properties.

Many epidemiological studies showed that red color juices such as those of pomegranates, grapes, and different berries have beneficial effects on human health due to their high anthocyanin content and antioxidant activity (2).

Probiotics are living microorganisms that, when administered in adequate quantities, confer health benefits to the host. Prebiotics are nonviable food components that confer health benefits to the host associated with the modulation of the microbiota. The association of probiotics and prebiotics in a food product result in a synbiotic product (3). The combination of these components has already been used in juices (4-6) and teas (7). The use of probiotics in anthocyanin-rich fruit juice has been investigated because of the high antioxidant activity of the juices.

One of the product innovations of processed mulberry is beverage of jelly. Beverage product of jelly to have the character of elastic with soft gel consistency so that easily to drink. This beverage can eliminate thirst overcome to feel hungry. Beverage product of jelly consumed by inhaled and direct swallowed. Commercial prebiotics like fructo-oligosaccharides (FOS), resistant dextrin, inulin, polydextrose and other related carbohydrates are known to beneficially affect host health by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon. It is known that the growth of probiotic strain is specific to the prebiotic concerned. It is hypothesized that a mixture of prebiotics can influence the intestinal bacteria in a synergistic manner (7).

Considering that the mulberry juice could be a new synbiotic matrix and that the addition of the probiotic culture in a lyophilized form has not been extensively studied. Study on shelf life of the jelly drink products should be consisted of physicochemical properties, sensory evaluation, and foodborne pathogen investigation. However, the number of probiotics was the most important properties as it was used to claim to be probiotic products. The objective of this study was to evaluate the effect of the addition of a probiotic culture (*Lactobacillus casei*) and/or prebiotic fiber (resistant dextrin or polydextrose) on the physicochemical characteristics, and probiotic survival of the probiotic and synbiotic mulberry jelly drink (MJ) during refrigerated storage.

2. MATERIAL AND METHODS

2.1 Materials

Mulberry, orange and pineapple were purchased from the local fruit contacted farm in Saraburi province. Resistant dextrin (Nutriose) and polydextrose was purchased from Vicchi, Enterprose Co., Ltd. *Lactobacillus casei* was provided by Brenntag Ingredients (Thailand) Public Company Limited.

2.2 Lactobacillus strain and growth condition

Lactobacillus caesi was obtained from Brenntag Ingredients (Thailand) Public Company Limited. From this culture, stock solution was prepared by adding sterile glycerol (50% v/v) to the activated culture. The glycerol stock culture was stored at -20°C in sterile screw cap tubes. The probiotic organism was grown individually and inoculated into 10 mL sterile de Man Rogosa and Sharp (MRS) broth and incubated at 37°C for 2 days under aerobic condition. The cells were harvested by refrigerated centrifuging (Sigma, Germany) at 1500g for 15 min at 4°C. Before inoculation into fruit juices, the harvested cells were washed twice with sterile saline water (0.85% w/v sodium chloride) to remove any residual MRS. The cell pellets were diluted with 0.85% sterile saline water to get a bacterial concentration of 10^{11}CFU/mL.

2.3 Preparation of probiotic mulberry jelly drink mixed with fruit juices and prebiotic

Mulberry, orange and pineapple fruits were squeezed using a domestic juicer and each the resulting juice was filtered through muslin clothes. There were four treatments in the current study including;

1. Mulberry jelly drink mixed with fruit juice (CMJ) as a control treatment

2. MJ added with probiotic culture (PRO-MJ)
3. MJ added with probiotic culture and resistant dextrin (SYN-MJ-RD)
4. MJ added with probiotic culture and polydextrose (SYN-MJ-PD)

Mulberry, orange, and pineapple juices were combined in a 70:15:15 ratio to create a mixed juice that was used as a raw ingredient for making jelly drinks. Using an electric stove, the blended juice was cooked to a temperature of 60°C for the CMJ sample. Following that, 1 g/100 g of carrageenan was gradually added. The resultant sample was then heated to 85°C for 5 minutes before being cooled to 70°C. The sample was then aseptically sealed inside a stand-up aluminium pouch featuring a spout.

Carrageenan was added for the PRO-MJ production process after the warm heating step (60°C). The resultant sample was then heated to 85°C for five minutes and cooled to 50°C. Subsequently, the probiotic bacteria were aseptically incorporated into the PRO-MJ sample to obtain a population of 10^{10} CFU/g. Additionally, a stand aluminium pouch with a spout was aseptically packed with the PRO-MJ sample. To produce the SYN-MJ-RD and SYN-MJ-PD samples, respectively, carrageenan was combined with 5 g/100 g of resistant dextrin (RD) and polydextrose (PD) prebiotics. The resulting mixture was then gradually added. The following step was like the PRO-MJ sample as well. All samples were stored under refrigerated temperature for 1 month and the qualities were determined at 1 week interval.

2.4 Determination of chemical properties

2.4.1 Proximate compositions

The proximate compositions (moisture, lipid, protein and ash) of all samples were determined in accord with AOAC (2000). The moisture content was gravimetrically measured using a vacuum oven at 60°C for 24 h. The ash content was determined using a muffle furnace at 550°C for 24 h. The protein and fat contents were determined by the Kjeldahl and Soxhlet extraction methods, respectively. The resistant dextrin and polydextrose contents were analyzed by using the method of AOAC (2000) (AOAC 2000.11).

2.4.2 pH and total soluble solid

The pH value was measured at ambient temperature with a pH meter (Satorious, USA) which calibrated at pH 4.0 and 7.0. The total soluble solid (TSS) was determined by using a digital refractometer (atago, Japan).

2.4.3 Total phenolic content

The total phenolic content (TPC) was determined with Folin–Ciocalteu reagent according to the method of Balange and Benjakul (8) with some modifications using gallic acid as standard. A mixture of 0.5 ml of the appropriate diluted sample and 0.5 ml of distilled water was prepared. Following that, 2.5 ml of a 2% sodium carbonate solution and 0.5 ml of the Folin-Ciocalteu reagent (1:1 with water) were added. After gently combining the mixture, it was left in the dark for forty minutes. The absorbance was then measured at 725 nm, and the TPC was calculated using the gallic acid standard curve and reported as mg of gallic acid (GAE) per g of sample.

2.4.4 Antioxidant capacities

DPPH radical-scavenging activity (DPPH-RSA) was determined by DPPH assay, as described by Binsan et al. (9) with a slight modification. 1.5 ml of the sample and 0.15 mM 2,2-diphenyl-1-

picrylhydrazyl (DPPH) in methanol were combined. After a thorough mixing, the mixture was let to stand for 60 minutes at room temperature in the dark. A UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan) was used to measure the absorbance of the resultant solution at 517 nm. The blank was prepared in the same manner, except that distilled water was used instead of the sample. The unit of measurement for the activity was mg Trolox equivalents (TE)/g sample.

2.4.5 Total anthocyanin content

The pH differential approach was used to estimate the total anthocyanin content (2). Briefly stated 0.2 M KCl and 0.2 N HCl solutions were used to create pH 1.0 buffer. Sodium acetate was used to create a buffer with a pH of 4.5, and acetic acid was used for adjusting the pH. Using the buffers, samples were diluted 25 times. Using the microplate reader, absorbance was measured at 510 and 700 nm. Cyaniding-3-glucoside equivalents (CGE), prepared with the buffers, act as the standard. The measurements were given in milligrams of cyaniding-3-glucoside equivalents per gram, or mg CGE/g. The absorbance of each sample was calculated using the following equation:

$$A = (A_{510nm}pH1.0 - A_{700nm}pH1.0) - (A_{510nm}pH4.5 - A_{700nm}pH4.5)$$

2.6 Probiotic viability

MRS agar medium (Hope Bio-technology CO. LTD, Qingdao, China) was used for the determination of viable counts. A 25 g sample was sequentially diluted 10 times to the appropriate gradient, then 0.1 mL of solution was spread to the MRS agar medium. The plates were incubated at 37 °C for 48 h. The results were repeated three times and the viable counts of were reported.

2.7 Statistical analysis

All the data were means (means ± SD) of determinations made in triplicate with standard deviations. ANOVA was performed and Duncan's multiple range tests ($p<0.05$) were used to identify significant differences between treatment means.

3. RESULTS AND DISCUSSION

3.1 Proximate compositions

Table 1 shows the proximate compositions of all MJ formulations. The MJ supplemented with either *L. casei* probiotic culture (PRO-MJ or SYN-MJ) had similar proximate compositions to the CMJ. In addition, supplementation with *L. casei* probiotic culture and the prebiotic types (resistant dextrin and polydextrose) did not change the proximate compositions among MJ samples. However, the addition of prebiotics (resistant dextrin and polydextrose) resulted in products with lower moisture values and higher carbohydrate values. Resistant dextrin and polydextrose are soluble oligosaccharides, which cause an increase in TSS and carbohydrate contents when added to foods. (10).

Table 1 Proximate compositions (g/100g) of mulberry jelly drink formulations

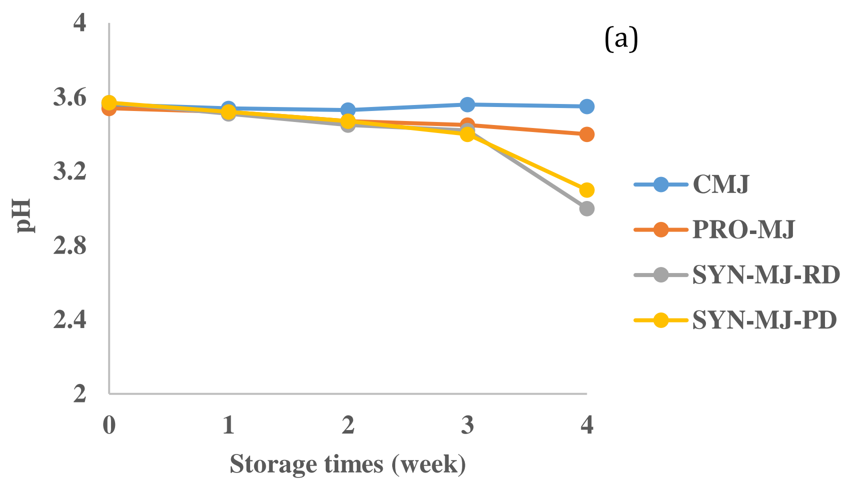
Parameters	Formulations			
	CMJ	PRO-MJ	SYN-MJ-RD	SYN-MJ-PD
Moisture (g/100g)	83.73±0.56^{a}	83.15±0.32^{a}	81.03±0.63^{b}	81.46±0.37^{b}
Protein (g/100g)	0.63±0.12^{a}	0.62±0.11^{a}	0.51±0.03^{b}	0.52±0.07^{b}
Lipid (g/100g)	0.46±0.06^{a}	0.41±0.02^{a}	0.32±0.03^{b}	0.31±0.04^{b}
Ash (g/100g)	0.54±0.02^{a}	0.52±0.02^{a}	0.43±0.03^{b}	0.40±0.03^{b}
Carbohydrate (g/100g)	14.64±0.62^{a}	14.73±0.47^{a}	16.67±0.51^{b}	17.12±0.44^{b}
Resistant dextrin (g/100g)	-	-	5.21±0.53^{b}	3.56±0.37^{b}
Polydextrose (g/100g)	-	-	5.11±0.43^{b}	3.89±0.11^{b}

*Different letters in the row indicate significant difference ($p < 0.05$)

3.2 pH and total soluble solid (TSS)

There was no effect of the probiotic and prebiotic additions on the initial pH value of all MJ samples (Figure 1a). However, a gradual decrease in pH was observed in all samples, except CMJ, during storage. Probiotics may produce organic acids from the consumption of citric acid and/or sugars or by the catabolism of amino acids (11, 3). The results indicated that the probiotic culture had a greater ability to metabolize the juice compounds, as evidenced by a decrease in pH found in either PRO-MJ or all SYN-MJ samples.

The reduction of TSS is related to the fact that, during refrigerated storage, these simple sugars are preferably consumed by the probiotic cultures, with reduction of the TSS (Figure 1b) (12, 3). Supplementation with probiotic cultures caused a slight increase in acidity as well as decreases in pH and TSS levels during refrigerated storage. The probiotic microorganisms may have metabolized the prebiotic or simple sugars present in the juice, resulting in consequent production of small quantities of organic acids; or the release of enzymes from dead bacteria may have hydrolyzed the juice sugars (13).



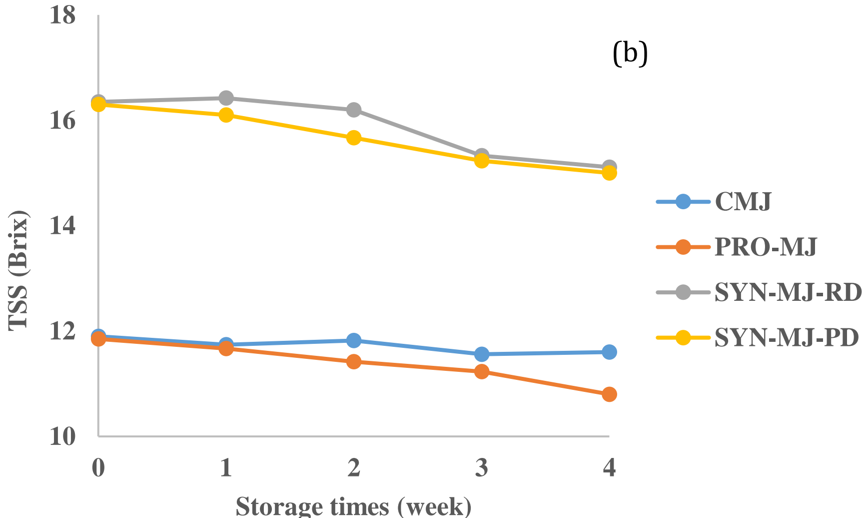


Figure 1 pH (a) and TSS (b) of the MJ samples during refrigerated storage for 4 weeks

3.3 Total anthocyanin content (TAC)

Anthocyanins are very sensible to adverse ambient and process conditions, such as light, oxygen, metal ions, high pH, high temperatures and processing times, which affect their stability**.** Of course, the role of internal properties (pH, chemical structure, and anthocyanin concentration) of the product, available enzymes and other color inducing substances, ionic metals, sugars, and processing conditions (intensity and duration of heating procedure, storage time and temperature, oxygen and light) is effective on their stability (14, 15). Mulberry fruits are a rich source of anthocyanins, which can scavenge free radicals (16). The total anthocyanin content of all the samples, except CMJ, increased progressively with increase in the storage time (Figure 2). Similar results were reported by Vivek *et al*. (16), where the total anthocyanin content of Sohiong juice increased after fermentation with *L. plantaru*m. β-glucosidase activity is shown by some lactic acid bacteria, which cleaves glycosidic linkages in the glycosylated anthocyanins complex to produce aglycone (anthocyanidins) compounds (17). This aglycone form of anthocyanins significantly increases the total anthocyanin content of fermented Sohiong juice with *L. plantarum.* In this research, higher total anthocyanin content was observed in the PRO-MJ compared to those of the SYN-MJ-PD and SYN-MJ-RD.

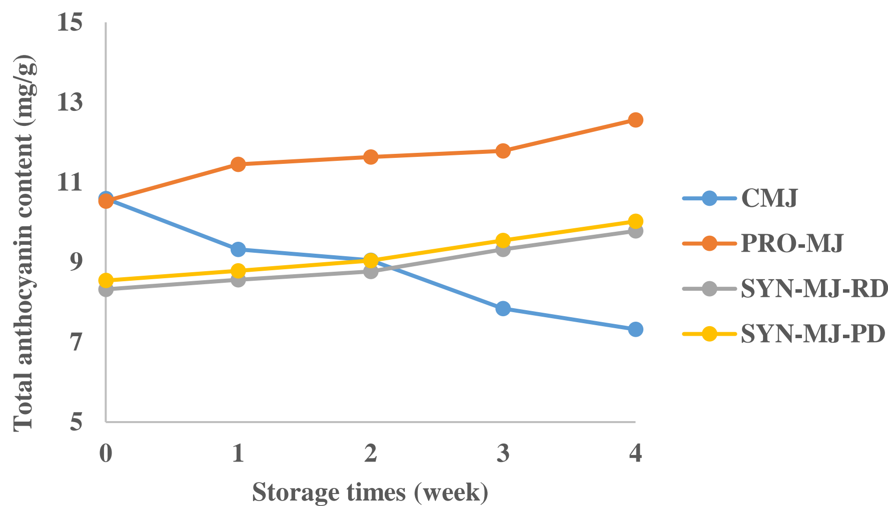


Figure 2 Total anthocyanin content of the MJ samples during refrigerated storage for 4 weeks

3.5 Antioxidant capacity

Using DPPH tests, the antioxidant properties of all MJ products were examined. Mulberry' antioxidant activities are generally linked to their phenolic compounds and anthocyanins, which are most likely caused by their redox characteristics, which enable them to function as reducing agents, hydrogen donors, and singlet oxygen quenchers. Stable free radicals of DPPH react with substances that can give hydrogen atoms. This technique works by scavenging DPPH by decolorizing the DPPH solution with the addition of an antioxidant or radical species. With greater storage times, the antioxidant activity increased in all samples that had probiotic cultures added (PRO-MJ, SYN-MJ-RD, SYN-MJ-PD). Nonetheless, a steady decrease in antioxidant activity was noted in the CMJ sample. The acylation of anthocyanins with phenolic acid or the diacylation of anthocyanins may be the cause of the increase in antioxidant capabilities (18).

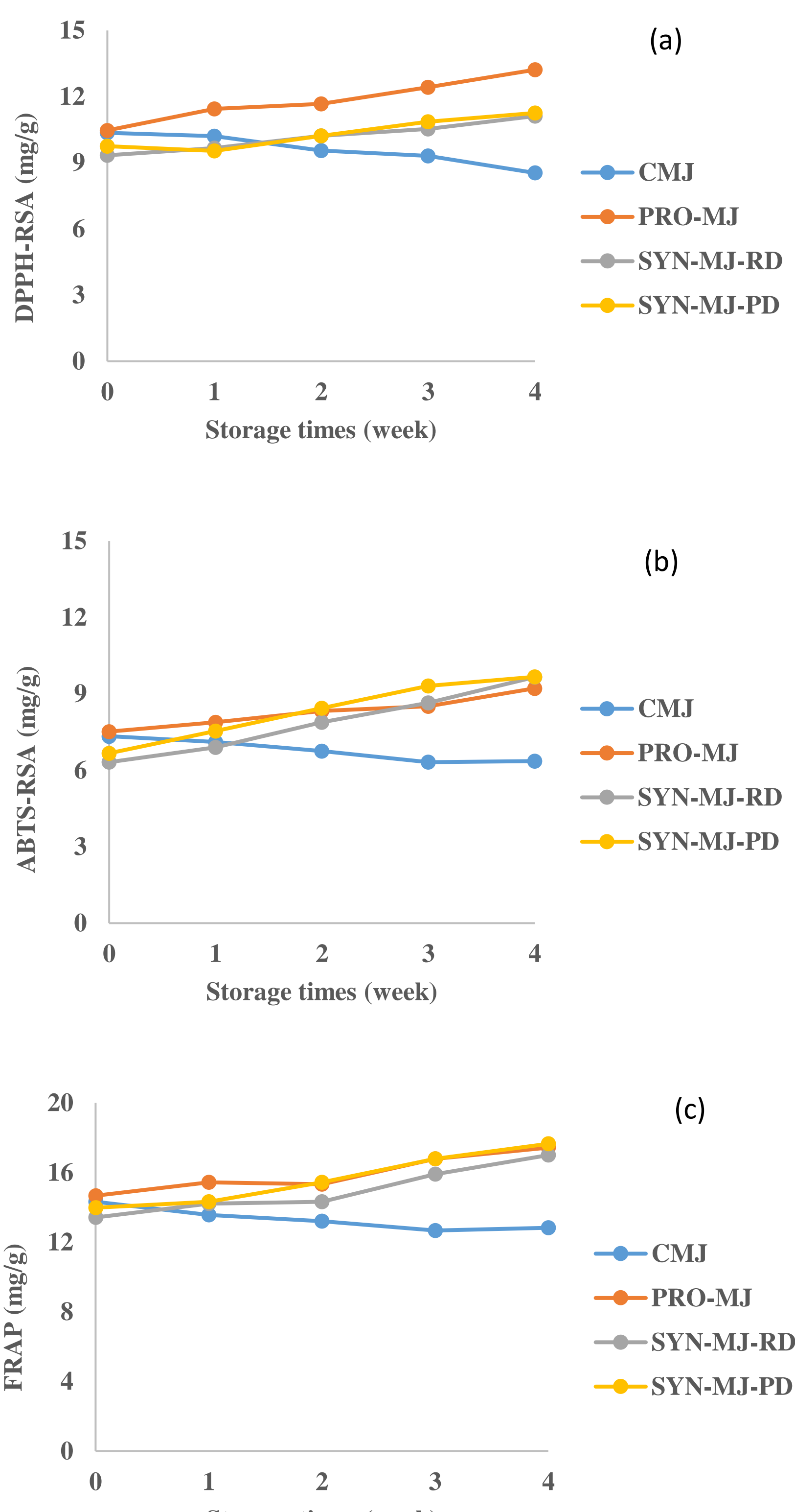


Figure 3 DPPH-RSA (a), ABTS-RSA (b) and FRAP (c) of all MJ samples during refrigerated storage for 4 weeks

3.6 Viability of probiotic

The probiotic drink should have a viable cell count of more than 6 log CFU/ml to obtain maximum health benefits. The survival of probiotic culture in all the MJ jelly formulations is shown in Figure 4. On the first day of storage, all of jelly formulations presented similar counts of initial viable of *L. casei*. The effect cold storage on survival of this probiotic bacteria in MJ jelly drink had performed for 4 weeks at 4°C. The results revealed the gradually reduced of the survival cells during the storage period. However, the MJ jelly drink still contained sufficient counts of *L. casei* to be termed a probiotic product after four weeks of storage. It is important to have significant number of viable probiotics at 6 log CFU/ml in the finish product until the product expiry date for maximum health benefits. The results of this study appear to agree with current literature, whereby the addition of fruit juice to a probiotic beverage can enhance or reduce probiotic viability depending on the formulations of beverages (19). Factors affecting the survival of probiotic including, pH, oxygen level, nutrient and presence of antimicrobial substance in the food matrix (20). The use of prebiotic is an interesting technique to extend survival rate and shelf life of probiotic products. Both resistant dextrin and polydextrose, prebiotics, may have a protective effect on probiotic cultures by increasing their survival and activity during food storage. This is because they are available as substrates for the metabolism of probiotic cultures serving as a carbon source for cell maintenance and preventing injuries caused by acidity; and can make a physical protection of bacterial cells from damages caused by the environment (13). Costa et al (21) reported that the high survival rate of the probiotic cultures in orange juice could be related to the high fiber content of this product. It was indicated that fibers could physically protect the probiotic cells damage from adverse environment by the adhesion of the bacterial cells to the oligosaccharide. The MJ probiotic jelly drink was found to maintain alive *L. casei* at 6 log CFU/g after 4 weeks of cold storage. While fortified the jelly drink with either resistant dextrin or polydextrose, the viability of probiotic was increased to around 7 log CFU/g. These results indicated that using resistant dextrin or polydextrose enhanced viability of *L. casei* in MJ jelly drink for at least 4 weeks in cold storage condition.

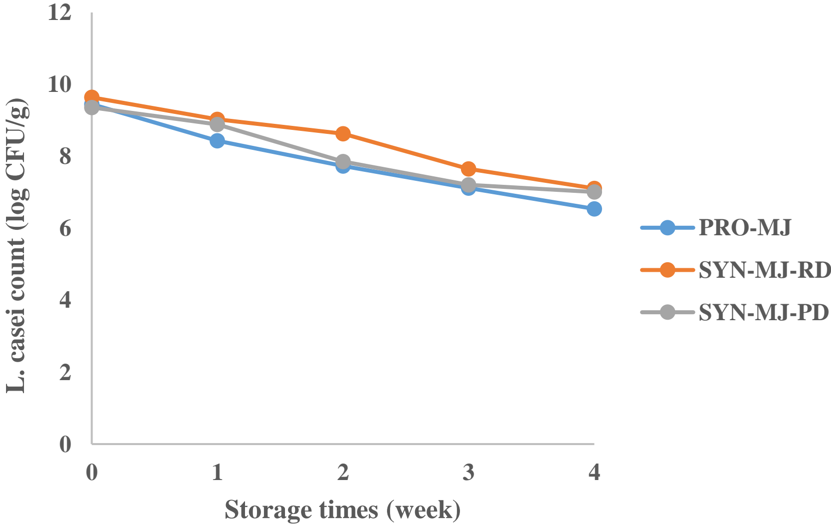


Figure 4 Viability of *L. casei* in all MJ samples during refrigerated storage for 4 weeks

4. CONCLUSIONS

Mulberry fruit, rich in vitamins, minerals, polyphenols, and anthocyanin, was explored for development of a possible probiotic product. The mulberry jelly drink mixed with fruit juice was incorporated using *L. casei*. The viability of cells and quality parameters were studied. The probiotic bacteria survived in the MJ matrix with final viable cell count above the minimum recommended value of 6 log CFU/ml at the end of the storage period and further storage at 4 ± 1 °C for four weeks. The antioxidant properties, total phenolic, and anthocyanin contents increased during probiotification, suggesting suitability of the juice for developing a probiotic product. A portion of 150 g of jelly drink had sufficient prebiotic content to be considered a prebiotic product to obtain the recommended daily intake. In addition, polydextrose and resistant dextrin can be used as a protector for probiotic culture, *L. casei*, in MJ, having no influence on the physicochemical characteristics. It is possible to develop synbiotic mulberry jelly drink mixed with fruit juices with added resistant dextrin or polydextrose, a prebiotic, and probiotic culture, in which prebiotics helps in maintaining the viability and stability of probiotic products.

5. ACKNOWLEDGEMENTS

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DP-0236

Application of Food Grade Shrimp Chitosan as a Preservative in Squeezed Orange Juice

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Keywords

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Chitosan
Natural preservative
Orange juice
Shelf-life extension

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ABSTRACT

Orange juice is one of the most popular fruit juices marketed and the most accepted for consumers. The problem of the orange juice industry is the limited shelf-life due to the growth of food spoilage and foodborne microorganisms. This study aimed to evaluate the antimicrobial activity of chitosan against spoilage microorganisms associated with orange juice and physicochemicals in orange juice. The result showed the minimum inhibitory concentration (MIC) values against *Alicyclobacillus acidoterrestris* and *Zygosaccharomyces bailii* were 0.004% (w/v), and their minimum bactericidal concentration (MBC) values were > 0.500% (w/v) and 0.006% (w/v), respectively. Moreover, spore germination of *A. acidoterrestris* was significantly inhibited at 0.500% (w/v). As *A. acidoterrestris* was more resistant to chitosan than *Z. bailii*, it was selected for artificial inoculation into orange juice. Adding chitosan at a concentration of 0.050 % (w/v) into orange juice retarded *A. acidoterrestris* cell growth and spore germination at both 10 and 35 °C throughout 14 days. Moreover, the overall characteristics of orange juice analyzed by pH, titratable acidity, and appearance showed little change after storage for 14 days. This study applied chitosan as a natural preservative to extend the shelf-life of orange juice instead of using chemical preservatives. It was an alternative method to extend the shelf-life of squeezed orange juice.

1. INTRODUCTION

Orange juice is one of the most popular fruit juices marketed. It is the most recognized and accepted because of its delicious taste and is a good source of vitamin C, antioxidants, carotenoids, and phenolic compounds. As a result, it can prevent several diseases and enhance the body's health [1, 2]. The major problem of the orange juice industry is the limited shelf-life due to the growth of food spoilage and foodborne microorganisms, which may cause harm to consumers. [1].

The foodborne microorganisms associated with orange juice include *Escherichia coli* and *Salmonella* [3, 4] It was reported in 2005 that there was a multistate outbreak of *S.* Typhimurium and *S.* Saintpaul in the USA associated with unpasteurized orange juice, causing 152 cases in 23 states [5]. Since then, most commercial orange juices have been pasteurized for pathogen inactivation. However, food spoilage microorganisms have always been a matter of great concern for the off-flavor and stability of fruit juices during storage. The most important ones are spore-form bacteria such as *Alicyclobacillus acidoterrestris,* which can resist acid and thermal pasteurization, yeast such as *Zygosaccharomyces bailii*, which is the most preservative-resistant yeast, able to resist high concentrations of acetic acid and ethanol and heat-resistant molds such as *Byssochlamys fulva* [6, 7]. Nowadays, there is a great demand for the safety of chemical preservatives, as these are considered to cause residual toxicity, carcinogenicity, and teratogenicity. For these reasons, consumers have become careful regarding the choice of products containing chemical preservatives, which has resulted in demand for natural and more adequate preservative options [8]. There is some interest in extending the shelf-life of beverages using natural preservatives, such as citric acid, erythorbic acid, bee glue, and chitosan [8, 9].

Chitosan is a modified natural carbohydrate polymer derived from deacetylated chitin, found in the exoskeletons of crustaceans, fungi, and other microorganisms. Chitosan has versatile bioactivity, including antimicrobial, antitumoral, antioxidant, and anti-inflammatory activities [10]. Currently, chitosan is applied to many food products as a preservative and additive to maintain food quality and extend the shelf-life of food products. In meat products, chitosan is used to reduce rancidity and spoilage and maintain the color of meat during storage [11]. In addition, chitosan was used as a natural preservative in orange juice. Ewis et al. [12] reported that using chitosan in orange juice exhibited improved physicochemical and shelf-life properties for up to 30 days based on the respective chitosan concentrations.

Moreover, chitosan showed antibacterial activity against *A. acidoterrestris* cells in orange juice storage at 50 °C for 7 days. It inhibited the production of guaiacol, an off-flavor in the products [13]. It was also reported that chitosan exhibited against *Z. bailli* in apple juice at 25°C for 9 days. This yeast was completely inactivated by chitosan glutamate at 0.1 g/L [14]. However, there has been no report on the effect of chitosan on spore germination of *A. acidoterrestris.* Therefore, this study focuses on the antimicrobial of shrimp chitosan against *A. acidoterrestris* cells, spores and *Z. bailli* which are spoilage microorganisms in acidic food products, and studied the application of shrimp chitosan to extend the shelf-life of squeezed orange juice at 10 °C and 35°C for 14 days.

2. MATERIAL AND METHODS

2.1 Tested microorganisms

A. acidoterrestris ATCC 49025 was bought from the American Type Culture Collection. *Z. bailii* TBRC-BCC 30635 was purchased from the Thailand Bioresource Research Center, Pathum Thani, Thailand.

2.2 Microorganism and spore preparation

A. acidoterrestris was grown in 10 mL of modified Alicyclobacillus Acidoterrestris medium (AAM) broth adjusted to pH 4.0 with 1 N sulfuric acid (H_2SO_4; RCI Labscan, Bangkok, Thailand) and incubated at 45 °C. While *Z. bailii* was grown in 10 mL of yeast malt extract (YM) broth and incubated at 25 °C. All cultures were grown in suitable media and temperature for two successive 24 h and 18 h transfers before use.

For *A. acidoterrestris* spore preparation, The bacterial was grown in AAM broth (pH 4.0) at 45 °C for 24 h. After that, 0.1 mL of the culture was inoculated on the surface of the AAM agar supplemented with 0.05% w/v of manganese sulfate monohydrate ($MnSO_4 \cdot H_2O$; Kemaus, New South Wales, Australia) and incubated at 45 °C for 7 days. Then, sterile deionized water (DI) was added to each plate to flood over the colonies, and the spores were scrapped off the agar with a sterile spreader. The spore solutions were collected, centrifuged at 4500 rpm for 10 min at 4 °C, washed twice with DI water, and then stored at -20 °C.

2.3 Preparation of chitosan solution

Shrimp chitosan (Food grade) with a molecular weight <500 kDa and % degree of deacetylation 95 was purchased from Bona Fides Marketing, Bangkok, Thailand. Chitosan stock solution (2% w/v) was prepared by dissolving 2 g of chitosan in 1% v/v acetic acid (CH_3COOH; RCI Labscan Limited, Bangkok, Thailand) and stirring continuously at room temperature for 24 h. After that, the chitosan solution was sterilized at 121 °C for 15 min.

2.4 Antimicrobial activity of chitosan

2.4.1 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of chitosan

A broth dilution assay was used to determine the MIC and MBC values of chitosan. Each tested microorganism was diluted with 0.1% peptone until microorganism concentrations were approximately 5-6 Log CFU/mL. The microorganism was mixed with suitable media at double strength concentration in Erlenmeyer flask (4-5 Log CFU/mL). After that, 5 ml of microorganism was pipetted and mixed with chitosan solution and distilled water in a test tube to a final volume of 10 mL. The test tubes were incubated at the optimum temperature of microorganisms for 24 h. The enumeration of microorganisms was verified using visual observation and the spread plate technique. The MIC value was determined as the minimum concentration of chitosan capable of inhibiting the microorganism growth or no growth by visual observation in test tubes after incubation for 24 h. The test tube with no growth observation was then taken at 0.1 mL and spread on suitable media before enumeration. The MBC value was determined as the minimum concentration of capable chitosan to kill the microorganism by more than 99.9% or a 3-Log reduction, as described by Davidson et al. [15, 16].

2.4.2 Sporicidal activity of chitosan

The effect of the chitosan on the germination rate of *A. acidoterrestris* spore was determined, as previously reported, with some modifications [17]. The suspensions were incubated at 80 °C for 10 min to kill vegetative cells. The spores were diluted in suitable media for each microorganism to create a spore suspension. Then, 500 µL of spore suspensions were added into 500 µL chitosan solution to give concentration at 0.050, 0.100-0.50% w/v and incubated at 45 °C for 24 h. The spore germination rate was evaluated using the spread plate technique and calculated as follows: (treatment group/control group) x100%.

2.4.3 Effect of chitosan on microorganism inhibition in orange juice

The squeezed orange juice was purchased from a department store in Bangkok, Thailand. The antimicrobial activity of chitosan was tested against *A. acidoterrestris* cells and spores in orange juice containing various concentrations of chitosan solution. The squeezed orange juice was mixed

with a chitosan solution. After that, orange juice with chitosan solution was inoculated with either cell or spore suspension of *A. acidoterrestris* (2-3 Log CFU/mL) and stirred continuously for 5 min. The final concentrations of the chitosan solution in orange juice were 0.050 and 0.100% v/v.

2.5 Effect of chitosan on physicochemical properties in orange juice

2.5.1 pH parameters

At room temperature and continuous stirring, the pH of the 10 mL sample was measured using a pH meter (Mettler Toledo model SevenDirect SD20, Greifense, Switzerland) according to AOAC (2000) method.

2.5.2 Titratable acidity (TA)

An Erlenmeyer flask was contained with 10 ml of orange juice and titrated with 0.1 M NaOH after adding two drops of phenolphthalein (as an indicator). Titration was continued until a pink color was noticed, and a burette reading was taken [18].

2.6 Statistical analysis

The data were subjected to analysis of variance (ANOVA) using the general linear model procedure of the SPSS statistical package (SPSS version 27.0). Duncan tests were used to compare means. All differences are reported as a significance level of 95%

3. RESULTS AND DISCUSSION

3.1 MIC & MBC values of chitosan

The antimicrobial activity of chitosan against spoilage microorganisms associated with orange juice, namely *A. acidoterrestris* and *Z. bailii*, was evaluated by MIC and MBC values. As shown in Table 1, the result showed the MIC values against *A. acidoterrestris* and *Z. bailii* were 0.004% (w/v). The MBC values showed that *A. acidoterrestris* was greater than 0.500%, while *Z. bailii* was 0.006%. This result is consistent with the report by Ulfadillah and Chang [13] that chitosan inhibited *A. acidoterrestris* cells by disrupting cells. Indeed, chitosans rapidly attack bacterial cell walls and membranes [19]. The interaction with the membrane proteins using hydrogen bonding through their hydroxyl groups would cause changes in membrane permeability and result in cell destruction. In addition, they interfered with membrane functions such as electron transport, nutrient uptake, protein and nucleic acid synthesis, and enzyme activity.

Table 1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of chitosan

Tested Bacteria	MIC (%w/v)	MBC (%w/v)
Alicyclobacillus acidoterrestris	0.004 ± 0.000	>0.500 ± 0.000
Zygosaccharomyces bailii	0.004 ± 0.000	0.006 ± 0.000

*Values are presented as the average mean ± standard deviation of two replicates

3.2 Spore germinaton inhibition of chitosan

Chitosan at the concentration of 0.500% (w/v) significantly inhibited the spore germination of *A. acidoterrestris*. It reduced the spore germination rate by 20% within 24 h. This result is consistent with Falcone et al. [20] who state that chitosan significantly reduced *A. acidoterrestris* spores in cooperation with pH and thermal processes. Contrary to the report by Mellegård et al. [21] chitosan did not inhibit *B. cereus* spore outgrowth and multiplication. The action of chitosan was found to be concentration-dependent and closely related to the average molecular weight and degree of deacetylation of the chitosan.

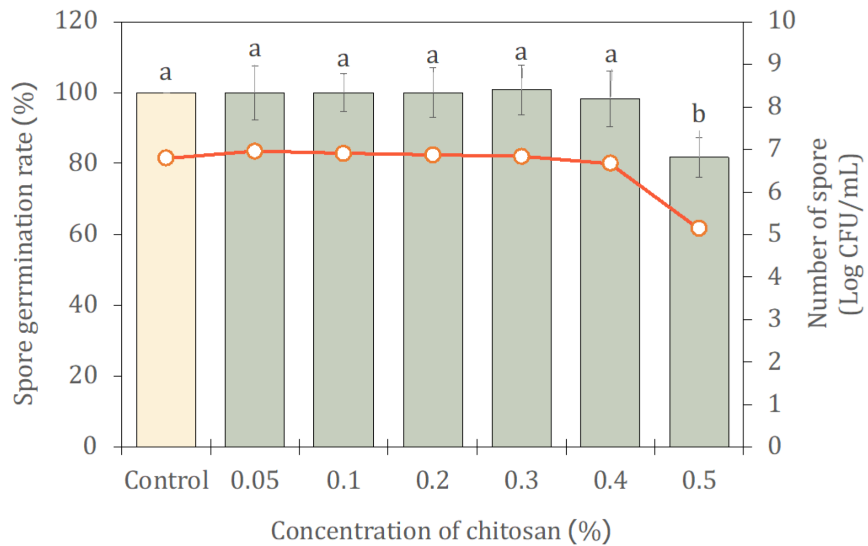

Figure 12. Sporicidal germination of *A. acidoterrestris* spore treated with chitosan at 0.05, 0.1, 0.2, 0.3, 0.4 and 0.50% w/v. Different letters were significantly different (p<0.05).

From the above results, it can be concluded that chitosan from shrimp effectively inhibits spoilage microorganisms associated with orange juice, namely *A. acidoterrestris* and *Z. bailii*. However, *A. acidoterrestris* was more resistant to chitosan than *Z. bailii*. Thus, *A. acidoterrestris* was selected to further investigation. Interestingly, *A. acidoterrestris* is a spore-forming bacteria that can resist acid and thermal pasteurization. It has been suggested as a target microorganism for the pasteurized process in the high-acidic fruit juice industry.

3.3 Evaluation of chitosan against microorganisms in orange juice

3.3.1 Changes in the number of A. acidoterrestris cells in orange juice

Numbers of *A. acidoterrestris* cells in the orange juice stored at 10 °C (Simulated conditions of a household refrigerator) and 35 °C (Simulated conditions for microbial growth) for 14 days were shown in Figure 13a-b. The result showed no significant difference in the amount of *A. acidoterrestris* cells in orange juice at 10 °C for 14 days (p>0.05). Comparing all treatments in orange juice at 35 °C for 14 days, the control significantly increased throughout 14 days and was significantly different (p < 0.05) to 0.050% and 0.100% chitosan. This result is consistent with the

report by Ulfadillah and Chang [13] who studied the antibacterial activity of chitosan against *A. acidoterrestris* in orange juice. The results showed that chitosan effectively retarded the *A. acidoterrestris* growth in orange juice during storage at 50 °C. The antibacterial activity of chitosan in orange juice was more effective than in microbiological media because the antibacterial property of chitosan was improved by acidic pH [22].

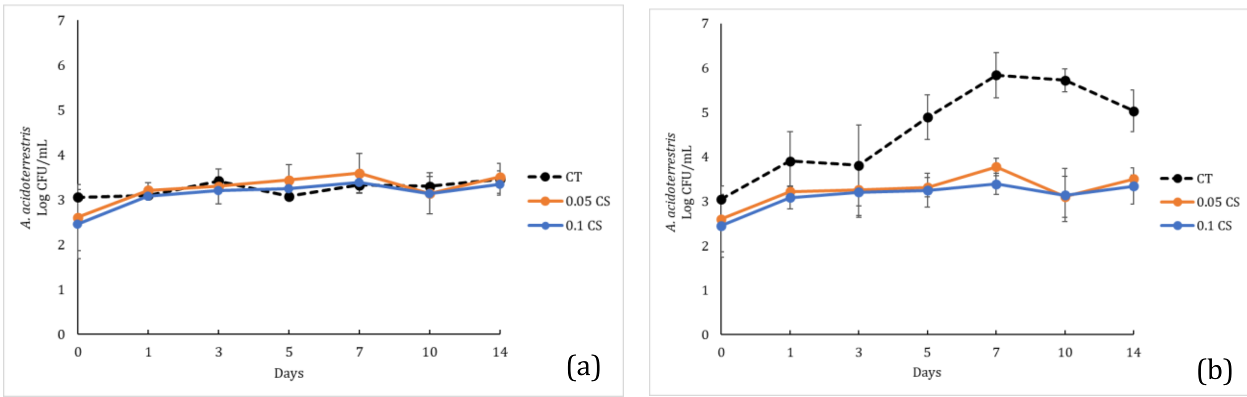

Figure 2. Antimicrobial activity of chitosan against *A. acidoterrestris* cells in orange juice at 10 °C (a) and 35 °C (b) for 14 days. The data are expressed as mean with SD (n = 2).

3.3.2 Changes in the number of A. acidoterrestris spores in orange juice

Numbers of *A. acidoterrestris* spores in the orange juice were shown in Figure 3a-b. The result showed no significant differences (p>0.05) in the amount of *A. acidoterrestris* spores in orange juice at 10 °C for 14 days. However, *A. acidoterrestris* spores in control germinated and increased to 6.76 Log CFU/mL after 14 days of storage at 35°C. Meanwhile, the number of spores in 0.050% and 0.100% chitosan did not significantly change throughout 14 days. This result is similar to the report by Falcon et al. [20] who studied the effects of chitosan concentrations and holding times to the pasteurization temperature on the survival of *A. acidoterrestris* spores, the result showed that combined with thermal processing, chitosan of low molecular weight (LMW; 50–190 kDa and 75–85% deacetylation degree) at a concentration of 1.4 g/L could effectively inhibit the germination of *A. acidoterrestris* spores. However, further investigation is needed into the mechanism of the spore germination inhibition of chitosan.

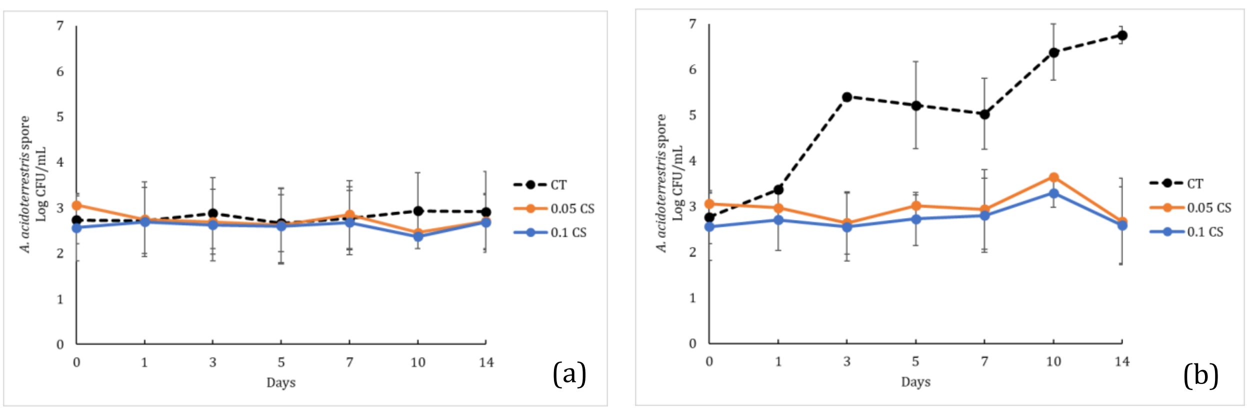

Figure 3. Antimicrobial activity of chitosan against *A. acidoterrestris* spores in orange juice at 10 °C (a) and 35 °C (b) for 14 days. The data are expressed as mean with SD (n = 2).

3.4 Impact of chitosan on physicochemical properties of orange juice

3.4.1 Changes in pH values of A. acidoterrestris in orange juice

The result from the pH values of orange juice at 10 °C and 35 °C for 14 days showed that they were not significantly different ($p>0.05$). On the initial day, the pH values of orange juice in control, 0.050% and 0.100% chitosan, were 3.90, 3.97, and 4.00, respectively (data not shown), and were relatively constant throughout the storage for 14 days. The pH values were in the range of 3.87-4.00. The result agrees with Ewis et al. [12] study to employ chitosan as a natural preservation ingredient instead of harmful preservatives to develop a functional drink. The results showed that pH increased with increasing chitosan concentration compared to the control. This effect might be attributed to chitosan having a positive charge when the pH is less than 6.5, which results in its ability to decrease fruit juice acidity due to its acid-binding ability [11]. Moreover, *A. acidoterrestris* does not produce gas during growth. The spoiled juice appears normal, with little or no change in pH [23].

3.4.2 Changes in the titratable acidity values in orange juice

Changes during storage in the titratable acidity values of *A. acidoterrestris* in orange juice at 10 °C and 35 °C presented in Table 2. The titratable acidity values of orange juice were significantly different ($p<0.05$) in control, 0.050%, and 0.100% chitosan from the initial day (0.50, 0.501, and 0.502%), respectively. There were significant differences when comparing the titratable acidity during storage for 14 days ($p<0.05$). There was an increasing trend of the titratable acidity in orange juice at both temperatures during storage. This result is consistent with the report by Tastan and Baysal [24] that the titratable acidity of pomegranate juice clarified with chitosan was observed from the 0 month (0.953%) to the 6th month (2.66%) during storage at 4 °C compared to the samples stored at 20 °C. During storage, there was an increasing trend of titratable acidity in pomegranate juice

Table 2. Changes in the titratable acidity values of *A. acidoterrestris* in orange juice at 10 °C and 35 °C for 14 days

Day	TA (%)					
	10 °C			35 °C		
	CT	0.05%	0.1%	CT	0.05%	0.1%
0	0.500 ± 0.00^{aA}	0.501 ± 0.00^{aA}	0.502 ± 0.00^{aA}	0.48 ± 0.00^{aA}	0.500 ± 0.00^{aB}	0.503 ± 0.00^{abC}
1	0.501 ± 0.00^{aA}	0.501 ± 0.00^{aA}	0.502 ± 0.00^{aB}	0.49 ± 0.00^{aA}	0.501 ± 0.00^{aB}	0.503 ± 0.00^{abC}
3	0.502 ± 0.00^{bA}	0.503 ± 0.00^{bB}	0.504 ± 0.00^{bB}	0.49 ± 0.00^{aA}	0.502 ± 0.00^{bB}	0.504 ± 0.00^{bcB}
5	0.502 ± 0.00^{bA}	0.504 ± 0.00^{bcAB}	0.504 ± 0.00^{bB}	0.49 ± 0.00^{aA}	0.502 ± 0.00^{bB}	0.506 ± 0.00^{dBC}
7	0.503 ± 0.00^{cA}	0.504 ± 0.00^{bcB}	0.504 ± 0.00^{bB}	0.49 ± 0.00^{aA}	0.503 ± 0.00^{cB}	0.503 ± 0.00^{abB}
10	0.503 ± 0.00^{cA}	0.504 ± 0.00^{bcB}	0.505 ± 0.00^{bcC}	0.500 ± 0.00^{bA}	0.505 ± 0.00^{dB}	0.502 ± 0.00^{aC}
14	0.504 ± 0.00^{dA}	0.505 ± 0.00^{cA}	0.506 ± 0.01^{cA}	0.500 ± 0.00^{bA}	0.506 ± 0.00^{dB}	0.504 ± 0.01^{bcC}

*Values are presented as the average mean ± standard deviation of two replicates.

a-d means within the same column and the same temperature followed by different letters were significantly different ($p<0.05$).
A-C means within the same row and the same temperature followed by different letters were significantly different ($p<0.05$).

3.4.3 Changes in the appearance of orange juice at 10 °C and 35 °C for 14 days.

The appearances of orange juice in different treatments on day 0 and treatments stored at 10 °C and 35 °C on day 14 are shown in Figure 4. They were shaken before being analyzed by visual observation. From the illustration, all treatments are not different.

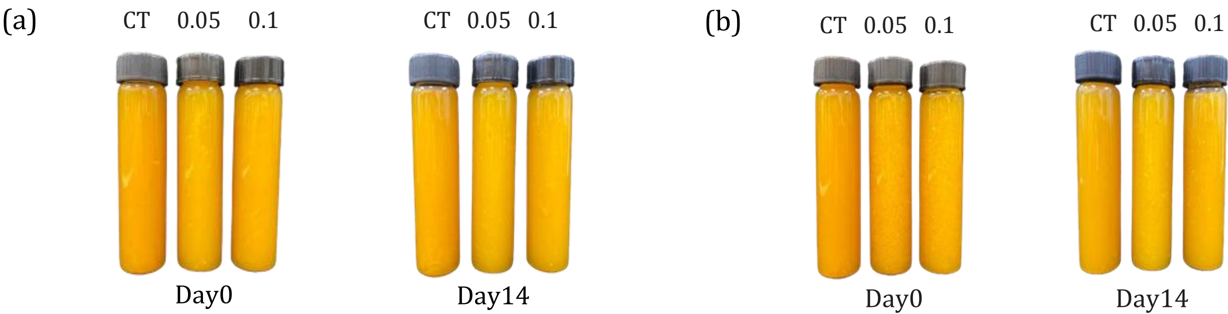

Figure 4. The appearance of orange juice with different treatments (Control, 0.05%, and 0.1% chitosan) and stored at 10 °C (a) and 35 °C (b) on days 0 and 14

4. CONCLUSIONS

Shrimp chitosan effectively inhibited microorganisms associated with orange juices, including *A. acidoterrestris* and *Z. bailii* cells. Moreover, it inhibited *A. acidoterrestris* spore germination. Adding chitosan at 0.050% w/v in orange juice inhibited the growth of *A. acidoterrestris* cells and spores. It did not significantly affect the pH and showed little change in titratable acidity after storage for 14 days. Therefore, shrimp chitosan could be used as a natural preservative in squeezed orange juice.

5. ACKNOWLEDGEMENTS

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Division E

(Related Topics)

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Detecting Chlortetracycline Residues in Milk Using a Lateral Flow Immunoassay

EP-P018

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Antibiotics residue
Chlortetracycline
Food safety
Lateral flow immunoassay
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ABSTRACT

The extensive use of antibiotics in animal husbandry for disease treatment, prevention, and growth promotion is a common practice. tetracyclines (TCs), a class of antibiotics that includes chlortetracycline (CTC), are widely used to inhibit bacterial growth. However, the use of antibiotics in animal husbandry may lead to residues in animal food products, posing risks to consumer safety. Therefore, the detection of antibiotic residues in food is important to mitigate the risk of consumption. Immunoassay methods are commonly used for detecting residue contaminants in food. Lateral flow immunoassay (LFIA) is favored for several advantages including rapid processing time; ease of use; and naked-eye readability. Additionally, it is a portable device allows for preliminary screening and surveillance on-site. In this study, LFIA was developed to detect CTC residues in pasteurized milk samples. Monoclonal antibodies against CTC were conjugated onto gold nanoparticles and immobilized on the conjugate pad. Bovine serum albumin conjugated with CTC (BSA-CTC conjugate) and goat anti-mouse antibodies were applied to the test line and control line of the LFIA, respectively. Under optimized conditions, the assay achieved cut off at 100 ng/mL by naked eye and a limit of detection (LOD) of 5.12 ng/mL determined using ImageJ software. The optimized LFIA required only 20 minutes for testing, easy to use with simple sample preparation, and exhibited specificity for CTC, making it suitable for screening CTC residues in milk

1. INTRODUCTION

Antibiotics are widely used in animal husbandry to treat and prevent diseases and promote animal growth. An antibiotic commonly used is chlortetracycline (CTC), which belongs to the tetracycline (TCs) group. Its role is to inhibit the growth of both gram-positive and gram-negative bacteria, such as bacilli streptococci, rickettsia bacteria, and spirochetes, by interfering with protein synthesis in bacterial cells [1].

However, the use of antibiotics in animal husbandry can lead to the presence of residues in animal food products, which may pose risks to consumers, such as nausea, anorexia, diarrhea, drug resistance, and potentially fatal reactions [2].

Therefore, to ensure consumer safety, the Codex Alimentarius Commission has established Maximum Residue Limits (MRLs) for CTC in milk, set at 100 μg/kg [3]. For that reason, detecting antibiotic residues in food is essential for global food safety, ensuring animal products meet established standards for international acceptance. The detection methods can be broadly categorized into two main approaches. The confirmation method involves verifying results, such as with high performance liquid chromatography (HPLC) and liquid chromatography tandem mass spectrometry. While these methods offer high sensitivity and specificity, require skilled personnel, and must be conducted in laboratory [4]. On the other hand, the screening method is used to screen the presence of residue contaminants. This approach relies on immunoassays, including enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassay (LFIA).

The LFIA, also known as a strip test, is a widely utilized method in the field of food safety for detecting contaminants in food. This method allows for rapid, single-step measurement, easy operation, and visual result interpretation. Additionally, LFIA can be used for on-site testing, enabling monitoring of food safety throughout the production chain, from raw materials to ready-to-eat products[5,6]. The detection process relies on the specific interaction between antigens and antibodies immobilized on a membrane. There are two reaction formats: the competitive format, suitable for small molecule detection, and the sandwich format, suitable for larger molecule detection. LFIA can report measurement results qualitatively and semi-quantitatively [7]. A typical LFIA consists of several components as shown in Figure 1A: 1) Sample pad, for accommodating the test sample; 2) Conjugate pad, where antibodies labeled with colloidal gold particles are located; 3) Nitrocellulose membrane, featuring test and control lines for displaying test results; and 4) Absorbent pad, used to draw the sample after reaction and prevent backflow [8].

In this study, LFIA was developed for detecting CTC residue in milk. Specific antibodies against CTC were conjugated to gold nanoparticles (GNPs-mAb-CTC conjugate) and applied to the conjugate pad. The nitrocellulose membrane was immobilized with GAM and BSA-CTC conjugate at the C-line and T-line, respectively. The optimization of various parameters inherent to the developed LFIA, such as the volume of GNPs-mAb-CTC conjugate and the concentration of BSA-CTC at the T-line, were thoughtfully conducted and undertaken by spiking CTC in pasteurized milk samples. The detection limits of CTC in milk were determined by naked-eye observation and ImageJ software analysis. Furthermore, the aim of the study was to optimize LFIA strips that provided rapid results, easy to use with simple sample preparation, and portable device for on-site screening of CTC antibiotic residues in various milk.

2. MATERIAL AND METHODS

2.1 Reagents

Chlortetracycline hydrochloride (CTC), Monoclonal antibodies against chlortetracycline (mAb-CTC) were obtained from our lab (Poungmalai et al. 2021), Goat anti mouse IgG secondary antibodies (GAM), Colloidal gold nanoparticles 40 nm (GNPs), 2-(N-morpholino) ethane Sulfonic Acid (MES), Borate buffer (BB), 0.01M Phosphate buffer saline (PBS), 0.01M Phosphate buffer (PB), Bovine serum albumin (BSA), Sodium carbonate (Na_2CO_3), 10% Sodium chloride (10% NaCl), 37% Formaldehyde, water deionized by a Milli-Q unit, Sucrose, Tween 20, Oxytetracycline (OTC), Tetracycline (TC), Doxycycline (DC), Streptomycin (STR), Lincomycin (LIN), and Neomycin (NEO)

2.2 Optimization and Preparation of GNPs-mAb-CTC conjugate

To determine the appropriate concentration of mAb-CTC for conjugation with GNPs, mAb-CTC solutions were prepared at concentrations ranging from 0 to 200 µg/mL in BB, pH 8.6. The pH of the 40 nm GNP solution was then adjusted to approximately 8.6 using Na_2CO_3, pH 9.0.

Subsequently, mAb-CTC and the GNPs solution were dispensed dropwise into 96-well plates in volumes of 20 and 200 µL, respectively. Then, 80 µL of 10% NaCl was added, and the mixture was mixed well. The color of the resulting solution was observed, and the absorbance at 520 nm was measured. Following the optimization, a suitable concentration of mAb-CTC and GNPs were mixed in a ratio of 10:1 (GNPs:mAb-CTC). The mixture was then incubated on a stirrer for 1 hour. 5% BSA was added and then incubated on a stirrer for an additional hour. Subsequently, the mixture was washed with BB using centrifugation at 25,000×g, 4°C, for 30 min, repeated 3 times. After that, the pellet was resuspended in 1% BSA, 2% sucrose in BB, and stored at 4°C. The conjugates were characterized for elemental composition using absorbance spectra obtained in the range of 300–700 nm from UV Vis spectroscopy [9].

2.3 BSA-CTC conjugate preparation

CTC was conjugated to a carrier protein, BSA, using the mannich reaction. The reaction involved mixing a solution of CTC (5 mg/ml in water) with BSA (10 mg/ml in 0.1M MES containing NaCl, pH 4.7), followed by thorough mixing. Then, 250 µL of 37% (v/v) formaldehyde was added, and the mixture was incubated in the dark at room temperature overnight to facilitate the conjugation reaction. Subsequently, the mixture was dialyzed against PBS (pH 7.4) at 4°C for 3 days, with PBS changed twice daily. The resulting BSA-CTC conjugate was quantified for protein content using the BCA assay for subsequent application on the T-line [10].

2.4 Fabrication and Interpretation of LFIA

The developed LFIA strip consists of four main components: 1) Whatman standard 17 as a sample pad, 2) glass fiber as a conjugate pad, 3) Whatman AE99 as a nitrocellulose membrane, and 4) an absorbent pad. Initially, the sample pad and conjugate pad were blocked with 1% BSA, 2% Sucrose in PB, pH 7.4 for 30 min and dried at 37°C. The nitrocellulose membrane was immobilized with various concentrations of BSA-CTC conjugate and 0.75 mg/mL of GAM at a dispense rate of 1 µL/cm at the T-line and C-line, respectively. GNPs-mAb-CTC conjugate was applied on the conjugate pad. Then, all membranes were dried at 37°C before assembly as shown in Figure 1A. For Interpreting the results, the presence of color at the test line indicates a negative result, while the absence of color at the test line represents a positive result as shown in Figure 1B [7].

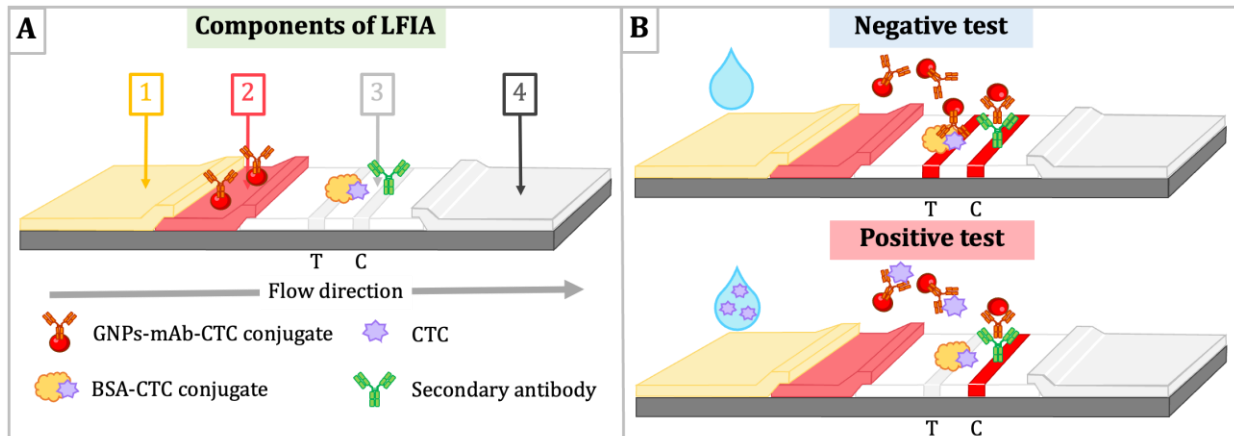

Figure 1. The composition (A) and interpretation (B) of LFIA

2.5 Sample preparation

To investigate the effect of milk sample composition on LFIA results, plain pasteurized milk samples were added with 50%Tween 20 to final concentration of 0.5%Tween 20 and subsequently tested on the LFIA strips. The results were then compared to those obtained using plain pasteurized milk.

2.6 Optimization of LFIA

The GNPs-mAb-CTC conjugate and the BSA-CTC conjugate on the T-line can significantly affect color intensity and sensitivity of the LFIA test. Therefore, optimization was necessary to establish suitable conditions. Optimization involved testing different volumes (2 and 4 µL) of GNPs-mAb-CTC conjugate and various concentrations (0.5, 1.0, and 1.5 mg/mL) of BSA-CTC conjugate on the T-line. The selection criteria considered the color intensity of both the C-line and T-line. Ideally, the T-line should disappear when testing milk samples containing 100 ng/mL of CTC. Following optimization, the LFIA strips were further evaluated for sensitivity and specificity. Additionally, the study investigated the performance of the LFIA with various types of plain milk, including UHT (Ultra-High-Temperature processed), pasteurized, and non-fat milk.

2.7 Sensitivity and Specificity test

Sensitivity will be determined based on the concentration of CTC that causes the T-line to disappear. The optimized LFIA will be tested with milk samples containing CTC concentrations ranging from 0 to 125 ng/mL. The results of the LFIA will be observed visually with the naked eye, and images will be captured using a smartphone to determine the limit of detection (LOD). Specificity will be evaluated based on the appearance of the test strip when the optimized LFIA is tested with milk samples containing antibiotics from the Tetracyclines (TCs) group, including TC , OTC , and DC , as well as antibiotics from the non-Tetracyclines (non-TCs) group, including STR, LIN, and NEO. The results of the LFIA will be observed.

2.8 Processing of data

The images of the LFIA were analyzed for the color intensity of the T-line using ImageJ software. Subsequently, the color intensity was calculated as Δ color intensity according to Equation 1, where I_S (Intensity of sample) represents the intensity obtained from the T-line when tested with milk sample containing CTC, while I_B (Intensity of blank) represents the intensity obtained from the T-line when tested with milk sample without CTC [11].

$$\triangle \text{ color intensity} = I_S - I_B \quad (1)$$

3. RESULTS AND DISCUSSION

3.1 mAb-CTC onto GNPs conjugate optimization

To identify the optimal concentration of mAb-CTC that effectively conjugates with GNPs without causing undesirable aggregation, solutions of mAb-CTC were prepared at concentrations ranging from 0 to 200 µg/mL. After that the solutions were mixed with a GNPs solution and NaCl. The absorbance at 520 nm was measured. It was found that at mAb-CTC concentrations ranging from 100 to 200 µg/mL, the solution exhibited a red-purple color and maintained a constant absorbance value. This indicated a sufficient concentration for saturating the surface of the GNPs. Meanwhile, at concentrations ranging from 0 to 80 µg/mL, the solution appeared as a grayish-purple color, resulting in empty spaces on the GNP surface due to insufficient mAb-CTC. When NaCl was added, the salt ions induced aggregation of the GNPs in these empty surface areas, as shown in Figure 2A.

Accordingly, considering both the color of the solution and the constant absorbance value, a concentration of 100 µg/mL of mAb-CTC was chosen as suitable for conjugation with GNPs to ensure stability. Subsequently, the GNPs-mAb-CTC conjugate was monitored by measuring its absorbance in the range of 300 to 700 nm using UV-Vis spectroscopy. As shown in Figure 2B, the GNPs exhibited a λ_{max} value of 522 nm. After conjugation with mAb-CTC, the λ_{max} value increased to 527 nm. This 5 nm red shift in absorbance, attributed to chemical interactions on the particle surface affecting the plasmon resonance of the GNPs [12], confirms the successful conjugation of mAb-CTC with GNPs.

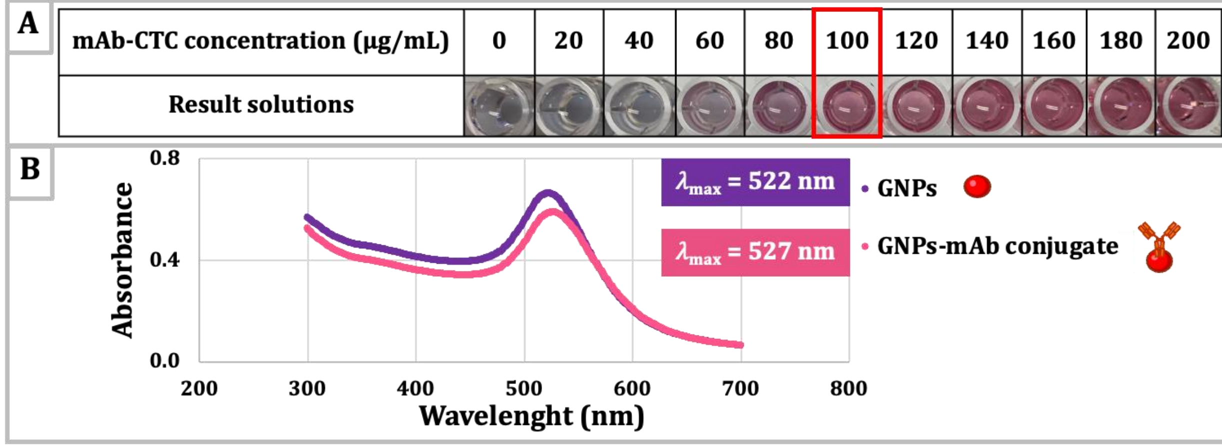

Figure 2. Optimization of mAb-CTC (A). UV–Vis spectra of GNPs and GNPs-mAb-CTC conjugate (B)

3.2 BSA-CTC conjugate

CTC has a molecular weight of 515.3 g/mol, which is considered to be a very small antigen known as a hapten. Therefore, it is difficult for antibodies to effectively capture CTC molecules on the T-line. To enhance antibody binding to CTC, its size is increased by linking it with a protein carrier such as BSA (BSA-CTC conjugate). The mannich reaction is employed to achieve this cross-linking. Formaldehyde is added to facilitate the reaction between the amino groups of the carrier protein and the phenol group of CTC [10]. Subsequently, the protein content of the BSA-CTC conjugates is quantified using the BCA assay, showing a concentration of 2.72 mg/mL. These BSA-CTC conjugates are then applied to the T-line for further investigation.

3.3 Sample preparation

The results showed that the sample solution had a slow flow rate (4.39 min) and produced faint lines on the LFIA test. This was attributed to matrix interference from the plain pasteurized milk. However, adding 0.5% Tween 20 to the milk significantly improved the flow rate (3.47 min) and resulted in noticeably more intense lines on the LFIA, as shown in the Figure 3. This improvement can be explained by Tween 20's ability to decrease the surface tension of the liquid sample, facilitating capillary force, and allowing smoother flow through the LFIA strip. Additionally, Tween 20 helps prevent non-specific binding and aggregation of molecules, which could otherwise impede flow and disrupt the accuracy of the results [13]. Therefore, the addition of Tween 20 provides a simple and effective sample pre-treatment method with no requirement centrifugation or dilution method.

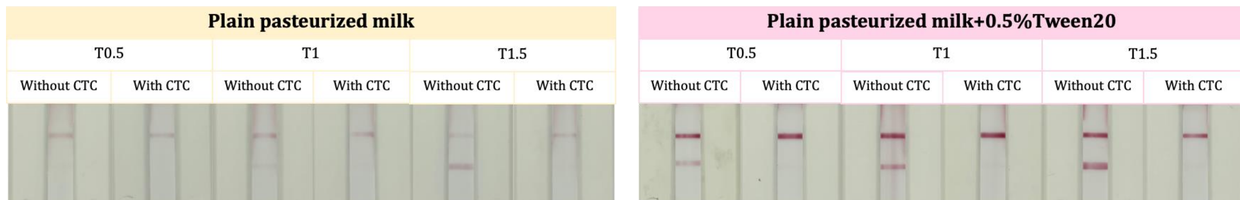

Figure 3. Results of LFIA tested with pasteurized milk

3.4 LFIA optimization

We optimized the LFIA by investigating the volumes of GNPs-mAb-CTC conjugate and the concentration of BSA-CTC conjugate at the T-line. The results revealed that the intensity of the color lines increased proportionally to the volume of GNPs-mAb-CTC conjugate and the concentration of BSA-CTC conjugate as shown in Figure 4A. However, the excessive proportions could lead to extremely intense color lines, affecting the reduced sensitivity of the LFIA. Finally, the optimal condition for LFIA was selected when a concentration of 1.5 mg/mL of BSA-CTC conjugate at the T-line and 2 µL of GNPs-mAb-CTC conjugate were used. Following this optimization, the LFIA will be further studied for sensitivity and specificity.

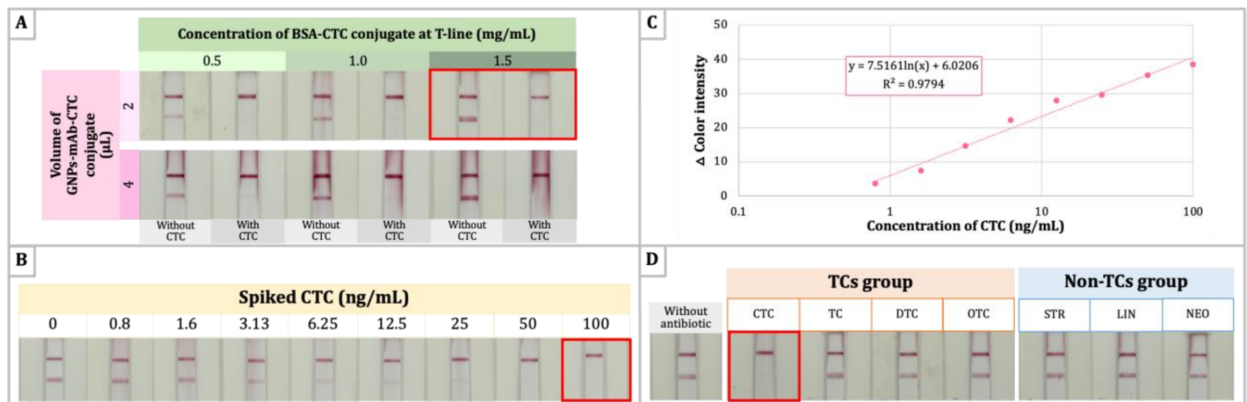

Figure 4. The optimization of LFIA (A). The sensitivity test (B) with linear plot of △ color intensity and various concentration of CTC (C), and the specificity test of the optimized LFIA (D)

3.5 Sensitivity and Specificity test

The sensitivity was determined by the cut-off value from visual detection and the limit of detection (LOD) using ImageJ software. The results will be observed visually and captured with a smartphone. After that, the images will be analyzed for color intensity using ImageJ software. The Δ color intensity will be calculated for plotting a graph, where the y-axis represents Δ color intensity and the x-axis represents the concentration of CTC (ng/mL), as shown in Figure 4C. The equation obtained from the linear regression analysis between Δ color intensity, and the concentration of CTC (ng/mL) is Y = 7.5161ln(x) + 6.0206 with an R^2 value of 0.9794. Then, the slope value will be used to calculate the LOD according to Equation 2, where SD represents the standard deviation [11]. The optimized LFIA exhibits the cut-off value and LOD of 100 and 5.12 ng/mL with the detection range of 0.8 to 100 ng/mL, respectively (Figure 4B). It can be eventually concluded that the optimized LFIA can be used for screening milk samples within the standards set by the Codex Alimentarius Commission for MRL of CTC in milk. Additionally, the optimized LFIA can be semi-quantify CTC residue in milk using linear regression equation. Furthermore, it shows specificity to CTC due to no cross-reactivity with other antimicrobial drugs (Figure 4D).

$$LOD = \frac{3SD}{Slope} \tag{2}$$

3.6 Various type of plain milk test

It was observed that all types of plain milk were successfully detected by the optimized LFIA, exhibiting sensitivity at the cut-off value of 100 ng/mL, as shown in Figure 5. This makes it suitable for use in detecting different milk samples for screening CTC residues to meet regulatory standards. Additionally, the sample preparation method is user-friendly with an assay duration of 20 minutes.

Figure 5. Results of LFIA testing with pasteurized milk, non-fat milk, and UHT milk

4. CONCLUSIONS

This study developed LFIA for detecting chlortetracycline antibiotic residues in milk using mAb-CTC labeled with GNPs for optical detection. The optimized LFIA obtained is user-friendly, allows for visual result interpretation, and provides rapid testing within 20 minutes. It demonstrated a cut-off value and LOD of 100 and 5.12 ng/mL, respectively, making it suitable for detecting CTC residues in milk to meet the MRL standards set by the Codex Alimentarius Commission. Additionally, it exhibits high specificity to CTC with no cross-reaction to other antibiotics. Furthermore, it can be used to test with pasteurized, UHT, and non-fat milk using simple sample preparation. In future studies, we intend to apply the developed LFIA for the detection of residual CTC in milk samples collected from various livestock species.

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Plastic bag bans policy: Behaviour and Attitude of People in Bangkok Metropolitan Region, Thailand

EP-O103

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ABSTRACT

Global warming is a global issue for sustainable development goals (SDGs). Furthermore, Thailand's policy banning 4 types of single-use plastics will be implemented. Plastic shopping bags with a thickness of less than 36 microns, plastic straws, foam boxes, and plastic glasses with a thickness of less than 100 microns are not to be sold or used in any cases to reduce environmental impacts. The objective of this research was to study the behavior, attitude, and decision-making on packaging design of Thai customers who shop at fresh markets in the Bangkok metropolitan region. From our "questionnaire" with 221 respondents, single-use plastic shopping bags are the most used of 4 plastic types. Most of the respondents (89.59 %) chose to agree with the policy and use other means of materials instead; however, the rest (10.41 %) are concerned about the necessity of these single-use plastic shopping bags in the local fresh market. The respondent who chose to bring a container of their own up to 91.4 % in this matter, 5.6 % chose to purchase a plastic bag from the shop and the last 3 % chose another solution and do not agree with a plastic ban. Most of the respondents chose a biodegradable plastic shopping bag green in color and preferred 2 baht for each bag.

1. INTRODUCTION

Single-use plastic shopping bags (SUPBs) are a significant cause of environmental pollution [1-2]. The magnitude of this problem has resulted in a growing tide of anti-plastic bag sentiment, characterised by the implementation of several interventions that include bans, taxes, nudges, and voluntary initiatives [3-4].

Thailand ranks 5th among the countries that generate the most plastic waste per capita in the world, with approximately 70 kilograms of plastic waste per person per year [5]. Over the past ten years, there has been a significant increase in the total amount of solid waste, surpassing 4 million tons, with an annual rise in the amount of mixed plastic waste. Only 25% of plastic waste is recyclable, with the remaining 75% consisting of single-use plastics (SUP) like food bags, straws, plastic cups, and foam boxes, according to the plastic waste management roadmap by 2018–2030 [6].

The COVID-19 pandemic situation in 2020 has led to a 15% increase in the amount of plastic waste, compared to the normal situation before 2019. During normal circumstances, the amount of plastic waste was about 5,500 tons/day, but during the COVID-19 period, it increased to about 6,300 tons/day [7-8] with the increase in plastic waste accounting for 62.3 %, while recycled plastic waste increased 33.3 % and plastic waste contaminated with food waste increased 71.1%.

Also, many countries in Asia and Africa implemented a plastic bag ban (PBB) such as Bangladesh, India, Taiwan, and China pioneered the implementation of a PBB in Asia. Thailand's government enacted a ban on free plastic bags in January 2020[9-10]. However, there is limited study on the consumer behavior on the PBB policy.

This study aims to enhance the understanding of Thai people's awareness and attitudes towards PBB in the Bangkok area. Furthermore, the research contributes to the environmental sustainability debate by addressing the following key objectives: (a) to understand considerations influencing the adoption and implementation of a PBB as a policy option; and (b) to suggest strategies for managing the problem of SUPBs litter.

2. MATERIAL AND METHODS

2.1 Qualitative Data

2.1.1 Consumer Recruitment:

This research was choosing consumers who shopped at fresh markets 10 participants, 2 males and 8 females, were interviewed for 15-20 minutes. The questionnaire consists of 3 parts: part 1: interviewee's profile, part 2: questions about experiences, opinions, behaviors, and attitudes towards the use of single-use plastic shopping bags and part 3: questions about perceptions and attitudes towards the policy to ban single-use plastic shopping bags. The interviewee has been recruited by simple random sampling from the buyer who is shopping at the fresh market on the weekend. If the shopper does not agree to participate, the researcher can skip them and choose only to agree. The customer insight data obtained were used to create a customer journey map that deeply understands the behaviors, problems/obstacles that arise from the target consumers. It was used as a guideline to design a solution that meets the needs of the consumers.

2.1.2 Customer journey map

The customer journey map was a method of describing consumers' views and feelings from the consumer experience received over a period (in Figure 1). To understand the behavior of each touchpoint of the consumer. This leads to identifying the root cause of the problem and using what has been achieved to develop products or services to be more efficient.

1. Define the representative of the model of the persona and the nature of use (context). If there are many forms representing the consumer, do it one by one and compare it again.
2. Identify the touch point where the consumer interacts with the service system such as meeting directly with the service provider (face-to-face contact) or using the service through the website or automatic service machines.
3. Activity information, emotions, or pain points where consumers have problems or dissatisfaction were recorded. That occurred at each touch point during that activity.
4. Comply the information obtained from personas into a customer journey map, which may incorporate images, videos, or key sentences (Quotes) that the service consumers have recorded to make the presentation clearer and add weight to persuade or make decisions on changes in the service system.
5. Analyze and conclusions from the data obtained at each touch point to identify problems that should be used to improve the existing service system for consumers to have a better experience.

2.2 Quantitative Data

2.2.1 The behavior of consumers who shop at fresh markets about the use of single-use plastic shopping bags

The consumer insight data was used to create a questionnaire. The questionnaire consists of questions to understand the single-use plastic shopping bag usage behavior of target consumers, attitudes about the policy to ban single-use plastic shopping bags, and factors regarding the purchase of biodegradable plastic shopping bags.

2.2.2 Data Analysis

The analysis started with data cleaning and data management by selecting the complete data and deleting the missing data. Data were reproduced by coding and characterized. Data were analyzed using SPSS 27. After assessing the sufficient reliability of the scale with Cronbach alpha (0.908). An average and percent of each variable were calculated for demographics, behavior, and attitude towards the packaging for food consumption. Codes with similarities were merged into higher-order codes, and the frequency of codes was analyzed statistically for differences between packaging categories.

1. Analysis of demographic characteristics of respondents
2. Analysis of consumer single-use plastic shopping bag usage behavior
3. Analysis of factors in consumer's decision to buy biodegradable plastic shopping bags

3. RESULTS AND DISCUSSION

3.1 Single-use plastic shopping bag usage behavior and target consumer's attitude towards plastic bag ban policy in Thailand

The in-depth interviews collected qualitative data from 10 target consumers who are customers and vendors selling using fresh markets in the Bangkok metropolitan region. The customer insight data obtained were used to create a customer journey map. Figure 1 shows the customer journey map of a customer (representative) who is female, 45 years old. She went shopping at the market 1-2 times a week by motor vehicle and using cloth bags.

The above data led to the design of the questionnaire. The questionnaire was used to survey the target consumers who are aged 20-59 years and shop at the fresh market in Bangkok metropolitan region. It makes known the habit of using single-use plastic shopping bags, and attitudes towards the government's ban single-use plastic shopping bags policy of target consumers as follows.

Process	Pre-service	Event time	Event time	Event time
Customer goals	Go to fresh market	Returning home	On the way home	Arrive at home
Touchpoints/ Emotional response	- Prepare a cloth bag, backpack, or basket. - Go to the market once, and get more than 5 plastic bags.	- Get a lot of plastic bags. - Put the purchased items in the prepared bag and put them in the basket in front of the motorbike.	- Ride with caution for fear of dropping your purchase.	- Separate purchased items - Put in the refrigerator or pantry. - Unpack the food bag. - Plastic Bags stained with food are disposed of. - Clean plastic bags to keep for things or garbage.
Customer thoughts	- Carrying heavy objects - Fear of torn bags - It's all very mixed up	- Heavy objects - Fear of torn bags - It is an obstacle to carry it home.	- Fear of dropping - Fear of torn bags	No worries about things not falling off during the trip.
Overall customer experience	☹	☹	☹	☺

Figure 1. Customer journey mapping of a customer who's going to shop at a fresh market

3.2 Demographic characteristics of respondents

Data from 221 respondents (see Table 1) showed that the majority of respondents were female (71.9%) and male (28.1%), with the majority of respondents aged 40-49 years (58.4%), followed by those over 50 years old (24%), 30-39 years old (16.7%), and 20-29 years old (0.9%), respectively. When considering the monthly income of the target consumers, it was found that 30,001 – 60,000 Baht (41.6%), 15,001 – 30,000 Baht (25.8%), 60,001 – 100,000 Baht (16.7%), below 15,000 Baht (8.1%), more than 100,000 (7.7%), respectively. When considering the number of the target consumers, they was found that 1 - 4 people (77.8%), 5 - 8 people (19.9%), 9 - 11 people (1.8%), and more than 12 people (0.5%), respectively.

Table 1. Demographic characteristics of target consumers

Demographic characteristics	**Number of people (%)**
Sex (sex)	
male	27.60
female	71.90
Not specified	0.50
Age (age)	
Under 20 years old	0.00
20 - 29 years	0.90
30 - 39 years	16.70
40 - 49 years	58.4
50 years or older	24.00
Monthly income (income)	
Under 15,000 Baht	8.10
15,001 – 30,000 Baht	25.80
30,001 – 60,000 Baht	41.60
60,001 – 100,000 Baht	16.70
More than 100,000 Baht	7.70
Number of family members (nfm)	
1 - 4 people	77.80
5 - 8 people	19.90
9 - 11 people	1.80
More than 12 people	0.50

3.3 Information on the behaviour of shopping in the fresh market of the target consumer

According to a study of the behavior of single-use plastic shopping bags of 221 target consumers who participated in the survey (see Table 2), it was found that the majority of respondents came to the fresh market 1-2 times a week 61.50%, followed by 3-4 times a week for 22.20%, most of them traveled to the market by car at 79.60 %, followed by motorbikes at 13.60%, bicycles at 2.70%, public vehicles at 5.40%, and walking to the market by themselves at 14.90%. Because of shopping at the market, it was found that most of the target consumers purchased products in large quantities at a time to use for several days, resulting in receiving more than 1-2 single-use plastic shopping bags per time, accounting for 38.90%, followed by 3-4 bags at 35.70% and more than 5 bags at 25.40%, respectively. (9) reported that average people in Bangkok use approximately 1.2 bags/person/day at supermarket and convenience store. When going shopping, most target consumers prepare their containers, such as cloth bags, baskets, and plastic bags, accounting for 68.80%, and another 31.20% do not prepare containers due to their previous behavior of often receiving single-use plastic shopping bags from the store every time after purchasing a product. From the data on the behavior of using single-use plastic shopping bags, most of the

target consumers are generation x consumers, which has a frequency of going to the market more than 1 time per week. In addition, containers for storing products were prepared instead of using single-use plastic shopping bags, and most of them were aware of the policy to ban plastic bags in Thailand.

Table 2. Characteristics of shopping behaviour at the fresh market of target consumers

Behaviour of using single-use plastic shopping bags when shopping	Percentage (%)
Weekly market visits (wmv)	
1 - 2 times	61.50
3 - 4 times	22.20
5 times	8.60
More than 5 times	7.70
Containers are prepared for storage. (prep)	
It is prepared.	68.80
No preparation	31.20
Number of single-use plastic shopping bags received (no.bag)	
1 – 2 Pieces	38.90
3 - 4 Pieces	35.70
More than 5 Pieces	25.40
How to get to the fresh market (vehicle)	
car	79.60
motorcycle	13.60
bicycle	2.70
public transport	5.40
walk	14.90

3.4 Characteristics of the attitude data of the target consumer towards the plastic bag cancellation policy using questionnaires.

As a result, the attitude towards the plastic bag cancellation policy of the target consumers using a questionnaire (see Table 3), it was found that most consumers perceived changes in the policy to stop using plastic will occur in 2022, accounting for 69.20%, and 30.80% are unaware of the policy to stop using plastic. 89.60% of the target consumers agreed with the policy because stopping the use of single-use plastic shopping bags helps reduce global warming, and 10.40% did not agree with the policy. Because 50% of respondents commented that some products need to use single-use plastic shopping bags, such as hot items, fresh food, vegetables, and fruits, and convenience of storage when purchasing goods 13.64%, another 9% commented that it would increase the burden on consumers and should focus on reuse rather than phase out. If the use of single-use plastic shopping bags is stopped, most of the target consumers, 91.4%, will be willing to bring their containers such as baskets, cloth bags, and others by themselves. However, the key question was whether, and to what extent, banning conventional plastic bags would trigger an offsetting increase in consumption of other bag types. (11)

From Table 4, the correlation coefficient can be summarized as follows:

1. Preparedness to bring your plastic bag substitutes is directly related to the policy agreement. That is, awareness of the policy to stop using single-use plastic shopping bags affects consumers who go to the fresh market to shop.
2. Preparedness for using containers instead of single-use plastic shopping bags is directly related to the frequency of using fresh market services.
3. Agreeing with the policy to stop using single-use plastic shopping bags and willingness to purchase alternative packaging products is an inverse relationship. As a result, people who agree with the policy to stop using single-use plastic shopping bags are unlikely to purchase

biodegradable plastic shopping bags. This is because consumers who use the fresh market's services prepare their containers to replace single-use plastic shopping bags until it becomes a habit. Therefore, there is no need to purchase biodegradable packaging.

Table 3. Policy perception, Opinions on the policy to stop using plastic, and guidelines for choosing packaging for the target consumers

Perception of the policy and opinions on the policy to stop using plastic	Percentage (%)
Perception of the plastic cancellation policy (percept)	
know	69.20
Don't know	30.80
Opinions on the policy to stop using plastic (agree)	
agree	89.60
disagree	10.40
Disagree due to	
Some products require single-use plastic shopping bags such as pork, meat, and fish.	50.00
Convenience when purchasing products and storing them.	13.64
The campaign should focus on reuse rather than cancelling use.	9.09
It increases the burden on consumers.	9.09
In an epidemic situation, disposable plastic bags are still necessary.	9.09
The focus should be on recycling rather than disuse.	9.09
Selected containers (solution)	
Cloth bags, boxes, baskets	91.4
Willing to buy single-use plastic shopping bags from stores.	5.6
Not interested in buying a replacement container.	0.5
Paper bags	0.5

Table 4. Correlation coefficient of data obtained from questionnaires

Pearson correlation											
	sex	age	income	nfm	wmv	prep	vehicle	no.bag	percept	agree	solution
sex	1.00	0.03	-0.165*	0.071	-0.068	-0.111	0.000	0.077	0.049	0.042	-0.060
age	0.03	1.00	0.108	0.047	0.074	0.038	0.153*	-0.048	-0.050	0.019	-0.078
income	-0.165*	0.108	1.00	-0.006	-0.025	0.028	-0.011	-0.061	-0.041	0.077	-0.107
nfm	0.071	0.047	-0.006	1.00	0.038	0.128	-0.024	0.142*	0.078	0.258**	-0.198**
wmv	-0.068	0.074	-0.025	0.038	1.00	0.218**	0.073	0.038	-0.032	-0.023	0.095
prep	-0.111	0.038	0.028	0.128	0.218**	1.00	0.035	0.311**	0.085	0.185**	-0.085
vehicle	0.000	0.153*	-0.011	-0.024	0.073	0.035	1.00	-0.107	-0.098	0.061	-0.008
no.bag	0.077	-0.048	-0.061	0.142*	0.038	0.311**	-0.107	1.00	0.085	0.151*	-0.130
percept	0.049	-0.050	-0.041	0.078	-0.032	0.085	-0.098	0.085	1.00	0.097	-0.114
agree	0.042	0.019	0.077	0.258**	-0.023	0.185**	0.061	0.151*	0.097	1.00	-0.830**
solution	-0.060	-0.078	-0.107	-0.198**	0.095	-0.085	-0.008	-0.130	-0.114	-0.830**	1.00

*. The correlation is significant at the 0.05 level.
**. The correlation is significant at the 0.01 level.

Summary of data correlations

From the 3 issues above, it is known that most of the target consumers, 89.60% agreed with the policy of preparing their containers to replace single-use plastic shopping bags when using the fresh market services. This group sees that there is no need to buy biodegradable packaging because they can use other containers instead. However, some consumers 10.40% disagreed because they expressed the opinion that single-use plastic shopping bags are still necessary for use. Recycling should be emphasized rather than discarded because this group views stopping the use of single-use plastic shopping bags as increasing the burden on consumers. From the policy awareness survey, 69.20% knew the policy and another 30.80% did not know the policy. The group that did not know the policy was mostly female, aged 40-49 years, accounting for 28.68 percent, and those over 50 years old accounting for 30.19 percent. The fresh market is used 1-2 times a week. Each time you go shopping, you use an average of 3-4 single-use plastic shopping bags. From the above information, it is found that the target consumers who do not know the policy is older. To encourage the target consumers to know about the plastic ban policy, there should be a campaign through advertising media channels such as news programs on television, radio news programs, social media, and signage at key locations. Because multi-channel campaigns are a good option to increase policy awareness among such target consumers. As for packaging, biodegradable plastic shopping bags are designed to replace traditional single-use plastic shopping bags, which will be discontinued. They are another option that will help solve the environmental problems currently being faced.

4 CONCLUSIONS

4.1 The behavior of single-use plastic shopping bags with handles in fresh markets in Bangkok and the vicinity

The study of behavior of using single-use plastic shopping bags when purchasing goods in the fresh market of the target consumers, it was found that most of the target consumer prepares containers for storing products instead of using single-use plastic shopping bags. While some target consumers do not prepare containers when they go shopping due to the habit of receiving single-use plastic shopping bags from stores. The purchase of products at a time is made in large quantities to be consumed for several days, resulting in many single-use plastic shopping bags being received. Even if you prepare your containers, single-use plastic shopping bags are needed anyway because some products still require single-use plastic shopping bags, such as hot items, meat, vegetables, and fruits. Single-use plastic shopping bags are still convenient to use and easy to store products. From this behavior, it was revealed that the target consumers still uses single-use plastic shopping bags when shopping. Because some products require single-use plastic shopping bags, the target consumers cannot put many types of products purchased together in one container.

4.2 Design and develop packaging according to consumer needs that are environmentally friendly and in accordance with government policy

From a study of the behavior of using single-use plastic shopping bags and the attitudes of target consumers who use fresh market services regarding the policy to stop using plastic, it was found that some target consumers commented that single-use plastic shopping bags are still necessary because some products require them such as hot items, meat, vegetables, and fruits.

From the necessity of using single-use plastic shopping bags, the focus group has proposed appropriate solutions for using alternative packaging. Most of them offer to bring their own container. This

was followed by buying single-use plastic shopping bags from stores and offering alternatives instead of declaring the ban on the use of plastic.

According to preliminary data on satisfaction with packaging characteristics, it was found that the suitable plastic bags to replace traditional single-use plastic shopping bags should be biodegradable plastic shopping bags with a thickness of not more than 36 microns with a green color. It has similar properties to traditional single-use plastic shopping bags and costs is not over 2 baht.

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Mechanical, physicochemical and heat sealing properties of banana flour films as affected by banana varieties and plasticizer types

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ABSTRACT

Banana flours prepared from Kluai Hin (KH) and Kluai Kha Nun (KKN) were used as film forming material for flour-based film. Mechanical, physicochemical and heat sealing properties of banana flour films from different banana varieties (KH and KKN), as influenced by different plasticizers (glycerol and sorbitol at 40% based on flour content), were characterized. Films prepared from both KH and KKN flours and plasticized with sorbitol had higher tensile strength (TS) but lower elongation at break (EAB), compared with those films plasticized with sorbitol ($p<0.05$). Films from KKN flour plasticized with glycerol showed higher EAB than those plasticized with sorbitol ($p<0.05$). Films plasticized with sorbitol had higher seal strength than those used with glycerol for both KH and KKN flours ($p<0.05$). The highest seal strength was observed in film prepared from KKN flour and plasticized with sorbitol ($p<0.05$). Water vapor permeability (WVP) of films plasticized sorbitol was lower than those films prepared with glycerol, regardless of banana varieties ($p<0.05$). When the same type of plasticizer was used, films prepared with KKN flour had lower WVP than those used with KH flour ($p<0.05$). Film plasticized with glycerol had higher moisture content but lower film solubility than those used with sorbitol as plasticizer for both KH and KKN flours ($p<0.05$). Films prepared from KH flour had higher L*, b* and transparency values but lower a*-value and light transmittance than those from KKN flour, regardless of types of plasticizers used ($p<0.05$). Films prepared KH flour had rough surface and cross-section, compared with those films prepared from KKN flour, irrespective of plasticizer types. Therefore, banana varieties and plasticizer types directly affected property properties of flour-based film.

1. INTRODUCTION

Nowadays, packaging has become important for food industry and is also related with global environment. The over-consumption of plastics is creating a global crisis of waste disposal. Plastic packaging waste disposal is one of urgent current environmental problem due to their non-biodegradability. Therefore, the developments of renewable and environmentally friendly bio-based materials have been received increasing attention.

Polysaccharide has been used as a material for preparing biodegradable films with several excellent properties [1, 2]. Generally, polysaccharide-based films have been widely used for coating or film production due to their relative abundance, good film-forming ability and nutritional qualities as well as excellent good barrier characteristics against gas, organic vapor and oil, compared to synthetic films [3].

Polysaccharides including starch, non-starch carbohydrates, gums, and fibers can be used as film forming material [4, 5]. The sequence of polysaccharides is simple, compared to proteins. However, the conformation of polysaccharide structures is more complicated and unpredictable. Although this electrostatic neutrality of carbohydrates may not affect significantly the properties of formed films and coatings, the occurrence of relatively large numbers of hydroxyl groups or other hydrophilic moieties in the structure indicate that hydrogen bonds may play significant roles in film formation and characteristics of resulting films [6]. Polysaccharides render transparent and homogeneous edible films with moderate mechanical properties.

Bananas and plantains (*Musa* spp.) are cultivated in the warm and high-rainfall of the tropical and subtropical regions. They originated in South-east Asia and have been spread to other regions of the world [7]. Banana is a potential source of high starch content (more than 60%). The application of flour and starch for various purposes is dependent upon its structural, physicochemical, and functional properties [8]. Flour and starch isolated from bananas and plantains have been wildly used as film forming material [3, 9, 10]. Additionally, there are many different types and species of bananas in Thailand. In a southern area of Thailand, Kluai Hin and Kluai Kha Nun are local banana belonging to the genus *Musa* from the family *Sapientum*, which are not economic bananas in the markets. However, they could serve as an excellent and interesting source of flour or starch for biodegradable film production. Moreover, banana films were developed for food applications. Salazar et al. [11] reported that green banana (*Musa acuminata* AAA) flour (whole banana and banana peel flours) film was used as a wrap for grilled chicken and showed an effect similar to that of conventional plastic (polystyrene bag) and the higher reduction of mesophilic aerobic microorganisms (*Enterobacteria* and *Staphylococcus aureus*) was developed when spices were added into the film. Furthermore, banana flour film was heat-sealable. Orsuwan and Sothornvit [12] reported that the shelf-life estimation of roasted peanuts packed in sachets from both plasticized banana flour nanocomposite film incorporated with garlic essential and commercial plastic packaging of polyethylene terephthalate/lowdensity polyethylene (PET/LDPE) based on the peroxide value (PV) was similar at high storage temperature (45 °C).

Plasticizers are required for edible films and coatings, especially for polysaccharides and proteins. Those films are often brittle and stiff due to extensive interactions between polymer molecules [13]. Plasticizers are low molecular weight agents incorporated into the polymeric film-forming materials, which decrease the glass transition temperature of the polymers. They are able to position themselves between polymer molecules and to interfere with the polymer-polymer interaction to increase flexibility and processability. However, no information on the properties of banana flour-based film from Kluai Hin and Kluai Kha Nun as affected by different plasticizers has been reported. Thus, the objective of this investigation was to determine the mechanical and physicochemical properties as well as heat seal ability of banana flour-based film.

2. MATERIAL AND METHODS

2.1 *Materials*

Bananas, Kluai Hin and Kluai Kha Nun (*Musa sapientum* Linn.) were obtained at 112-116 days after petal fall from Mueang Pattani district, Pattani province, in the southern part of Thailand. Banana flours were prepared according to Sothornvit and Pitak [8]. The proximate composition of the banana flour powder from Kluai Hin was: 8.53 ± 0.10% moisture content, 3.42 ± 0.01% protein, 0.34 ± 0.04% fat, 2.93 ± 0.04% ash and 0.80 ± 0.01% crude fiber. The proximate composition of the banana flour powder from Kluai Kha Nun was: 6.84 ± 0.10% moisture content, 2.78 ± 0.01% protein, 0.50 ± 0.08% fat, 2.33 ± 0.06% ash and 1.41 ± 0.05% crude fiber.

2.2 Chemicals

Glycerol and sorbitol were obtained from Sigma-Aldrich (St. Louis, MO, USA). Phosphorus pentoxide (P_2O_5) were purchased from Merck (Darmstadt, Germany). All chemicals are of analytical grade.

2.3 Preparation of banana flour-based film plasticized with glycerol and sorbitol

2.3.1 Preparation of film forming solution (FFS)

The film-forming solutions from Kluai Hin (KH) and Kluai Kha Nun (KKN) flours were prepared using the solution casting method proposed by Sothornvit and Pitak [14] with a slight modification. Banana flour was added with 3 volumes of distilled water and heated to 90°C for 20 min with vigorous stirring. The flour concentration of the mixture was fixed at 3.5% (w/v). Glycerol and sorbitol at 40% (w/w) of flour content were used as plasticizers. The mixture was stirred gently for 30 min at room temperature. The solution obtained was filtered through two layers of cheese-cloth to remove undissolved debris. The dissolved air in was removed by a vacuum pump (Diaphragm vacuum pump, Wertheim, Germany) for 30 min at room temperature.

2.3.2 Preparation of film

To prepare the films, the film-forming solution (10 g) was cast onto a plastic petri dish (90 × 15 mm^2) and air-blown for 12 h at room condition (27 ± 2 °C and 75 ± 10 % relative humidity (RH)). The films were further dried at 25 °C and 50 ± 5 % RH for 24 h in an environmental chamber (WTB Binder, Tuttlingen, Germany). The resulting films were manually peeled off and subjected to analyses.

2.3.3 Determination of film properties

Prior to testing of film thickness, mechanical properties and seal strength, films were conditioned for 48 h at 50 ± 5% relative humidity (RH) and 25 ± 0.5 °C. For scanning electron microscopy (SEM), films were conditioned in a desiccator containing dried silica gel for 2 weeks, followed by placing for 1 week in a desiccator containing P_2O_5 at room temperature (25-30 °C) to obtain the most dehydrated films.

2.3.3.1 Film thickness

The thickness of films was measured using a micrometer (Mitutoyo, Model ID-C112PM, Serial No. 00320, Mitutoyo Corp., Kawasaki-shi, Japan). Five random locations around each film of ten film samples were used for average thickness determination.

2.3.3.2 Mechanical properties

Tensile strength (TS) and elongation at break (EAB) were determined as described by Iwata et al. [15] with a slight modification using the texture analyzer (Model TA-XT2, Stable MicroSystems, Surrey, UK) equipped with load cell of 50 kg. Ten film samples (20 × 30 mm^2) with initial grip length of 30 mm were used for testing. Cross-head speed was set at 30 mm/min.

2.3.3.3 Seal strength

Film samples were cut into strips of 25 × 20 mm^2. One strip was placed on the top of another. Those two strips were heat-sealed using impulse sealer with magnet Model ME-300HIM (S.N.MARK Ltd., Park, Nonthaburi, Thailand) at 150 ± 0.5 °C for 1.50 s of heating time and 1.50 s of cooling time. The width of seal area was 2 mm.

All sealed film samples were conditioned at 25 ± 0.5 °C and 50 ± 5% relative humidity (RH) for 48 h before testing seal strength. The heat-seal strength was estimated using the peel tests. The peel strength of the heat-sealed films was determined according to Standard ASTM F-88 (ASTM., 2001) with slight modifications, using the texture analyzer (Model TA-XT2, Stable MicroSystems, Surrey, UK) equipped with load cell of 50 kg at 25 ± 0.5 °C and 50 ± 5% RH. Each leg of the sealed film was clamped to the machine, in which each end of the sealed film was held perpendicularly to the direction of the

pull. The distance between the clamps was 50 mm. A 100 N static load cell and cross-head speed of 30 mm/min were used. The maximum force required to cause seal failure was reported as seal strength in newtons/meter (N/m). Ten specimens of each sample were used for testing. Seal strength and seal efficiency were calculated as follows: (see Equation (1))

$$\text{Seal strength} = \text{Peak force/film width} \tag{1}$$

2.3.3.4 Water vapor permeability (WVP) and moisture content (MC)

WVP was measured using a modified ASTM method (ASTM, 1989) as described by Shiku et al. [16]. The film was sealed on an aluminum permeation cup containing dried silica gel (0 % RH) with silicone vacuum grease and a rubber gasket to hold the film in place. The cup was placed in a desiccator containing the distilled water at 30 °C. The cup was weighed at 1 h intervals over a 10 h period. WVP of the film was calculated as follows: (see Equation (2))

$$WVP\ (\mathrm{gm^{-1}s^{-1}Pa^{-1}}) = wlA^{-1}t^{-1}(P_2 - P_1)^{-1} \tag{2}$$

where w is the weight gain of the cup (g); l is the film thickness (m); A is the exposed area of film (m^2); t is the time of gain (s); $P_2 - P_1$ is the vapor pressure difference across the film (Pa).

The moisture content (MC) of film was determined by calculating the mass loss of the samples (20 × 50 mm^2) after drying in an oven (Memmert, Model UM 500, Schwabach, Germany) at 105°C for 24 h [17].

2.3.3.5 Film solubility

Film solubility was determined according to the method of Gennadios et al. [18]. The conditioned film samples (20 × 40 mm^2) were weighed and placed in a 50 mL centrifuge tube containing 10 mL of distilled water with 0.1% (w/v) sodium azide. The mixture was shaken at a speed of 250 rpm using a shaker (Heidolth Inkubator 10000, Schwabach, Germany) at 30 °C for 24 h. Undissolved debris was removed by centrifugation at 3000 g for 20 min using a centrifuge (model J-E Avanti, Beckman Coulter, Inc., Palo Alto, CA, USA). The pellet was dried at 105 °C for 24 h and weighed. The weight of solubilized dry matter was calculated by subtracting the weight of insolubilized dry matter from the initial weight of dry matter and expressed as a percentage of the total weight.

2.3.3.6 Color

Film samples were subjected to color measurement using a CIE colorimeter (Hunter associates laboratory, Inc., VA, USA). D65 (day light) and a measure cell with opening of 30 mm was used. The color of the films was expressed as L^*-value (lightness), a^*-value (redness/greenness) b^*-value (yellowness/blueness) and total difference of color (ΔE^*). The color parameters, L^*, a^* and b^*-values of the white standard are 93.78, −0.87 and 0.35, respectively.

2.3.3.7 Scanning electron microscopy (SEM)

Morphology of surface and cross-section of film samples were visualized using a scanning electron microscope (SEM) (Quanta 400, FEI, Eindhoven, the Netherlands). For cross-section of film, samples were fractured under liquid nitrogen prior to visualization. Then, the samples were mounted on bronze stub and sputtered with gold (Sputter coater SPI-Module, West Chester, PA, USA) in order to make the sample conductive. The photographs were taken at an acceleration voltage of 15 kV.

2.4 Statistical analysis

All experiments were run in triplicate with different three lots of films. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out by Duncan's multiple range test. Analysis was performed using the SPSS package (SPSS for windows, SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1 Thickness and mechanical properties

Thickness and mechanical of films based on Kluai Hin (KH) and Kluai Kha Nun (KKN) flours plasticized with glycerol and sorbitol is shown in Table 1. Film from KH flour plasticized with sorbitol had higher thickness than those added with glycerol ($p<0.05$). However, films from KKN flour plasticized with glycerol and sorbitol had no difference in thickness ($p>0.05$). When the same type of plasticizer was used, films prepared with KKN flour had lower thickness than those prepared with KH flour ($p<0.05$). The difference in thickness between films from both flours might be due to the differences in chemical compositions of flours, which determined the ordered alignment of starch chain forming the film network. KKN flour had higher starch content but lower protein content than KH flour, might be more prone to interaction and alignment of starch molecules in film matrix with higher degree of compactness. The higher bulky structure of film from KH flour was formed, in comparison with film from KKN flour and higher protrusion of film network was obtained. Among all films, the highest tensile strength (TS) and elongation at break (EAB) was observed in film prepared from KKN flour plasticized with sorbitol and glycerol ($p<0.05$), respectively. Films from both KH and KKN flours plasticized with sorbitol exhibited higher TS than those plasticized with glycerol ($p<0.05$). However, no difference in TS of films from both flours was observed ($p>0.05$), when the same plasticizer was used. Higher TS of edible films is generally necessary in order to withstand the normal stress encountered during their application. Films plasticized with glycerol had higher EAB than those used with sorbitol, regardless of types of banana flours used ($p<0.05$). When glycerol was used as plasticizer, EAB of film from KKN flour was higher than those from KH flour ($p<0.05$). Nevertheless, films plasticized with sorbitol had no difference in EAB between those prepared from KH and KKN flours ($p>0.05$). The lower EAB reflected the decreased flexibility. Generally, flexibility of edible films should be adjusted according to the intended application of film.

This result suggested that the difference in molecular weight of glycerol and sorbitol had the influence on mechanical properties of banana flour-based film. The molecular structure of glycerol is smaller than sorbitol, as indicated by the molecular weight of glycerol and sorbitol are 92 and 182 g/mol, respectively. Glycerol can more easier penetrate into biopolymer matrix, thereby increasing the volume of empty spaces between the chains of molecules and causing a decrease in the strength of intermolecular forces along the matrix [2, 19]. Moreover, the smaller size of hydrophilic plasticizer could disrupt intra- and inter-molecular interactions of starch, leading to the decreased amount of hydrogen bound in the starch chains and increased chain mobility of starch in film matrix. It was noted that glycerol might be more plasticizing effect than sorbitol as evidenced the lower TS and higher EAB of resulting films. This result was in accordance with Orsuwan and Sothornvit [3] who reported that sorbitol yielded the films with higher TS than those using glycerol or mixture (glycerol: sorbitol at ratio 1 : 1) as plasticizer, when both banana flour and starch from Kluai Namwa were used as film forming material.

3.2 Heat sealing properties

Heat sealing properties of films based on Kluai Hin (KH) and Kluai Kha Nun (KKN) flours plasticized with glycerol and sorbitol is shown in Table 1. Seal strength of films plasticized with sorbitol was higher than those used with glycerol, regardless of types of banana flours ($p<0.05$). When the same type plasticizer was used, film prepared with KKN flour had higher seal strength than those prepared with KH flour ($p<0.05$). KKN flour film plasticized sorbitol showed the highest seal strength than others ($p<0.05$). This might be due to the stronger seal, which was in agreement with higher TS of film from KKN flour plasticized sorbitol. During sealing, plasticized banana flour-based film could form the molecular interdiffusion and stabilized mainly by hydrogen bond. This result suggested that the higher hydroxyl groups in sorbitol (6-OH groups) could more interact with starch molecule in film matrix via hydrogen bond than glycerol (3-OH groups). Plasticizer such as glycerol or sorbitol could act as heat

sealing promoters, thereby forming an adhesive force [20]. Orsuwan and Sothornvit [3] also reported that sorbitol which was used as plasticizer showed the highest significant influence on seal strength of banana flour film or banana starch film, followed by mixed plasticizer (glycerol : sorbitol) and glycerol, respectively. Mode of failure for peel test of those films is summarized in Table 1. There were more than one failure modes occurred for films from various types of banana flours and plasticizers. The quality of heat sealing correlated with mode of failure. For film prepared with KH flour as film forming material, the adhesive seal failure was found for films plasticized with both glycerol and sorbitol. This indicated that the incomplete fusion of starch molecules at seal area was generated and the weaker of seal was developed. This result was associated with the lowest seal strength of films, especially for KH flour film plasticized with glycerol. However, KKN flour film plasticized with glycerol exhibited adhesive seal failure or cohesive seal failure, while either the cohesive seal failure was observed when sorbitol was used as a plasticizer. It was found that types of banana flours affected heat sealability of film differently. This might be due to the different characteristics of flours from two different banana varieties. The plasticizer also directly affected the mode of failure of films. This result indicated that films plasticized with sorbitol had stronger seal than those using glycerol, irrespective of banana flour. Thus, types of plasticizers in banana flour films from two different banana varieties directly determined mechanical and heat sealing properties of resulting films.

Table 1. Thickness, mechanical properties, seal strength and mode of failure of films based on Kluai Hin and Kluai Kha Nun flours plasticized with glycerol and sorbitol.

Varieties	Plasticizers	Thickness (mm)	TS (MPa)	EAB (%)	Seal strength (N/m)	Mode of failure
KH	Glycerol	0.089 ± 0.001^{b*}	2.40 ± 0.19^{b}	14.03 ± 0.61^{b}	39.96 ± 4.46^{c}	I
	Sorbitol	0.091 ± 0.001^{a}	8.55 ± 1.00^{a}	4.95 ± 0.72^{c}	68.03 ± 17.52^{b}	I
KKN	Glycerol	0.086 ± 0.002^{c}	2.46 ± 0.04^{b}	26.82 ± 1.34^{a}	66.88 ± 4.87^{b}	I/II
	Sorbitol	0.087 ± 0.001^{c}	9.18 ± 0.75^{a}	5.14 ± 0.84^{c}	150.85 ± 26.26^{a}	II

*Mean ± SD (N=3). The different superscript lowercase letters in the same column indicate the significant differences ($p<0.05$).
KH = Kluai Hin; KKN Kluai Kha Nun.
(I) Adhesive seal failure; (II) Cohesive seal failure; (III) Failure at seal edge; (IV) Failure at film body.

3.3 Water vapor permeability and moisture content

WVP of films based on Kluai Hin (KH) and Kluai Kha Nun (KKN) flours plasticized with glycerol and sorbitol is shown in Table 2. In general, films prepared from both KH and KKN flours plasticized with sorbitol had lower WVP than those added with glycerol ($p<0.05$). For films added with the same plasticizer, those prepared from KKN flour had a lower WVP than those prepared from KH flour ($p<0.05$). This might be due to the different chemical compositions between KH and KKN flours, particularly in protein content of flours. The increasing amount of hydrophilic substance such as proteins more likely increased the hydrophilicity of film [1]. As a result, the adsorption and permeation of water vapor through the films prepared from KKN flour was lowered. This might be associated with the lower protein content of KKN flour (2.78 %), compared with KH flour (3.42%). The lowest WVP was observed in film prepared from KKH flour and using sorbitol as plasticizer ($p<0.05$). It was noted that KKN flour and sorbitol yielded the film with higher barrier property against water vapor than KH flour and glycerol, respectively. This result indicated the alignment of film structure, which varied, depending on banana flours and plasticizers used. The different hydrophilic plasticizers (glycerol and sorbitol) distributed and interacted with starch molecules in film structure in different ways, thereby providing the different film networks. Moreover, the higher plasticizing effect of glycerol in film

network might be more reduced the interaction between polymer chains and increased an empty space between the chains of molecules in film matrix, in which decreased in barrier property to water vapor was developed. Orsuwan and Sothornvit [3] who reported that the banana flour/starch-based film from Kluai Namwa showed the lower WVP when sorbitol was used as plasticizer, in comparison which those used other plasticizers. Thus, the using of plasticizer, particularly from sorbitol, could enhance water vapor barrier property of banana flour-based edible film.

Moisture content of all film sample is shown in Table 2. For banana flour films prepared using sorbitol as plasticizer, those from both KH and KKN flours had lower moisture content than those prepared using glycerol ($p<0.05$). Moreover, when comparing the effect of flour from different banana varieties on moisture content of resulting film, film prepared from KKN flour had the lower moisture content than those from KH flour when both glycerol and sorbitol were used as plasticizer ($p<0.05$).

Table 2. Water vapor permeability, film solubility and moisture content of films based on Kluai Hin and Kluai Kha Nun flours plasticized with glycerol and sorbitol

Varieties	Plasticizers	WVP ($\times10^{-11}$ gm^{-1} s^{-1} Pa^{-1})	Film solubility (%)	Moisture content (%)
KH	Glycerol	$2.91 \pm 0.07^{a*}$	21.40 ± 1.51^{c}	28.93 ± 0.32^{a}
	Sorbitol	1.63 ± 0.03^{c}	33.72 ± 0.76^{a}	11.41 ± 0.48^{c}
KKN	Glycerol	2.43 ± 0.08^{b}	17.57 ± 1.87^{d}	24.32 ± 0.36^{b}
	Sorbitol	1.10 ± 0.05^{d}	26.08 ± 1.19^{b}	9.71 ± 0.40^{d}

*Mean ± SD (N=3). The different superscript lowercase letters in the same column indicate the significant differences ($p<0.05$).
KH = Kluai Hin; KKN Kluai Kha Nun.

3.4 *Film solubility*

Film solubility of films based on Kluai Hin and Kluai Kha Nun flours plasticized with glycerol and sorbitol is presented in Table 2. The solubility of films from KH flour was higher than those of films from KKN flour for both glycerol and sorbitol as plasticizer ($p<0.05$). For both films prepared from KH and KKN flour, films added sorbitol as plasticizer had the higher solubility than those added with glycerol ($p<0.05$). The lowest film solubility was observed in film prepared from KKN flour plasticized with glycerol ($p<0.05$). It was noted that both banana flours and plasticizers had an impact on solubility of resulting films. As a result, the lower solubility of films plasticized with sorbitol when compared with glycerol might be associated the different interactions between them and starch chains. Due to the larger molecular size of sorbitol, it caused difficulty penetrates itself into biopolymer matrix. However, the smaller size of glycerol can easier increase the volume of empty spaces between the chains of molecules and might bind with film matrix to a higher extent. This result suggested that sorbitol in banana flour films was more leached out than glycerol from film matrix.

3.5 *Color*

Table 3 presents the color (L^*-, a^*-, b^*- and ΔE^*-values) of films based on Kluai Hin and Kluai Kha Nun flours plasticized with glycerol and sorbitol is presented in Table 3. KH flour had higher L^*- and b^*-values but lower a^*- *and* ΔE^*-values than KKN flour ($p<0.05$). Films prepared from KH flour showed the higher L^*- and b^*- values but lower a^*- *and* ΔE^*-values than those films from KKN flour, regardless of types of plasticizers ($p<0.05$). This result suggested that the color of banana flour directly affected the color of resulting film. However, when glycerol and sorbitol were used as plasticizer, no difference in L^*-, a^*- *and* ΔE^*-values was observed in films prepared from KH flour ($p>0.05$). Moreover, L^*-, b^*- *and* ΔE^*-values of films from KKN flour had no significant different when both plasticizers were used. This result suggested that type of plasticizer had no effect on color of resulting films. Photographs films from Kluai Hin and Kluai Kha Nun flours plasticized with glycerol and sorbitol are shown in Figure 1.

Table 3. Color of flours and films based on Kluai Hin and Kluai Kha Nun flours plasticized with glycerol and sorbitol

Varieties	Plasticizers	*L**	*a**	*b**	*ΔE**
KH	Glycerol	86.80 ± 0.03^{a*}	0.50 ± 0.04^{c}	10.63 ± 0.56^{a}	11.42 ± 0.48^{b}
	Sorbitol	86.83 ± 0.13^{a}	0.55 ± 0.04^{c}	10.23 ± 0.19^{b}	11.38 ± 0.21^{b}
KKN	Glycerol	85.44 ± 0.42^{b}	1.56 ± 0.14^{a}	9.44 ± 0.20^{c}	11.82 ± 0.31^{a}
	Sorbitol	85.61 ± 0.34^{b}	1.48 ± 0.05^{b}	9.23 ± 0.34^{c}	11.62 ± 0.47^{ab}
KH flour		84.40 ± 0.00^{A}	1.93 ± 0.00^{B}	12.57 ± 0.02^{A}	14.71 ± 0.01^{B}
KKN flour		80.31 ± 0.01^{B}	2.88 ± 0.00^{A}	10.07 ± 0.01^{B}	16.31 ± 0.01^{A}

*Mean ± SD (N=3). The different superscript lowercase letters in the same column indicate the significant differences ($p<0.05$). The different superscript uppercase letters in the same column indicate the significant differences ($p<0.05$).
KH = Kluai Hin; KKN Kluai Kha Nun.

Figure 1. Photographs of flours and films based on Kluai Hin (KH) and Kluai Kha Nun (KKN) flours plasticized with glycerol and sorbitol

3.6 Film morphology

SEM micrographs of the surface and freeze-fractured crosssection of films based on Kluai Hin and Kluai Kha Nun flours plasticized with glycerol and sorbitol are shown in Figure 2. The surface of films prepared from KKN flour were smoother and more homogeneous than those of films prepared from KH films, regardless of type of plasticizer. Films prepared with KH flour had rougher surface than those from KKN flour for both glycerol and sorbitol used. This results suggested that the different chemical composition between KH and KKN flours plausibly effected on film structure. Moreover, the different amount of insoluble matter or nonstarchy component in flours could also affect the microstructure of resulting film differently. The cross-section of films prepared from KKN flour were smoother and more compact than those found in films prepared from KH films for both plasticizers used. The similar cross-section between glycerol and sorbitol was observed, particularly from KKN flour. However, film from KH flour plasticized with glyecrol became rougher, compared with that

plasticized with sorbitol. As a result, type of banana flour had the marked impact on microstructure of films.

Varieties	Plasticizer			
	Glycerol		Sorbitol	
	Surface	Cross-section	Surface	Cross-section
KH				
KKN				

Figure 2. SEM micrographs of surface (1000x) and cross-section (800x) of films based on Kluai Hin (KH) and Kluai Kha Nun (KKN) flours plasticized with glycerol and sorbitol. In cross-sectional images, the upper surface represents the film surface exposed to the air during film casting.

4. CONCLUSIONS

Properties of banana flour-based film was influenced by Kluai Hin flour and Kluai Kha Nun flour as well as different types of plasticizers (glycerol and sorbitol). Different types of banana flours and plasticizers directly had the impact on mechanical properties of film. In general, glycerol yielded the film with higher EAB than sorbitol, while sorbitol enhanced the films with higher TS and seal strength than glycerol, regardless of types of banana flours used. Both Kluai Hin flour and Kluai Kha Nun flour films were heat sealable. The seal strength and mode of failure of film varied with the film compositions. Generally, Kluai Kha Nun flour yielded the film with higher seal strength than Kluai Hin flour for peel test, regardless of plasticizer types used. Sorbitol was an appropriated plasticizer, which potentially enhanced the heat sealing properties of banana flour films. Water barrier property was effectively improved when film was plasticized with sorbitol. However, glycerol used decreased the solubility of films for both Kluai Hin and Kluai Kha Nun flours. Therefore, use of appropriate banan flour and plasticizer could not only enhanced mechanical and heat sealing properties of banana flour film, but also provide water vapor barrier property for resulting films.

5. ACKNOWLEDGEMENTS

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Exploring Student Attitudes and Behaviors on Reducing Plastic Straw Usage in Srinakharinwirot University, Ongkharak

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ABSTRACT

Single-use plastics (SUP) are becoming a serious environmental issue. Plastic straws, a type of SUP, can be harmful to the environment and marine wildlife. In university settings, the use of plastic straws is primarily observed in coffee shops. Therefore, this study aims to explore students' attitudes and behaviors regarding the reduction of plastic straw usage when purchasing beverages at coffee shops at Srinakharinwirot University, Ongkharak. A questionnaire was distributed to students at Srinakharinwirot University, Ongkharak. Descriptive statistics and Multiple Correspondence Analysis (MCA) were employed to analyze the data. Fourth-year students constituted the majority of respondents. The predominant monthly expenditure on food and beverages exceeded 5,000 baht. The typical price range for purchasing a drink was between 50-60 baht for a cold beverage. Respondents indicated that using personal cups and buying from coffee shops that do not provide plastic straws, especially those offering promotions, are the most effective strategies for reducing plastic straw usage in their daily routines. Nevertheless, the primary outcome of the current waste management system for existing plastic cups and straws was waste segregation. Motivating factors that could encourage reduced plastic straw consumption at coffee shops included convenience (such as using sip lids or paper straws) and environmental concerns (such as educating consumers about the environmental impact of plastic straws). The MCA analysis results revealed the identification of two distinct groups of respondents with different attitudes and behaviors. Tailored approaches that align with the attitudes and behaviors of each group should be implemented.

1. INTRODUCTION

Single-use plastics (SUP) are becoming a serious environmental issue, especially during and after the COVID-19 period [1]. In 2018, 1.91 million metric tons (MMT) of SUP were estimated in Thailand [2]. The amount increased to 2.15 MMT in 2019. Drinking straws, a type of SUP, are commonly

used in food packaging, particularly in the beverage, restaurant, and cafeteria industries.

Despite being lightweight, this characteristic also makes it challenging for waste segregation and management. A study revealed that 9% of plastic straws (by pieces) were among the plastic wastes collected along Mandvi Beach in India [3]. Plastic straws pose a significant threat to marine life due to their small size, which can be mistaken for food by birds, fish, and sea creatures, leading to entanglement, ingestion, and potential death.

There are at least three approaches that can be used to reduce the usage of plastic straws, including government policies [2], the adoption of alternative straws (e.g., paper, seaweed, rice, pasta straws, or sip lids) [4], and interventions to change consumer behavior [5]. Although changing consumer behavior may be the least likely to succeed in reducing plastic straw usage, promoting sustainability through this approach can be implemented by environmentally-conscious coffee shop owners. Understanding the factors that influence consumer behavior and preferences is crucial for successful implementation. For example, initiatives and norms in Pakistan's universities have influenced students' intentions to use reusable cups [6]. In rural areas, the reduction of SUP usage is mainly influenced by morality, whereas in urban areas, it is driven by rationality [7]. Students with a better understanding of the environmental impact of plastic packaging exhibit more environmentally friendly behavior [8]. Interventions like separating drinking straws from cups by default have been shown to decrease straw consumption [9].

University coffee shops heavily depend on students as a major consumer base. In Thailand's warm climate, students often prefer cold beverages served in PET plastic cups with lids and plastic straws at these coffee shops. While there is a push to replace plastic straws with paper ones to reduce environmental impact, it is essential to note that paper straws can degrade when submerged in beverages for extended periods, potentially leading to increased operational costs for shop owners. Encouraging the use of personal cups could be an effective strategy to reduce single-use plastic consumption. However, practical challenges such as carrying personal cups while attending classes or commuting may impede its implementation. This study aims to explore students' attitudes and behaviors regarding the reduction in plastic straw usage when purchasing beverages at coffee shops at Srinakharinwirot University, Ongkharak. The research utilized a Google Forms questionnaire to gather insights that can inform efforts to reduce plastic straw usage at coffee shops in confined areas, such as university campuses.

2. MATERIAL AND METHODS

2.1 Sampling

The sample used in this study was collected through purposive sampling, consisting of 401 undergraduate students enrolled in various faculties at Srinakharinwirot University, Ongkharak, although 385 participants would have been sufficient. The sample size was determined using Taro Yamane's formula [10], with a 95% confidence level and a 5% margin of allowable error.

2.2 Questionnaire and Survey

A survey was designed to explore the attitudes and behaviors of respondents regarding reducing plastic straws usage at Srinakharinwirot University, Ongkharak. The survey consisted of 13 questions spread across three sections:

Section 1: The initial part aimed to collect demographic details about the participants, such as gender, age, educational level, and monthly expenses on food and beverages.

Section 2: The second part focused on the beverage consumption habits of students at Srinakharinwirot University, Ongkharak. It covered topics including the usual price range spent on a drink and preferred drink types.

Section 3: The third part examined behaviors and attitudes toward purchasing cold beverages from coffee shops, the use of plastic straws, as well as recommendations for reducing the use of plastic

straws. This section delved into participants' practices of adopting personal cups, accepting plastic straws from coffee shops, their perceptions of the environmental impact of plastic straws, their use of plastic straws after knowing the impact on wildlife, strategies for reducing plastic straw usage in daily routines, motivating factors that could encourage reduced plastic straw consumption at coffee shops, and the current management of existing plastic cups and straws.

The survey was conducted using a Google Form and its data were nominal. To assess its accuracy, the Item-Objective Congruence (IOC) was used to evaluate by a panel of three experts. Each question was rated on a scale of -1 (irrelevant), 0 (inconclusive), or 1 (satisfactory) based on its alignment with the study objectives. Questions with an average IOC value exceeding 0.5 were considered suitable for inclusion, while those falling below the threshold underwent revision until meeting the criterion. Once the questionnaire achieved an IOC value above 0.5, it was distributed to students studying various disciplines at Srinakharinwirot University, Ongkharak.

2.3 Statistical analysis

The data collected through the questionnaire was subjected to analysis with R statistical language [11] version 4.3.3 in conjunction with the factominer and factoextra packages to process and interpret the gathered data utilizing descriptive statistics and Multiple Correspondence Analysis (MCA). MCA represents a form of multivariate statistical method that facilitates the graphical exploration of relationships among various categorical variables [12].

3. RESULTS AND DISCUSSION

3.1 Demographic data

A breakdown of respondents in Table 1 shows that they were grouped according to gender, with males accounting for 42.40%, females for 44.14%, and those with unspecified gender for 13.46% of the sample. The majority of respondents were 22 years and older, making up 48.40% of the total sample. In terms of education, the highest proportion of respondents were at the Year 4 level (35.65%), closely followed by Years 1, 2, and 3 with proportions ranging from 23.20% to 20.20%. When it comes to monthly expenditure, the data indicates that the largest portion of the budget was allocated to food and beverages, with 45.14% of respondents spending over 5,000 baht in this category. This was followed by expenditures of 3,001-5,000 baht (30.67%), 2,001-3,000 baht (16.71%), and 2,000 baht and below (7.48%), respectively.

Table 10. Demographic details of participants

Questions and answer choices	Frequency	Percentage
Gender		
Male	170	42.40%
Female	177	44.14%
Gender not specified	54	13.46%
Age		
Under 18 year old	5	1.24%
18 year old	12	3.00%
19 year old	44	10.97%
20 year old	62	15.46%
21 year old	84	20.93%
22 year old	95	23.70%
Over 22 year old	99	24.70%
Educational level		
1st year	93	23.20%
2nd year	81	20.20%

3rd year	84	20.95%
4th year and over	143	35.65%
Monthly expenses on food and beverages		
2,000 baht and under	30	7.48%
between 2,001 and 3,000 baht	67	16.71%
between 3,001 and 5,000 baht	123	30.67%
more than 5,000 baht	181	45.14%

3.2 Beverage consumption habit

The data from Table 2 reveals that the most common expenditure range for beverages among respondents was between 50 to 60 baht, accounting for the largest proportion at 43.39%. Following this, the expenditure range of 65 to 75 baht represented 21.45% of the total, while the range of 35 to 45 baht accounted for 19.70% of respondents. Expenditure exceeding 75 baht was at 8.23%, and expenditure below 30 baht constituted 7.23% of the total. A large majority of respondents, totaling 88.78% (as depicted in Table 2), had a preference for cold beverages. This strong preference for cold drinks is expected due to the hot climate in Thailand. It also led to a significant use of plastic straws.

Table 2. Beverage consumption habits of participants

Questions and answer choices	Frequency	Percentage
The usual price range spent on a drink		
30 baht and under	29	7.23%
between 35 and 45 baht	79	19.70%
between 50 and 60 baht	174	43.39%
between 65 and 75 baht	86	21.45%
more than 75 baht	33	8.23%
Preferred drink types		
hot	45	11.22%
cold	356	88.78%

3.3 Attitude and behavior toward plastic straw reduction

Table 3 displays the outcomes of a survey concerning strategic approaches to minimize the use of plastic straws, along with primary motivating factors and existing waste management methods. When participants were questioned about what strategies they would use to reduce plastic straw consumption in their daily activities, the most prevalent responses were using personal cups (32.23%) and coffee shops that do not provide plastic straws (32.23%). Other options included purchasing beverages from coffee shops that utilize sip lids instead of plastic straws (24.27%) and waste segregation (20.97%).

Regarding motivating factors for reducing plastic straw usage, respondents ranked campaigns aimed at educating consumers about the environmental impact of used plastic straws as the most significant (31.66%), followed by coffee shops adopting sip lids (26.64%), the use of paper straws (21.76%), and providing price reductions for customers who bring their own cups (19.94%).

The survey findings unveiled a contrast between existing waste management practices and the envisioned strategies for decreasing plastic straw usage. While waste segregation was the preferred current waste management practice, the adoption of personal cups and participation in coffee shop initiatives supporting straw reduction were the favoured strategies. A similar finding was reported that environmental concerns did not impact enhancing the use of reusable cups [6].

Furthermore, the survey emphasized that, although educating consumers on the effects of plastic straws on the environment was the highest motivating factor (31.66%), respondents exhibited a stronger preference for coffee shop practices such as providing sip lids (26.64%) and using paper straws (21.76%). The cumulative effect of these two factors exceeds the influence of the educating campaigns, implying that convenience might be another factor influencing respondents' behaviors in reducing plastic straw usage apart from individuals' environmental awareness. There was clear evidence that an increase in obstacles (a decrease in convenience) for consumers by separating straws from cups by default significantly reduced straw usage [9]. A similar outcome was also observed using the straw-upon-request-only ordinance strategy [13]. As the use of sip lids as well as paper straws in establishments does not raise complications, this may explain our results. In general, it is also possible that there are two groups of respondents, convenience and environmental concern-oriented.

Table 3. Strategies, motivating factors, and current waste management for reducing usage of plastic straws*

Questions and answer choices	Frequency	Percentage
Strategies for reducing plastic straw usage in daily routines		
waste segregation to enhance waste management	108	20.97%
the use of personal cups	166	32.23%
purchasing from a coffee shop using the sip lid	125	24.27%
purchasing from a coffee shop offering a promotion on not receiving plastic straw	116	32.23%
Motivating factors that could encourage reduced plastic straw consumption at coffee shops		
discounting from the use of personal cups	143	19.94%
educating consumers on the effects of plastic straws on the environment	227	31.66%
a coffee shop using the sip lid	191	26.64%
a coffee shop switched to paper straws	156	21.76%
The current waste management of existing plastic cups and straws		
waste segregation	232	36.48%
cleaning of plastic waste prior to discarding	119	18.71%
use personal cups	175	27.52%
use waste for DIY projects	110	15.72%

*Participants can select more than one answer in each question.

3.4 MCA analysis

Figure 1 shows the percentage of the total variance of the overall dimensions. There are seven dimensions that can be used to explain four variables, including the use of personal cups, the use of plastic straws for cold drinks, perception of the impact of plastic straws on environmental issues, and the use of plastic straws after known environmental impact, respectively. The main idea of this figure is not to show the influence of all the variables but to display the percentage of explained variance in every dimension. The first two dimensions account for 43.2% of the total variance (all dimensions = 100%). Although there are seven dimensions, only the first two dimensions were used for data exploration in this study.

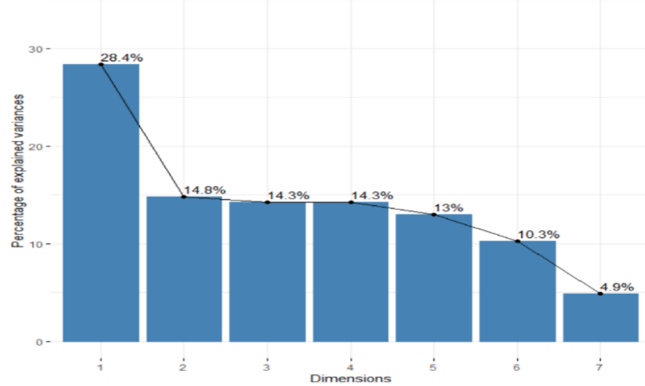

Figure 1. Percentage of explained variances of the 4 variables including the use of personal cups, the use of plastic straws for cold drinks, perception of the impact of plastic straws on environmental issues, and the use of plastic straws after a known environmental impact

Figure 2 illustrates the percentage of contribution for each variable categories on dimension 1 (Figure 2a) and dimension 2 (2b). Five variable categories that are of the most importance to dimension 1 (above a red dot line or a Kaiser line) include use plastic straw because of convenience, not use plastic straws because of environmental issues, use personal cups, use plastic straws after known (environmental issues), and do not use personal cups. Vary high impact, vary low impact, and medium impact are the most important to dimension 2. Variable categories that contribute the most to dimension 1 and dimension 2 are the most important in explaining the variability of this survey. It is important to note that there is no relationship between the perception of the impact of plastic straws on environmental issues (contributes the most to dimension 2) and the other three variables (the use of personal cups, the use of plastic straws for cold drinks, and the use of plastic straws after known environmental impact).

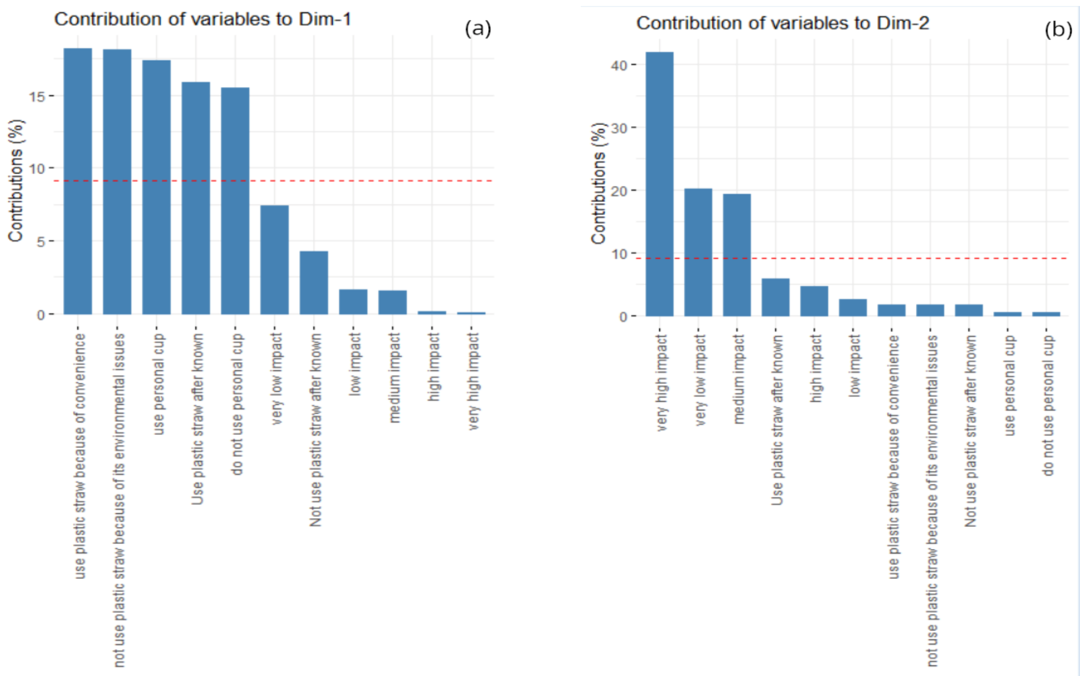

Figure 2. Percentage contribution of each variable categories on dimension 1 (a) and dimension 2 (b)

Figure 3 illustrates a square cosine graph representing the degree of association between variable categories and a particular dimension, as explained in the previous section (Figure 2). The more orange the letters, the stronger the association with the dimension. In addition, the variance obtained for dimension 1 was 28.4%, and 14.8% for dimension 2. The total inertia was 43.2%. The correlation between each variable category can be observed by their closed coordinates. Two groups of attitudes/behaviors can be clearly separated. The first group, located on the right-hand side of dimension 1 in the graph, shows correlations among those who do not use personal cups, use plastic straws after being aware of environmental issues, and use plastic straws for convenience. On the opposite side, the second group with a close relationship consists of those who use personal cups, do not use plastic straws due to environmental concerns, and do not use plastic straws after being aware of environmental issues. There was no relationship between the perception of plastic straw impacts on the environment (very high impact, high impact, medium impact, low impact, very low impact) and other variable categories as associated with dimension 2. The conclusion drawn from the results confirms findings in the previous section that the two groups of respondents exist: one more focused on environmental concerns and the other on convenience, each exhibiting different attitudes and behaviors.

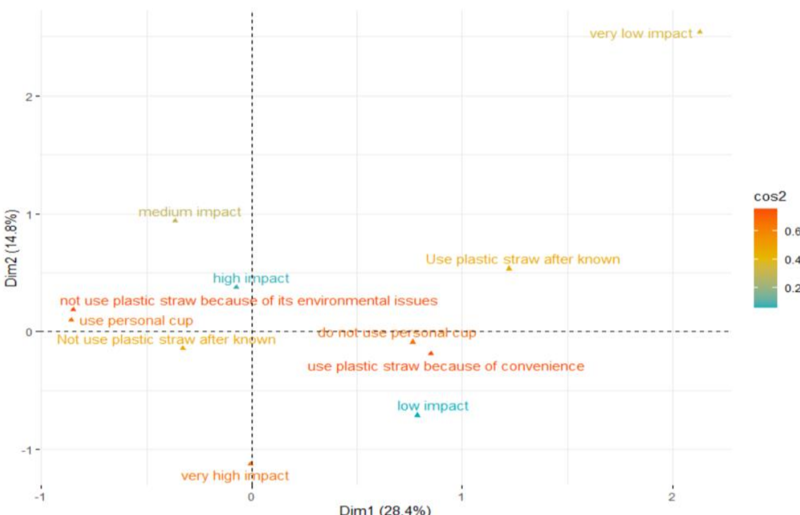

Figure 3. MCA plot of square cosine (cos2) of variable categories on dimension 1 (28.4%) and dimension 2 (14.8%) of MCA.

It was reported that environmental concerns and task-specific benefits were the keys to success, while lack of knowledge, lack of opportunity, inconvenience, and task difficulty were the barriers to plastic waste reduction [14]. Students with better knowledge about the negative impacts of plastic waste on the environment behave better in managing plastic waste [8]. In another research, having environmental concerns without environmental social norms and campaigns did not impact behavior [6]. In China, due to the plastic restriction ban policy, cultivating the environmental awareness of consumers increased the chance for the success of plastic waste reduction. This would lead to changes in retails as well as reduced policy costs [15]. Visualizing the consequences of marine life affected by plastic waste significantly reduced plastic waste [5]. Any interventions on plastic waste reduction contribute more or less. However, knowing the factors influencing their behavior and using appropriate implementation can enhance the success of plastic waste reduction. From our study, as there are two groups of respondents with differences in attitudes/behaviors, two different approaches for plastic straw reduction suitable for each group should be implemented. The use of sip lids or paper straws for the convenience group seems to be an appropriate approach. The implementation can help reduce the use of plastic straws in both groups automatically. Increasing campaigns on the effects of plastic straws on the environment and wildlife as well as the use of personal cups should be carried out in parallel. Apart from enforcing the impact of plastic straws on the environment and wildlife to the environmental concern group, it may also have some effects on another.

4. CONCLUSIONS

In conclusion, the fourth-year students were the predominant respondents. The high percentage of monthly expenses on food and beverages was more than 5,000 baht. The usual price range for purchasing a drink was typically between 50-60 baht for a cold drink. Respondents ranked the use of personal cups and purchasing from a coffee shop that offers promotions, rather than receiving plastic straws, as their best strategies for reducing plastic straw usage in their daily routines. However, the main result of the current waste management system for existing plastic cups and straws was waste segregation. Motivating factors that could encourage reduced plastic straw consumption at coffee shops appeared to stem from both conveniences, such as a coffee shop using sip lids or paper straws, and environmental concerns, like educating consumers on the effects of plastic straws on the environment. The results from the MCA analysis revealed that two groups of respondents with different attitudes and behaviors were classified. Different approaches that suit each group of students in reducing plastic straw usage at the Srinakharinwirot University, Ongkharak should be implemented in parallel. This

includes using sip lids or paper straws and launching campaigns on the effects of plastic straws on the environment and wildlife. Despite the fact that the university has the ability to enforce regulations that mandate coffee shops to utilize environmentally-friendly product like paper cups or paper straws, implementing such measures is likely to lead to increased costs for business owners without effectively promoting environmental conservation awareness among students. Promoting environmental stewardship among students is anticipated to generate significant societal benefits in the future.

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