PSU Prince of Songkla University | PSU Agro-Industry | PSU Science | Universiti Kebangsaan Malaysia

2024

ISSTUP

ABSTRACTS and PROCEEDINGS

Food and Science for Sustainable Future

The 2nd International Symposium on Science & Technology UKM-PSU 2024

Conference Date
May 3, 2024

The 2nd International Symposium on Science & Technology

UKM-PSU 2024

"Food and Science for Sustainable Future"

May 3, 2024 Crystal Hotel Hat Yai ,Thailand

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CONFERENCE PROGRAM

May 3, 2024	
Time	Program
08.00 - 09.00 am	**Registration Room: Crystal Grand Ballroom (1st floor)** (Note: For poster presentation, all the posters must be installed at the allotted place before 8:45 am)
09.00 - 09.15 am	**Opening Ceremony** by Asst. Prof. Dr. Thakerng Wongsirichot, Vice President for Academic and International Affairs, PSU, Thailand
09.15 - 09.45 am	**Plenary Speaker 1** : Assoc. Prof. Sunton Wongsiri, M.D. Vice President for Research and Innovation,Prince of Songkla University (PSU), Thailand **Title** "Driving Sustainable Development: PSU's Vision and Research Strategy"
09.45 – 10.15 am	**Plenary Speaker 2** : Assoc. Prof. Ts. Dr. Lim Seng Joe Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM), Malaysia **Title** "Functional Food: An ASEAN perspective"
10.15 - 10.45 am	**Poster Session 1** **Room:** Crystal Grand Ballroom (1st floor)
10.45 am - 12.15 pm	**Roundtable discussion** : Sirirat Room (3rd floor)
10.45 - 11.15 am	**Keynote Speaker 1 Session: Food and Nutrition** **Room:** Sorrapong Room (3rd floor) **Prof. Dr. Soottawat Benjakul** **Title "** Exploitation Of Seafood Processing Byproducts As Functional Ingredients, Nutraceuticals And Food Additives**"**
	Keynote Speaker 1 Physical and Computational Science/Life Science **Room:** Channapa Room (3rd floor) **Asst. Prof. Dr. Pruet Kalasuwan** **Title "** Quantum technology, a cutting-edge technology derived from a 125-year-old theory, in the context of Thailand and PSU.**"**
	Keynote Speaker 1 Session: Life Science **Room:** Chanmanee Room (3rd floor) **Assoc. Prof. Dr. Mohd Shazrul Fazry Sa`ariwijaya** **Title "** Current progress on herbal research on solving modern metabolic diseases**"**

Time	Program
11.15 am - 12.15 pm	**Oral Session 1** **Oral Session: Food and Nutrition** **Room:** Sorrapong Room (3rd floor) **Oral Session: Session: Food and Nutrition /Life Science** **Room:** Channapa Room (3rd floor) **Oral Session: Life Science** **Room:** Chanmanee Room (3rd floor)
12.15 - 01.15 pm	**Lunch** (2nd floor)**/ Muslim prayer time** (3rd floor)
01.15 - 01.45 pm	**Poster Session 2** **Room:** Crystal Grand Ballroom (1st floor)
01.45 - 03.30 pm	**Oral Session 2** **Oral Session: Food and Nutrition** **Room:** Sorrapong Room (3rd floor) **Oral Session: Session: Food and Nutrition /Life Science** **Room:** Channapa Room (3rd floor) **Oral Session: Life Science** **Room:** Chanmanee Room (3rd floor)
03.15 - 03.30 pm	**Keynote Speaker 2 Session: Food and Nutrition** **Room:** Sorrapong Room (3rd floor) **Dr. Hafeedza Abdul Rahman** **Title "** Decoding the key phytochemical compounds and anti-obesity mechanisms of selected Malaysian herbs**"**
03.30 - 04.00 pm	**Keynote Speaker 2 Session: Food and Nutrition /Life Science** **Room:** Channapa Room (3rd floor) **Prof. ChM. Dr. Ishak Ahmad** **Title "** Bio-Nanocomposite Film as Active and Intelligent Food Packaging**"**
	Keynote Speaker 2 Session: Life Science **Room:** Chanmanee Room (3rd floor) **Assoc. Prof. Dr. Ekkasit Kumarnsit** **Title** "Screening of kratom plant extract potential for further development and therapeutic use"

Time	Program
04.00 – 04.15 pm	Session break
04.15 – 05.00 pm	Awards and Closing Ceremony **Room:** Crystal Grand Ballroom (1st floor)

ORAL PRESENTATION PROGRAM

SESSION: Food and Nutrition

Time	Code	Title
Room: Sorrapong Room (3rd floor)		
11.15 - 11.30 am	OF1 : ID60	EFFECT OF *Moringa oleifera OLEIFERA* LEAVES EXTRACT ON COGNITIVE FUNCTION IN LIPOPOLYSACCHARIDE (LPS)-INDUCED RATS : Dr.Hamizah Shahirah Hamezah
11.30 - 11.45 am	OF13 : ID24	EFFECT OF FREEZE-THAW CYCLES ON QUALITY CHANGES IN BREAST AND LEG MEAT FROM SPENT DUCKS : Miss.Pitchaporn Ungkusonmongkol
11.45 - 12.00 am	OF3 : ID54	UTILIZATION OF BY-PRODUCT OF PROCESSED-SHRIMP UTILIZATION FOR UNMISSED FOOD CONDIMENT, KAPI AND ITS PROCESSED IMPROVEMENT : Mr.Chanonkarn Rujirapong
12.00 - 12.15 am	OF4 : ID65	DEVELOPMENT OF PROTEIN FORTIFIED PLANT ICE CREAM BASED ON SOY AND PEANUT MILK FOR THAI ELDERLY : Mr.Nattakorn Promvikorn
01.45 - 02.00 pm	OF5 : ID1	CHARACTERISATION OF SACHA INCHI OIL MICROCAPSULE OBTAINED VIA COMPLEX COARCEVATION : Dr.Noor Soffalina Sofian Seng
01.45 - 02.15 pm	OF6 : ID2	EFFECT OF DEHYDRATION ON THE PHYSICOCHEMICAL AND ANTIOXIDANT PROPERTIES OF STINGLESS BEE HONEY : Dr.Arnida Hani The.
02.15 - 02.30 pm	OF7 : ID6	MULTIFUNCTIONAL GELATIN FILM INTEGRATED WITH CHITOSAN CARBON DOTS AND BUTTERFLY-PEA FLOWER ANTHOCYANINS: PROPERTIES AND BIOACTIVITIES : Mr.Arunachalasivamani Ponnusamy
02.30 - 02.45 pm	OF9: ID 50	IMPROVEMENT FLAVOR OF TEA FROM GNETUM GNEMON USING DIFFERENT PRETREATMENTS AND ADDING PANDANUS POWDER AND CHRYSANTHEMUM POWDER : Miss.Rungnapa Anankarnkit
02.45 – 03.00 pm	OF9 : ID22	EFFECTS OF SOURSOP LEAF EXTRACT, PULSED ELECTRIC FIELD AND VACUUM IMPREGNATION ON MELANOSIS INHIBITION AND SHELF-LIFE EXTENSION OF REFRIGERATED SHRIMP : Mr.Abubakar Ahmad
03.00- 03.15 pm	OF12 : ID49	OPTIMIZATION OF EXTRACTION IN TORCH GINGER INFLORESCENCE WITH A BOX-BEHNKEN DESIGN : Miss.Amila Firdhauzi

SESSION: Physical and Computational Science/Life Science

Time	Code	Title
Room: Channapa Room (3rd floor)		
11.15 - 11.30 am	OL5 : ID3	ANTIDIABETIC POLYSACCHARIDES FROM VARIOUS MALAYSIAN HERBS : Dr.Babul Airianah Othman
11.30 - 11.45 am	OL6 : ID11	CELLULOSE BASED HYDROGEL FOR PLANT GROWING MEDIA : Prof. Dr.Sarani Zakaria
11.45 - 12.00 am	OL7 : ID62	EFFECT OF SYNTHETIC CANNABINOIDS ON β-GLUCAN-INDUCED TRAINED IMMUNITY IN MACROPHAGES : Miss.Intan Mukti Pratiwi
12.00 - 12.15 am	OL8 : ID58	CHITIN EXTRACTION FROM SQUID PEN WASTE THROUGH LACTIC ACID FERMENTATION AND POSTTREATMENT WITH ALKALINE PROTEASE : Miss Jariya Ruangwicha

SESSION: Food and Nutrition

Time	Code	Title
Room: Channapa Room (3rd floor)		
01.45 - 02.00 pm	OF14 : ID16	DEBITTERING OF PROTEIN HYDROLYSATE AND PLASTEIN FROM SALMON FRAME USING MAILLARD REACTION : Mr.Kartik Sharma
01.45 - 02.15 pm	OF15 : ID17	PREPARATION AND CHARACTERIZATION OF PROTIEN HYDROLYSATE FROM ULTRASONICATED SHRIMP SHELL PROTIEN ISOLATE : Miss.Akanksha Rajeev Gautam.
02.15 - 02.30 pm	OF16 : ID20	PROPERTIES OF FILM BASED ON CO-PRECIPITATED BAMBARA GROUNDNUT PROTEIN ISOLATE AND FISH SKIN ACID-SOLUBLE COLLAGEN : Mr.MD.Shihabul Awal
02.30 - 02.45 pm	OF17 : ID7	POTENTIAL APPLICATION OF POLY-LACTIC ACID (PLA) ANTIOXIDANT ACTIVE FILMS (*Alpinia mutica* LEAF EXTRACT) AS FOOD PACKAGING : Dr.Hamizah Shahirah Hamezah
02.45 – 03.00 pm	OF18 : ID10	SENSORY ACCEPTANCE AND GLYCEMIC INDEX OF SELECTED COMMERCIAL BREAKFAST BAR PRODUCTS : Dr.Zalifah M Kasim
03.00- 03.15 pm	OF19 : ID13	TEMPERATURE INFLUENCE ON BIOACTIVE COMPOUND EXTRACTION FROM MORINDA CITRIFOLIA VIA SUBCRITICAL WATER EXTRACTION : Dr.Nurfatimah Mohd Thani
03.15- 03.30 pm	OF20 : ID14	EVALUATION OF PHYSICOCHEMICAL PROPERTIES OF RED AND GREEN CHILLIES DURING POSTHARVERST STORAGE : Dr.Maimunah Mohd Ali

SESSION: Life Science

Time	Code	Title
Room: Chanmanee Room (3rd floor)		
11.15 - 11.30 am	OL1 : ID15	CASHEW LEAF EXTRACT: ANTIFUNGAL ACTIVITY AND APPLICATION FOR EXTENDING SHELF-LIFE OF DRIED SALTED FISH : Miss.Pitima Sinlapapanya
11.30 - 11.45 am	OL2 : ID41	IN SILICO AND IN VITRO STUDIES OF SELECTED FLAVONOIDS ON WOUNDHEALING AND ITS UNDERLYING MECHANISMS VIA WNT/**B**-CATENIN PATHWAY : Dr.Murni Nazira Sarian
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03.15- 03.30 pm	OL16 : ID61	EXPLORING THE ANTIOXIDANT CAPACITY OF RICE-DERIVED BIOACTIVE PEPTIDES IN *Saccharomyces cerevisiae* : Miss.Simran Kaur

POSTER PRESENTATION PROGRAM

SESSION 1 **Time:** 10.15 - 10.45 am

Room: Crystal Grand Ballroom (1st floor)

Code	Title
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PF1 : ID5	BONE DENSITY, PHYSICAL ACTIVITY AND CALCIUM INTAKE OF INDIAN CHILDREN FROM TWO SCHOOLS OF SELANGOR, MALAYSIA
PF2 : ID9	PROTEOLYTIC FERMENTATION OF EDIBLE BIRD'S NEST GLYCOPROTEINS FOR ENHANCED PHYSICOCHEMICAL PROPERTIES AND BIOACTIVITIES
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PF6 : ID32	UPSCALING THE PRODUCTION OF PROTEIN HYDROLYSATE FROM TUNA VISCERA FOR PETS
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PL8 : ID48	ANTIBACTERIAL EFFECT OF BLACK SOLDIER FLY LARVAE EXTRACTS AGAINST *V. CHOLERAE* AND *V. PARAHAEMOLYTICUS*
PL9 : ID53	BIODEGRADATION OF PHENOLIC COMPOUNDS IN PALM OIL MILL EFFLUENT BY WHITE ROT FUNGI

SESSION 2 **Time:** 01.15 - 01.45 pm
Room: Crystal Grand Ballroom (1st floor)

Code	Title
Food and Nutrition	
PF11 : ID51	DEVELOPMENT OF FISH SKIN GELATIN FILM INCORPORATED WITH EPIGALLOCATECHIN GALLATE PRODUCED BY THERMOCOMPRESSION MOLDING PROCESS
PF12: ID55	SENSORY ATTRIBUTES AND ANTIOXIDANT ACTIVITY OF MANGO AND CHIA SEEDS FORTIFIED JELLY
PF13 : ID63	TEXTURE SOFTENING OF ENZYME-TREATED CHICKEN MEAT FOR SENIORS WITH CHEWING DIFFICUTIES
PF14 : ID67	EFFECT OF SOLVENTS EXTRACTION ON PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY OF SOUTHERN THAILAND TORCH GINGER
PF15 : ID111	QUALITIES AND CHARACTERISTICS OF GREEN MUSSEL TREATED WITH ACID ELECTROLYZED WATER AND SUBJECTED TO HIGH-PRESSURE PROCESSING
PF14 : ID67	EFFECT OF SOLVENT EXTRACTION ON PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY OF TORCH GINGER FROM SOUTHERN THAILAND
PF15 : ID111	QUALITIES AND CHARACTERISTICS OF GREEN MUSSEL TREATED WITH ACID ELECTROLYZED WATER AND SUBJECTED TO HIGH-PRESSURE PROCESSING
PF16 : ID114	FUNTIONAL PROPERTIES OF MUNG BEAN-BROWN RICE EXTRUDED SNACKS
PF17 : ID115	DESALINATION OF FERMENTED FISH (BUDU) AND EFFECT ON PROBIOTIC MODULATION
PF18 : ID120	MECHANISM OF POLYSACCHARIDES FROM SPLIT GILL MUSHROOM (SCHIZOPHYLLUM COMMUNE) IN THE STIMULATION OF GLUCOSE UPTAKE IN MUSCLE CELLS
PF19 : ID121	METABOLOMICS APPROACH TO ASSESS METABOLITE PROFILE OF MODIFIED TEMPE AND EVALUATION OF ITS ANTIOXIDANT ACTIVITIES
PF20 : ID122	EFFECT OF *CLITORIA TERNATEA* FLOWER SUPPLEMENTATION ON THE PHENOLIC METABOLITE PROFILE AND ANTIOXIDANT ACTIVITY OF TEMPE
PF21 : ID123	COMPARATIVE ANALYSIS OF METABOLITE RELEASE FROM CONVENTIONAL AND ORGANIC TEMPE DURING *IN VITRO* DIGESTION, EXAMINING THEIR ANTIOXIDANT PROPERTIES
Life Science	
PL10 : ID57	WHOLE GENOME SEQUENCING REVEALS THE MUTATIONAL LANDSCAPE FROM DISEASE DIAGNOSIS TO RELAPSE IN PATIENTS WITH CHILDHOOD ACUTE MYELOID LEUKEMIA
PL11 : ID61	EXPLORING THE ANTIOXIDANT CAPACITY OF RICE-DERIVED BIOACTIVE PEPTIDES IN *SACCHAROMYCES CEREVISIAE*
PL12 : ID62	EFFECT OF SYNTHETIC CANNABINOIDS ON β -GLUCAN-INDUCED TRAINED IMMUNITY IN MACROPHAGES
PL13 : ID66	SCREENING OF SOME PROBIOTIC PROPERTIES OF YEASTS ISOLATED FROM THAI TRADITIONAL KOMBUCHA SAMPLES
PL14 : ID69	ANTIMICROBIAL POTENTIAL OF ENDOPHYTIC FUNGAL EXTRACTS FROM VEGETABLES AND MEDICINAL HERBS AGAINST FOODBORNE PATHOGENS
PL15 : ID116	EFFECTS OF MYCORRHIZAL FUNGI ON THE GROWTH OF DURIO ZIBETHINUS SEEDLINGS
PL16 : ID119	ISOLATION AND CHARACTERIZATION OF DOMINANT ACETIC ACID BACTERIA AND YEAST ISOLATED FROM KOMBUCHA SAMPLES
PL17 : ID133	OPTIMIZING PRODUCTION OF PROBIOTIC KOMBUCHA FROM COCOA HONEY USING RESPONSE SURFACE METHODOLOGY
PL18 : ID134	DEVELOPMENT OF PROBIOTIC KOMBUCHA FROM COCOA HONEY

OPENING REMARK

by Assistant Professor Dr. Thakerng Wongsirichot,

Vice President for Academic and International Affairs of Prince of Songkla University, Thailand.

Good morning ladies and gentlemen, distinguished invited speakers, honorable colleagues, and participants.

It is my distinct pleasure to welcome you to the opening ceremony of the 2nd International Symposium on Science and Technology UKM-PSU (ISSTUP2024).

First, may I thank each honorable speaker for taking part in this event. I also want to thank all committees for organizing this event.

With the theme of "Food and Science for Sustainable Future", you will have the chance to exchange opinions and come up with new, creative, and future-oriented suggestions for the mutual challenges. I believe what you earn here will surely benefit what you do in the future.

Therefore, it is my sincere hope that you seize the opportunity this symposium offers you and participate fully in this experience.

On behalf of the administration, PSU will continue to support this international symposium in the future.

Last but not least, I wish the 2nd International Symposium on Science and Technology UKM-PSU (ISSTUP2024) a great success.

Thank you.

Abstracts of Plenary Speakers and Keynotes Speakers

DRIVING SUSTAINABLE DEVELOPMENT: PSU'S VISION AND RESEARCH STRATEGY

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ABSTRACT

Prince of Songkla University (PSU), established in 1967, stands as a beacon of academic excellence and innovation in southern Thailand. Guided by its vision to spearhead sustainable development globally, PSU is committed to building academic and innovation leadership, fostering human resources development, and advancing research to address regional challenges and societal needs. Through interdisciplinary collaboration, PSU endeavors to drive innovation and create solutions that propel sustainable development forward. Embracing international collaboration and aligning research efforts with the UN Sustainable Development Goals, PSU seeks to bring global perspectives and expertise to local issues. The university's research flagship areas, spanning agriculture, food, medical and health, tourism, marine sciences, disaster management, language, peace, and creative economy, serve as pillars for knowledge creation and application. Leveraging a three-pronged approach of "Think, Create, and Serve," PSU strategically develops and disseminates research outcomes to address market demands and societal needs. Notable achievements include the internationally acclaimed works of distinguished researchers like Professor Dr. Soottawat Benjakul in seafood research, and recognition at prestigious research competitions such as the International Exhibition of Inventions Geneva. As PSU continues to support and promote research endeavors, it remains dedicated to driving sustainable development and societal advancement at the forefront of the world.

FUNCTIONAL FOOD: AN ASEAN PERSPECTIVE

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ABSTRACT

With a population exceeding 688 million, ASEAN comprises ten nations—Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Singapore, Thailand, and Vietnam. Timor-Leste is under consideration as the potential 11th member. The culinary landscape in ASEAN, shaped by diverse cultural influences, showcases an array of traditions and fusions, making food a central element of exploration in the region. Southeast Asia's historical role in the spice trade, dating back to 1500 BC and peaking in the 15th century, contributed to flourishing economies and empires. In the contemporary era, spice utilisation in local cuisines persists, adapting to current needs. The field of food science, technology, and nutrition in ASEAN is rapidly evolving to tackle global challenges like food security, sustainability, and public health. This dynamic context gives rise to novel advancements specifically tailored to the region's needs. Functional food, delivering added benefits beyond basic nutrition, has become a focus. Our research, spanning the last decade, centres on key Malaysian resources—seaweeds, edible bird's nests, and tropical fruit vinegars. Despite their distinct characteristics, our research follows similar objectives, employing innovative methods to develop functional food and ingredients. By studying physicochemical characteristics and bioactivities, particularly in brown seaweed-derived fucoidan, glycoproteins from edible bird's nests, and the fermentation of tropical fruit vinegars, our work contributes to both fundamental and applied research. The translational nature of our research has led to significant findings, facilitating the commercialisation of products and technologies.

EXPLOITATION OF SEAFOOD PROCESSING BYPRODUCTS AS FUNCTIONAL INGREDIENTS, NUTRACEUTICALS AND FOOD ADDITIVES

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ABSTRACT

Fish and shellfish have been very crucial income generator for Thailand. During processing, a large amount of byproducts, e.g. frame, skin, bone from fish and cephalothorax and shell from shrimp is generated. Proper discard and treatment are associated with high cost. Utilization of those byproducts by conversion to the marketable high-valued products has gained augmenting interest for seafood industry as well as nutraceutical market. Fish processing byproducts, especially fish skin can serve as potential raw material for production of hydrolyzed collagen (HC) rich in bioactive peptides. However, lowering fishy odor/flavor by pretreatment or removal of fat from HC is required. In addition, the liposome can be employed to load HC as the core, thus masking fishy odor/flavor. HC is able to induce skin nourishment and bone strengthening. Fish bone can be used to produce biocalcium with high solubility and bioavailability in simulated gastrointestinal tract. Biocalcium can be fortified in several foods as calcium supplement. Shrimp shell is basically converted to chitooligosaccharide (COS) via enzymatic or chemical reactions. However, redox pair reaction (H_2O_2 + ascorbic acid) has been shown as the potential method to produce COS than H_2O_2 alone. COS can be applied as natural food additive due to antioxidant and antimicrobial activities. COS could retain color of raw tuna slices because of its reducing power. In term of nutraceutical, COS could prevent fatty liver disease in Wistar rats. Furthermore, shrimp oil rich in astaxanthin and polyunsaturated fatty acids (PUFAs) can be extracted from either cephalothorax or hepatopancreas. Shrimp oil promoted cognitive function in chronic cerebral hypoperfusion (CCH) rats. It also prevented coronary artery disease in cell line study. Therefore, the manufacture of value-added products with bioactivities and nutraceutical properties can be a promising approach to gain the better benefit. Also, the marine resources can be better exploited with sustainability.

Keywords: Byproducts; utilization; functional ingredient; food additive; seafood

QUANTUM TECHNOLOGY, A CUTTING-EDGE TECHNOLOGY DERIVED FROM A 125-YEAR-OLD THEORY, IN THE CONTEXT OF THAILAND AND PSU.

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Abstract

Quantum physics was discovered by notable physicists at the beginning of the 20th century. It unveils the veracity of nature on a minuscule level and in a manner that contradicts common intuition. Despite presenting itself as the opposite of the common notion, it has been empirically demonstrated to exist in nature over the course of the century. By comprehending the properties of the electromagnetic spectrum and atomic structures, significant advancements in science and technology can be achieved. Furthermore, the practical utilization of quantum physics in fields such as NMR, laser science, and microelectronics offers numerous advantages. This emerging paradigm holds immense potential and is supported by substantial evidence. However, many quantum experts considered this evolution to be the first instance of quantum evolution, based on quantum technology. It encompasses the period from the early 1900s to just before the late 1900s. Thank you for the significant advancements in several scientific and technological fields. As the 21st century commenced, the quantum era ushered in a new level of influence, commonly referred to as the second generation of quantum technology. The primary enhancement of the second generation is the capacity to manipulate and implement individual quantum units. In the near future, we anticipate the emergence of second generation quantum technology, which will be characterized by more sensitive sensors, highly secure communication, and powerful computers.

In this presentation, I will provide an overview of the origins of quantum technology, its current advancements, and the potential implications it may have for humanity.

CURRENT PROGRESS ON NATURAL RESEARCH EXTRACTS ON SOLVING MODERN DISEASES

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ABSTRACT

Metabolic diseases, including obesity, type 2 diabetes, and cardiovascular disorders, continue to pose significant global health challenges. The escalating prevalence of these conditions necessitates innovative and sustainable approaches for prevention and management. In recent years, extracts from natural resources has emerged as a promising avenue, garnering attention for its potential in mitigating metabolic diseases. The exploration of natural remedies is rooted in the rich tradition of traditional medicine and has gained momentum with advancements in scientific methodologies. Researchers are actively investigating the bioactive compounds present in various herbs, fungus, microbes, plants and animals elucidating their mechanisms of action and potential therapeutic effects. Polyphenols, alkaloids, flavonoids, proteins and saccharides are among the key chemicals that were investigated under scrutiny, with studies highlighting, not limited to their anti-inflammatory, antioxidant, and insulin-sensitizing properties. Despite promising findings, challenges such as standardization of chemicals in extract, variability in bioavailability, limited long-term studies, such as toxicity and side effects, and clinical efficacies have impede these extracts market success. Collaborative efforts between traditional medicine practitioners, pharmacologists, and clinicians are imperative to bridge the gap between traditional knowledge and evidence-based medicine. In conclusion, as scientific understanding of the bioactive components and their mechanisms deepens, the integration of natural extracts interventions into mainstream healthcare may offer novel and effective strategies for the prevention and management of metabolic disorders.

Keywords: Natural extracts; metabolic diseases; modern diseases

DECODING THE KEY PHYTOCHEMICAL COMPOUNDS AND ANTI-OBESITY MECHANISMS OF SELECTED MALAYSIAN HERBS

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ABSTRACT

Malaysian herbs have garnered attention for their rich phytochemical content and potential health benefits including flavonoids, phenolic acids, stilbenes, and lignans. To unravel their potential, scientists are employing advanced analytical techniques like nuclear magnetic resonance (NMR) spectroscopy and liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify and characterise their key bioactive compounds. Furthermore, the study delves into elucidating the specific mechanisms through which these phytochemicals exert anti-obesity effects. This involves investigating their interactions with molecular targets involved in metabolic pathways associated with obesity, such as inhibition of pancreatic lipase and α-glucosidase enzymes, modulation of adipocyte differentiation, and regulation of lipid metabolism. The implications of these findings extend to both pharmaceutical and nutraceutical industries, offering valuable insights for developing natural-based anti-obesity interventions. Understanding the bioactive compounds and their mechanisms within Malaysian herbs can guide the formulation of novel therapeutic agents or dietary supplements targeting obesity and its associated health risks including diabetes and hypertension. Furthermore, this research highlights the relevance of traditional herbal medicine in addressing contemporary health challenges, emphasizing the importance of integrating traditional knowledge with modern scientific approaches.

Keywords: Anti-obesity; herbs; mechanism; secondary metabolites

SCREENING OF KRATOM PLANT EXTRACT POTENTIAL FOR FURTHER DEVELOPMENT AND THERAPEUTIC USE

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Kratom plant has been use in traditional medicine. It's claimed to have multiple therapeutic properties. However, scientific proof is needed for confirmation. Rats and mice are used in neuroscience research of kratom plant extract. Kratom leaves were collected from natural sources in southern parts of Thailand for extraction. The extract containing mitragynine, a major component, was used for the experiments. The results showed that kratom extract significantly reduced rat's body weight without an overeating rebound effect after cessation of kratom treatment. Moreover, kratom extract was found to significantly attenuate the severity of ethanol, morphine and methamphetamine withdrawal symptoms. A preliminary also showed that movement deficit and abrupt neural signaling in a mouse model of Parkinson's disease were successfully treated by kratom extract. A long term treatment of kratom extract for 19 weeks exhibited safety effects seen on biochemical and hematological parameters. Altogether, kratom plant extract appeared to have beneficial properties for further development to be used in clinical levels.

Keywords: Mitragyna speciosa korth, beneficial effects, drug dependence, safety test

Proceeding and Abstracts

EFFECT OF *Moringa oleifera* LEAVES EXTRACT ON COGNITIVE FUNCTION IN LIPOPOLYSACCHARIDE (LPS)-INDUCED RATS

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ABSTRACT

Neuroinflammation is one of the factors leading to neurodegenerative conditions including cognitive impairment and increased risk of Alzheimer's and Parkinson's diseases. Medicinal plants such as *Moringa oleifera* possess high polyphenolic content known to exert immunomodulatory effects for neuroinflammatory prevention. Hence, this study aims to investigate the effect of *M. oleifera* leaves extract on cognitive status and locomotor function in the lipopolysaccharide (LPS)-induced neuroinflammation in rats. In this study, Sprague Dawley rats were induced with LPS to develop neuroinflammatory conditions. The LPS-induced rats received oral administration of *M. oleifera* leaves extract (100, 200, and 400 mg/kg), ibuprofen (40 mg/kg), and 5% Tween-20 (vehicle control) for 28 days. Behavioral changes were evaluated by conducting open field, novel object recognition, and Morris water maze tests. *M. oleifera* leaves extract treatment showed slight improvement in the exploratory behavior. The *M. oleifera* leaves extract-treated rats showed lower escape latency ($p<0.05$) in the Morris water maze test compared to the non-treated group, indicating improved cognitive function. Hence, *M. oleifera* was able to alleviate the cognitive impairments in LPS-induced rats, suggesting the potential of *M. oleifera* leaves extract against neuroinflammatory conditions.

Keywords: Neurodegeneration, neuroinflammation, *Moringa oleifera*, polyphenol, memory

EFFECT OF FREEZE-THAW CYCLES ON QUALITY CHANGES IN BREAST AND LEG MEAT FROM SPENT DUCKS

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ABSTRACT

This study investigated the effect of multiple freeze-thaw cycles (0, 1, 3, and 5) on the chemical and physical properties of breast and leg meat derived from spent ducks. With increasing number of freeze-thaw cycles, both breast and leg meat showed a reduction in moisture content and solubility of sarcoplasmic proteins. Conversely, myofibril fragmentation index (MFI), TCA-soluble peptides content (TCA), Thiobarbituric acid reactive (TBARS), and protein oxidation levels increased with repeated cycles. Total protein solubilities and pH increased during the third freeze-thaw cycle but declined at fifth. Metmyoglobin content in breast and leg meat initially increased after the first cycle, followed by a slightly rise up to the fifth cycle. The color a* value significantly decreased, while L* and b* values showed an upward trend with increasing freeze-thaw cycles. Thawing loss experienced a dramatic increase, and cooking loss and shear force initially rose after the first cycle but declined after the third cycle. In conclusion, the freeze-thaw process had a substantial impact on the quality of spent duck meat.

Keywords: Freeze-Thaw, Spent duck, Meat quality, Chemical properties, Physical properties.

UTILIZATION OF BY-PRODUCT FROM PROCESSED-SHRIMP FOR THAI ESSENTIAL FOOD CONDIMENT, KAPI AND ITS PROCESS IMPROVEMENT

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ABSTRACT

Generally, shrimp processing generates by-products around 50% of total volume. All by-products were not well utilized and often caused burden management, despite shrimp head and shell containing astaxanthin, polyunsaturated fatty acid (eicosapentaenoic acid; EPA and docosahexaenoic acid; DHA) giving various health benefits. In this study, shrimp head was fermented by mixing with salt at ratio of 12:1, 14:1 and 6:1 (w/w) and fermentation for 90 days. During fermentation, decreasing of unsaturated fatty acid (UFA) and DHA was found while EPA increased. Fatty acid profile of all fermented treatments seemed to not difference, and all treatments have rough texture, inferior appearance such as non-homogeneity and dark color, and long fermentation time because of high physical and chemical resistance of shrimp by-product's structure. Therefore, the acid pretreatment process was adopted to soften the shrimp by-products. Organic acids, namely citric and acetic acids, were used at concentrations of 0% and 5% (w/v) and conducted at room temperature (30±3^{o}C) for 50 h. Total soluble solid (TSS) and pH of the mixture were monitored. TSS of all treatments gradually increased and the sample treated with acetic acid showed higher TSS than other treatments ($p<0.05$). The pH of acetic acid treatment remained constant while pH of citric acid treatment gradually increased ($p<0.05$). Based on pH change and unsatisfactory smell of citric acid treatment, the acetic acid was selected. Thereafter, the samples treated with acetic acid at concentration were studied at 0%, 2.5%, 5%, 7.5% and 10% with solid to acid ratio 1:0.6 and fermented for 48 h. This experiment found that TSS gradually increased while pH constantly remained during fermentation. In conclusion, acetic acid at 10% is the proper condition for softening shrimp by-product, however, using only TSS and pH may not be good determinant for by-product softening, and fermentation therefore, deep information must be further investigated.

Keywords:Acid;fatty acid profile;Shrimp by-product;softening

DEVELOPMENT OF PROTEIN FORTIFIED PLANT ICE CREAM BASED ON SOY AND PEANUT MILK FOR THAI ELDERLY

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ABSTRACT

The requirement for food and nutrition by the elderly is demanding urgent attention. Beside the main course, plant-based ice cream could be developed using protein to improve its nutrition. The plant-based ice cream was prepared with different soy milk (SM) and peanut milk (PM) ratios (100:0, 75:25, 50:50, 25:75, and 0:100). The chemical, physical, and sensory properties of plant-based ice cream were investigated. The total solid content of ice cream with different blends ranged between 24.17 and 26.88%. The 100:0 sample had the highest protein content (5.05%). As the amount of PM increased, the protein content decreased ($p<0.05$). Rheological measurement indicated that all ice cream mixes exhibited pseudoplastic flow ($n<1$). The viscosity and consistency coefficients of the mixes declined, whereas the n value rose with increasing PM up to 50%. In addition, the overrun and melting rate of the samples tended to increase, but the hardness decreased with increasing PM proportions. The acceptance test by the elderlies ($n = 50$) revealed that the different SM to PM ratios did not affect the liking score of all attributes except mouth feel, where the 0:100 sample obtained the highest ($p<0.05$). Therefore, this ice cream was chosen and added with pea protein concentrate (PPC 2, 3, and 4%). All samples were compared with 2% soy protein concentrate (control). The addition of PPC to PM ice cream positively influenced the protein content ($p<0.05$). Apparent viscosity, consistency coefficient, and overrun increased, while flow behavior decreased with increasing PPC levels ($p<0.05$). Nevertheless, PPC had no effect on melting rate ($p\geq0.05$). The acceptance test demonstrated that the ice cream added with 4% PPC gained the highest flavor (7.38±1.36) and overall liking (8.09±0.73) scores ($p<0.05$). Therefore, this PM-based ice cream with 4% PPC could be a potential ice cream that could enhance nutrition for older adult consumers.

Keywords: elderly acceptability; peanut milk; plant-based ice cream; rheological properties; soy milk

CHARACTERISATION OF SACHA INCHI OIL MICROCAPSULE OBTAINED VIA COMPLEX COARCEVATION

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ABSTRACT

Sacha inchi oil, renowned for its high content of beneficial unsaturated fatty acids such as linolenic acid (omega-3) and linoleic acid (omega-6), serves as a promising alternative to fish oil, offering numerous health benefits. However, its susceptibility to oxidative degradation poses challenges, resulting in undesirable odor and reduced shelf life. The study delved into optimizing the microencapsulation process of sacha inchi oil, investigating the interplay between gelatin and gum arabic at different pH levels (2.0, 4.0, and 7.0) to form a biopolymer complex, with varying ratios (1:4, 1:2, 1:1, 2:1, and 4:1, gum arabic:gelatin w/w) of these materials to sacha inchi oil. Spectrophotometric transmittance (%T) measurements unveiled nuanced interactions between gelatin and gum arabic at distinct pH levels, with the highest turbidity recorded with pH 7.0, ratio 1:2. The increase in turbidity as pH was increased indicated the initiation of the electrostatic interaction between protein and polysaccharide, leading to the formation of soluble complexes. The encapsulation process, followed by freeze-drying and particle size reduction, showcased varying outcomes based on core-to-coating material ratios. Notably, the lowest ($p<0.05$) microcapsule oil retention (OR%) was obtained with a 1:1 ratio and pH 2.0, whilst highest ($p<0.05$) OR% (90.17± 3.00%) occurred at a 1:2 ratio at pH 5.5. Increasing the ratio of protein and polysaccharide will increase the pH value to a higher one, by providing more protein to neutralize the polysaccharide and form coacervation. These findings underscore the pivotal influence of core-to-coating material ratios on encapsulation efficiency, oil content, and microcapsule morphology, offering valuable insights into refining sacha inchi oil microencapsulation for improved stability and diversified applications in functional foods and nutraceuticals. Ongoing research efforts include evaluating oxidative stability and in vitro gastric digestion of the microcapsules.

Keywords: oxidation, encapsulation, omega-3, biopolymer

EFFECT OF DEHYDRATION ON THE PHYSICOCHEMICAL AND ANTIOXIDANT PROPERTIES OF STINGLESS BEE HONEY

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ABSTRACT

High moisture content can lead to undesirable fermentation and growth of microbes, which can negatively impact the quality of stingless bee honey. This study was conducted to determine the effect of dehydration on the physicochemical and antioxidant properties of stingless bee honey. Three dehydration treatments were carried out including thermal (temperature 90-95°C, 60 s), microwave oven (power 60 Power Level, 60 s) and food dehydrator (temperature 55°C, 18 h). The samples were analysed to determine the proximate content (moisture content, protein, ash, fat, and energy), colour intensity, pH value, total soluble solids, viscosity, hygroscopicity, total phenolic, total flavonoid and antioxidant activity, compared with raw stingless bee honey without dehydration treatment as a control sample. Microwave treatment gave a significant effect ($p<0.05$) on the physicochemical of stingless bee honey sample by producing the highest protein content (0.12%), soluble solids content (73 ºBrix), viscosity (15.01Pa·s) and hygroscopicity (0.19 g of absorbed water per 100 g dry solids), compared to other dehydration treatments. All treatments resulted in lower ($p<0.05$) moisture content (13.66 to 20.26%), compared to control (22.95%). While microwave (pH 3.57) and food dehydrator (pH 3.62) treatments recorded lower ($p<0.05$) pH, compared to control (pH 3.75). For the antioxidant properties, food dehydrator treatment produced the highest antioxidant (Ferric Reducing Antioxidant Power) activity value (371.55µg $FeSO_4$/g), microwave treatment produced the highest total phenolic content (514 mg Gallic Acid Equivalent/g), and both microwave (39.32mg Routine Equivalent/g) and food dehydrator (36.54 mg Routine Equivalent/g) treatments produced the highest total flavonoid content ($p<0.05$). Overall, dehydration treatment is strongly encouraged in stingless bee honey to maintain the quality and antioxidant content, so, that its shelf-life lasts long, and its high nutritional content can be obtained by the consumer.

Keywords: antioxidant, dehydration, physicochemical, stingless bee honey

MULTIFUNCTIONAL GELATIN FILM INTEGRATED WITH CHITOSAN CARBON DOTS AND BUTTERFLY-PEA FLOWER ANTHOCYANINS: PROPERTIES AND BIOACTIVITIES

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ABSTRACT

Food packaging sector has grown dramatically in recent years with innovative and active food packaging technology, which minimizes the food loss without compromising consumer safety and providing high-quality food. Hence, this study was focused on developing an active and pH-responsive intelligent film based on fish gelatin by incorporating chitosan carbon dots (CS-CDs) and butterfly pea flower (*Clitoria ternatea)* anthocyanins (BA). The effects of CS-CDs at different amounts (1, 3, 5; wt%) on antibacterial, antioxidant, and physicochemical characteristics were investigated. CS-CDs significantly enhanced the mechanical properties of the polymeric matrix ($p<0.05$). The film containing 5wt% CS-CDs showed an excellent UV barrier property and exhibited higher antioxidant and antimicrobial properties than those incorporated with lower amounts of CS-CDs. The BA incorporated films also underwent varying color changes, depending on pHs (pH 2 to 12). When the developed film was used for wrapping whole shrimp (*Litopenaeus vannamei*) and stored at 4°C, CS-CDs incorporated films retarded the bacterial growth and retained the shrimp color to a greater extent than the control (wrapped with polyethylene film). Shelf life of shrimp wrapped with film containing 5% CS-CDs delayed the quality degradation during storage compared to control ones. The end of storage coincided with the increased pH, which was associated with color alteration of film to purple blue. Therefore, fish gelatin film incorporated with CS-CDs and BA could be used to extend the shelf life and monitor quality loss via visualization of color changes before consumption.

Keywords: Gelatin; chitosan; carbon dots; anthocyanins; pH

IMPROVEMENT FLAVOR OF TEA FROM *GNETUM GNEMON* USING DIFFERENT PRETREATMENTS AND ADDING PANDANUS POWDER AND CHRYSANTHEMUM POWDER

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ABSTRACT

Gnetum gnemon var. *tenerum* is the queen of local vegetable in southern Thailand due to its mild flavor, sweet and umami taste besides containing less chemicals or pesticide when compared with other commercial vegetables. It contains polyphenols, tannins, flavonoids and other phytochemical compounds. From a previously studied of *G. gnemon* var. *tenerum* powder for making drinking like tea, it was found the unique flavor with less acceptability. Hence, this study aimed to pre-treat the leaves before drying including withering, steaming, rolling and steamed-rolling treatment, and using pandanus leaves and chrysanthemum flower to improve the flavor. Total polyphenol compounds and antioxidant activity were also determined. The study result indicated that withering showed the highest total polyphenols compounds of 26.95±0.47 mg GAE/g DW ($p<0.05$). However, DPPH scavenging activity of steamed-rolling treatment was higher than other treatment ($p<0.05$). There was no significant difference in the sensory score of any var *tenerum* tea ($p>0.05$). The suitable pretreatment of Liang powder was steamed-rolling treatment (steaming 1 min with rolling 15 min) based on antioxidant activity. In addition, adding 40% chrysanthemum powder gave the highest acceptance score with 6.38±1.65 based on 9-point Hedonic scale assay. Therefore, *G. gnemon* var. *tenerum* tea mixture of *G. gnemon* var. *tenerum* powder 60% with chrysanthemum powder 40% was optimum ratio and yielded total polyphenol compounds of 25.07±0.92 mg GAE/g DW and DPPH scavenging activity of 39.10±0.81 mg Trolox/g DW.

Keywords: Gnetum gnemon var. *tenerum powder; Pre-treatment process; Flavor; Antioxidant*

EFFECTS OF SOURSOP LEAF EXTRACT, PULSED ELECTRIC FIELD AND VACUUM IMPREGNATION ON MELANOSIS INHIBITION AND SHELF-LIFE EXTENSION OF REFRIGERATED SHRIMP

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ABSTRACT

Pacific white shrimp were subjected to pulsed electric field (PEF) followed by soaking in soursop leaf extract (SLE) at 0.5% and 1% with the aid of vacuum impregnation (VI) for 2 cycles. The effects of these treatments on the quality and shelf life of the shrimp were investigated. Notably, the sample with prior PEF treatment and soaked in 1% SLE along with VI (PEF-SLE1-VI) had the highest total phenolic content (27.68 ± 1.49 mg GAE/g sample) ($p < 0.05$). Thirteen phenolic compounds were detected in the shrimp meat, as identified by LC-MS. During 15-day storage period, the quality changes in various shrimp samples were monitored. The PEF-SLE1-VI sample exhibited the lowest alterations in quality, compared to others ($p < 0.05$). Remarkably, this sample had total bacterial count below the acceptable limit (6-log CFU/g sample) after 15 days of refrigerated storage. Additionally, it effectively mitigated melanosis and maintained the highest textural and sensorial properties. Furthermore, the PEF-SLE1-VI sample demonstrated the lowest chemical changes including total volatile basic nitrogen (TVB-N) content (19.41 mg N/100g sample), and thiobarbituric acid reactive substances (TBARS) (3.30 mg MDA/kg sample) after 15 days. Overall, prior PEF treatment of whole

shrimp with subsequent soaking in 1% SLE in conjunction with VI, therefore, contributed to quality maintenance and shelf-life extension of the refrigerated Pacific white shrimp. These findings clearly indicated the potential of integrating PEF, VI and natural extracts like SLE in shrimp preservations. This technology can be properly implemented in shrimp during post-harvest handling and storage.

Keywords: Pacific white shrimp; pulsed electric field; vacuum impregnation; soursop leaf extract; melanosis; shelf-life

OPTIMIZATION OF EXTRACTION IN *Etlingera elatior (Jack) R.M. Sm.* INFLORESCENCE WITH A BOX-BEHNKEN DESIGN

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ABSTRACT

Etlingera elatior (Jack) R.M. Sm. flower could be developed into tea. The drying process is the first step in making a brewed herbal tea. Proper drying will enhance herbal tea's active ingredients, enhancing their characteristics and healthcare advantages. Therefore, an optimal extraction process is needed to analyze the phytochemicals in dried *Etlingera elatior (Jack) R.M. Sm.* This research used a Box-Behnken Design (BBD) for optimization extraction. A Box-Behnken Design (BBD) is an experimental design that can assess several variables and their interaction. BBD uses three or more factors with three levels of factor values (-1, 0, 1). Box-Behnken Design (BBD) was used for the optimization of ultrasound-assisted extraction (UAE) with several extraction factors including solvent concentration (70, 85, 100%), solid-to-solvent ratio (1:20, 1:15, 1:10), and temperature (30, 50, 70 °C). The total unit experiment is 15 with 3 center points. Controlled variables were amplitude of 50%, power of 50 W, and extraction time of 15 mins. Once the BBD was completed, Minitab software (Minitab Ltd, Brandon Curt, UK) was used for data analysis. The result showed that temperature, solvent concentration, and solid-to-solvent ratio affected phytochemical extraction. The suggested optimum extraction condition was 71.23% ethanol in water as the extraction solvent, 50 °C of extraction temperature, and a 1:20 solid-to-solvent ratio. The total phenolic content obtained from extraction at optimum conditions is 9.14 ± 0.11 mg GAE/g dried sample. The total flavonoid content obtained from extraction at optimum conditions is 11.09 ± 0.21 mg QUE/g dried sample.

Keywords: BBD, optimization, *Etlingera elatior (Jack) R.M. Sm.*, extraction, phytochemical.

ANTIDIABETIC POLYSACCHARIDES FROM VARIOUS MALAYSIAN HERBS

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ABSTRACT

Antidiabetic drugs are used to mitigate hyperglycemia. They, however, could cause several side effects when used over a long period, being inconvenient and expensive. As an alternative, traditional medicine from plant extract with antidiabetic properties is a promising area in diabetes therapy. *Centella asiatica*, *Cosmos caudatus* and *Oenanthe javanica* have been reported to show great potential to manage diabetes mellitus in the last decade.

Triterpenoids and many other phenolic compounds extracted from them have been identified as the major bioactive metabolites in regulating glycemia. Although polysaccharides and their derivatives have been reported to cause the same effect, no attention has been given to them despite the number of polysaccharides per gram sample being much higher than the amount of phenolic content in their crude extract. Thus, we preliminarily screened polysaccharide extracts from those herbs for antidiabetic properties. Out of the three herbs, the 50% ethanol precipitate of *Centella asiatica* (CA50) at 5 mg/mL demonstrated the highest inhibition of α-amylase and α-glucosidase with 68.3±0.04% and 62.3±0.08% inhibition, respectively. The CA50 fraction also exhibited the highest percentage of total sugars and glucuronic acid, with the monosaccharide's constituent in CA50 predominated by galacturonic acid (94.55 mg/g) followed by galactose (40.55 mg/g) and arabinose (14.79 mg/g). These polysaccharides of interest will later be further identified and characterised based on their composition and structure through a series of analyses. These identified and characterised polysaccharides will be the hallmark for further developing nutraceutical ingredients that can be an adjunct treatment for diabetes or a supplement for prediabetic individuals.

Keywords: Antidiabetic; Polysaccharides; Centella asiatica, Cosmos caudatus; Oenanthe javanica

CELLULOSE BASED HYDROGEL FOR PLANT GROWING MEDIA

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ABSTRACT

The application of hydrogel in agriculture has gained attention due to their availability to store water and fertiliser. However, currently most hydrogel are produced from petroleum based and are no biodegradable that could affect the environment. Hydrogel is a hydrophilic three-dimensional structure, possesses multifaceted properties including a high capacity for water retention and a reduced toxicity to the environment. Hence, the utilisation of biodegradable hydrogel is on the high demand in order to overcome the obstacles and long-term effect from the use of non-biodegradable hydrogel.
This study focuses to evaluate the performances of cellulose hydrogel application as a plant growth medium. The assessments included material characterization for two types of hydrogel (Hydrogel-Epichorohydrine 10%) and (Hydrogel-Epichorohydrine 10% - CarboxylmethylCellulose 2%], the effect of hydrogel application on seed germination, plant growth and physiology in three plant species *(Brassica juncea, Lactuca sativa, Solanum lycopersicum)*. This study also evaluated the effect of plant-growth promoting rhizobacterium (PGPR) on *Lactuca sativa* grown in hydrogel with Epichorohydrine 10%, CarboxylmethylCellulose 2%. The hydrogel with Epichorohydrine 10% cross-linking strength is at 92.49% and the hydrogel swelling analysis showed that the swelling increased by 65% within ten days. The field emission scanning electron microscopy (FESEM) analysis showed the hydrogel has a porous structure. The seed germination rate in hydrogel was comparable to that of soil and perlite. Hydrogel application as the growth medium have enhanced the plants' physical growth performances in general for all plant species.

Keywords: precool method, dissolution, aerogel, absorption, swelling

EFFECT OF SYNTHETIC CANNABINOIDS ON B-GLUCAN-INDUCED TRAINED IMMUNITY IN MACROPHAGES

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Abstract

Innate immunity is well known as the first line defense against pathogens. This immunity is characterized by a fast response, limited specificity, and not have memory for future insults. However, there is evidence that innate immunity such as monocytes, macrophages, and NK cells, also has memory phenotypes, one of them is called trained immunity. Trained immunity relies on metabolic reprogramming and epigenetic modification to regulate inflammatory-related gene expression. Maladaptive of it can be the cause of diseases, which makes it a novel target therapy. Nowadays, numerous biological compounds can affect the innate immune response. Cannabinoids are known to have anti-inflammatory effects, but in some studies, they were reported to induce pro-inflammatory response. Cannabinoids have been reported to decrease the paw inflammation induced by zymosan, which can be used to train macrophages. Thus, in this study, we investigated the effect of cannabinoids on trained immunity. We found that Δ^8-tetrahydrocannabinol (Δ^8-THC), Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD), cannabigerol (CBG), and cannabinol (CBN) were not toxic to bone marrow-derived macrophages (BMDMs) at concentration of 20 μM. Moreover, pretreatment with Δ^8-THC (20 μM) followed by stimulation with β-glucan (50 μg/mL) in the presence of Δ^8-THC induced more TNF-α production, compared to β-glucan (50 μg/mL) stimulation alone. On the other hand, Δ^8-THC, Δ^9-THC, and CBD at concentration of 20 μM significantly reduced both TNF-α and IL-6 production in β-glucan-trained macrophages when added before LPS stimulation. These results indicated the differential impact of cannabinoids on trained immunity which may have long-lasting effects on innate immune memory.

Keyword : Cannabinoids, ELISA, Macrophages, Trained Immunity

Introduction

Immunity in vertebrates is characterized as adaptive and innate immunity. Adaptive immunity is characterized by the role of T cells and B cells, which exhibit specificity in pathogen recognition and possess the capacity to retain memory for subsequent encounters. In contrast, innate immunity is widely recognized as the first line of defense that lacks specificity and the ability to develop memory against future invasions (Netea et al., 2011). However, recent evidence found that innate immunity also has memory after exposure to certain pathogens or stimuli.

In humans, the finding of innate immune memory was initiated by the non-specific protection from BCG vaccine in children in West Africa. BCG vaccination reduced overall mortality caused by infections other than tuberculosis (Garly et al., 2003). Another evidence was the immune response of human monocytes ex vivo elevated when stimulated with bacterial and fungal pathogens after BCG vaccination (Kleinnijenhuis et al., 2012). This memory that showed an increased state of activation in innate immune response after primary stimulation is called trained immunity (Netea et al., 2011). Train immunity has been known to be affected by epigenetic reprogramming such as DNA methylation, noncoding RNA, and histone modification (Benjaskulluecha et al., 2022). The metabolism in innate immune cells also shifted in trained immunity from oxidative phosphorylation into glycolysis through activation of the Akt/mTOR pathway (Chen et al., 2023).

Trained immunity can be found in innate immune cells, including macrophages. Compared to the naïve macrophages, the trained macrophages give a faster response (Zubair et al., 2021). Until 2021,

numerous stimuli can train immunity, such as β-glucan, BCG vaccine, oxidized low-density lipoprotein (ox-LDL), lipopolysaccharides (LPS), aldosterone, High Mobility Group Box 1 (HMGB1), lysophosphatidylcholines (LPC), fungal chitin, cytomegalovirus (CMV), western diet (WD), and uric acid (Drummer et al., 2021). This knowledge is beneficial because trained immunity is found to be involved in the pathogenesis of some diseases, such as cardiovascular diseases, autoimmune diseases, and atherosclerosis.

Because there is still a growing need of knowledge in innate immune memory, this research will contribute to investigating the effect of synthetic compounds from the plant, *Cannabis sativa*, which has gained interest for medical use. This plant may have the potential to be applied in the medical field because the study observed that cannabinoids have an immune-modulating effect that involves the endocannabinoid system (Yekhtin et al., 2022). Regardless of the cannabinoid potential, variations of the cannabinoid composition in plant extract can have different effects. The study performed by Namdar et al., 2017 showed that different locations of inflorescence samples, the polarity of solvents, and the separating methods affected the composition of the yielded compounds. Because of the variation in extract composition, the synthetic method to produce the cannabinoids will help to obtain a certain amount of compounds and also produce some derivatives that may have a better effect on human health. This research used five synthetic cannabinoids, which are Δ^8- tetrahydrocannabinol (Δ^8-THC), Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD), cannabigerol (CBG), and cannabinol (CBN). The effect of those synthetic compounds was observed in mouse bone marrow-derived macrophages (BMDM) whether they can repress or enhance the trained immunity. The result of this research is expected to provide knowledge on the effect of the synthesized cannabinoids in innate immune memory which can further be a candidate of target treatment in innate immune memory-associated diseases.

Materials and Methods

1. Cannabinoids

The five cannabinoids included in this study were chemically synthesized, and their identity and purity were confirmed by Nuclear Magnetic Resonance (NMR). They were prepared as stock solutions in DMSO. The details about molecular weight are Δ^8-Tetrahidrocannabinol (Δ^8-THC, 314.5 g/mol), Δ^9-Tetrahidrocannabinol (Δ^9-THC, 314.5 g/mol), Cannabidiol (CBD, 314.5 g/mol), Cannabigerol (CBG, 316.49 g/mol), and Cannabinol (CBN, 310.44 g/mol).

2. Generation of Bone Marrow Derived Macrophages (BMDM)

DMEM (Dulbecco' Modified Eagle Medium) Complete Media, which consists of DMEM High Glucose (Cytiva, Canada) media with 1% Sodium Pyruvate, 1% HEPES, and 1% Penicillin-Streptomycin and 10% Fetal Bovine Serum (Gibco, USA) were used for this experiment. Bone Marrow-Derived Macrophage Media (BMDM Media), which contained DMEM complete media (DMEMC) supplemented with 20% L929 cells supernatant containing M-CSF and 5% horse serum. C57Bl/6 mice (8-10 weeks old) were purchased from Nomura Siam International (Bangkok, Thailand). The mice were sacrificed using isoflurane inhalation and cervical dislocation. The femur, tibia, and humerus were dissected and the bone marrow was flushed and strained using 70 μM cell strainer in Serum Free Media + 1% Penicillin/Streptomycin and centrifuged. The bone marrow cells were resuspended in BMDM media and differentiated on the Hyclon™ plate for 7 days. After 7 days, the cells were harvested and used for the experiment.

3. Cytotoxicity test of compounds

Cytotoxicity test was performed using MTT assay (Thiazolyl blue tetrazolium bromide, Alfa Aesar). For observing the cytotoxicity of synthetic compounds, 2 x 10^4 BMDM cells were cultured in 96-well plate for 20 hr, then incubated using the compounds at concentrations of 20 μM and 50 μM. The detail scheme and calculation are shown in Figure 2A and B respectively.

4. β-glucan-induced Trained Immunity in Macrophages

To induce β-glucan trained immunity in macrophages, BMDM were cultured in DMEMC overnight

and primed with 50 µg/mL Pachymann BG (Megazyme, USA). After 24 h of priming, the medium was replaced with fresh DMEMC, and the cells were rested for 48 h. The resting step was followed by *Escherichia coli* LPS (L2880, Sigma Aldrich, USA) (10 ng/mL) stimulation before collection of the supernatant.

5. Investigating the synthetic cannabinoid effect on β-glucan-trained macrophages

To investigate the effect of synthetic cannabinoids on β-glucan-trained macrophages, the 2 x 10^5 BMDM were cultured in a 48-well plate overnight. There were two timelines for observing the effect of synthetic cannabinoids, which are shown in Figure 1A and 1B. After the LPS stimulation, the supernatant was collected and subjected to ELISA (Biolegend, USA) to examine the cytokine level for TNF-α and IL-6. ELISA was performed based on the manufacturer's suggestion.

6. Statistical analysis

All of the experiments were performed in duplicates except the cytotoxicity test (triplicates), and the data were presented with SEM. Statistical analysis was performed by using GraphPad Prism (San Diego, USA). One-way analysis of variance (ANOVA) was chosen to determine statistically significant differences between the results ($p<0.05$ considered significant).

Results and Discussion

Before investigating the effect of synthetic cannabinoids, the cytotoxicity assay was performed using compounds with the concentrations of 20 µM and 50 µM and 0.1% DMSO in DMEM media as the control. As shown in Figure 5, all cannabinoids were not toxic at both concentrations, except for the CBD which had some cytotoxicity at 50 µM. It was consistent with the other studies which found that CBD had a cytotoxic effect at higher concentrations, starting from 40 µM in various immune cells from PBMC (Jindaphun et al., 2024). However, other studies reported various cytotoxicity of CBD in different cell types, but immune cells were found to be more sensitive to cannabidiol. The cytotoxicity of CBD varies depending on several factors such as the cell type, the dose, CB receptor presentation, and time of exposure (Yeisley et al., 2021). Thus, for further experiment, the concentration at 20 µM of each cannabinoid was used.

To investigate the effect of synthetic cannabinoids, the experiment was conducted in two different conditions to see whether different exposures could have different effect or not. In the first scheme (Figure 1A), the morphology of the cells was more rounded, had more pseudopodia compared to the cell without stimulation, and showed an increasing number of cells after 24 hr of incubation (Figure 3). It may be caused by the activation of dectin-1 (β-glucan receptor) which activates the mTOR pathway that plays role in cell growth (Wang and Levine, 2010). Furthermore, the treatment of cannabinoids also affected cytokine production. It can be seen in Figure 6 that Δ^8-THC can increase TNF-α in β-glucan trained macrophages, but it did not affect the IL-6 level. The increase of TNF-α may be caused by the downstream effect of mTORC1 activation by the signaling pathway of the CB1/CB2 receptor through activation of PI3K phosphatidylinositol-3 kinase (McCoy K. L., 2016), which then enhances the pro-inflammatory gene expression.

The other stimulation scheme, which is shown in Figure 1B, revealed the different effect on β-glucan induced trained macrophages. In terms of cell morphology, cannabinoid-treated cells have more pseudopodia compared to unstimulated cells, β-glucan-stimulated cells, and LPS-stimulated cells (Figure 4). Interestingly, the effect of Δ^8-THC was different if it was given 1 hr before LPS stimulation compared to 1 hr before β-glucan treatment. The result showed that it lowered the cytokine production of both TNF-α and IL-6, as shown in Figure 7A and 7B. Δ^9-THC and CBD also had a suppressing effect on both TNF-α and IL-6 production (Figure 7A and 7B). The effect of CBD was determined by which receptor is activated by it. A study reported that CBD can lower TNF-α and IL-6 cytokine production mediated by CB2 receptor and Adenosine 2A receptors (Anil et al., 2022). These differences may be caused by different activated signaling pathways in macrophages immune response that should be studied further.

Conclusion

All synthetic cannabinoids showed no toxic effect in BMDM at concentration of 20 μM. The synthetic cannabinoids have different effect on the cytokine production in β-glucan-induced trained immunity in macrophages either enhancing or dampening effect.

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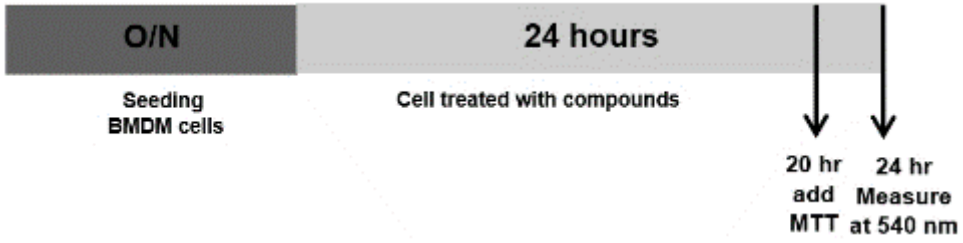
A

B

Figure 1: This timeline shows the experimental procedure to examine the effect of synthetic cannabinoids. A) In the priming step: cannabinoid (20 μM) was given 1 hour ahead in the priming step, then the media changed into cannabinoid 20 μM and β-glucan (50 μg/mL) for 23 hr,. After that, cells start to rest in freshly changed DMEMC for 48 hours. Finally, LPS stimulation was 10 ng/mL for 24 hr, and the supernatant was harvested. B) In the stimulation step: Firstly, the cells were only stimulated with β-glucan (50 μg/mL) for 24 hr, then the media changed into DMEMC followed by 48 hr resting. Next, the synthetic cannabinoids (20 μM) were added 1 hr before LPS stimulation. In the end, the media was changed into cannabinoids 20 μM and LPS (10 ng/mL) for 23 hr before the supernatant was harvested.

A



B

$$\% \text{ Cell Viability} = \frac{(\text{Absorbance at 540 nm of samples}) - (\text{Absorbance of Blank at 540 nm})}{(\text{Absorbance at 540 nm of control}) - (\text{Absorbance of Blank at 540 nm})} \times 100$$

Figure 2 : A) The timeline for MTT Assay. B) Calculation of cytotoxicity test. Control was the cells with 0.1% DMSO in DMEMC media and DMEMC media only was used as the blank. The viability ≥80% is considered as non-toxic to the cells.

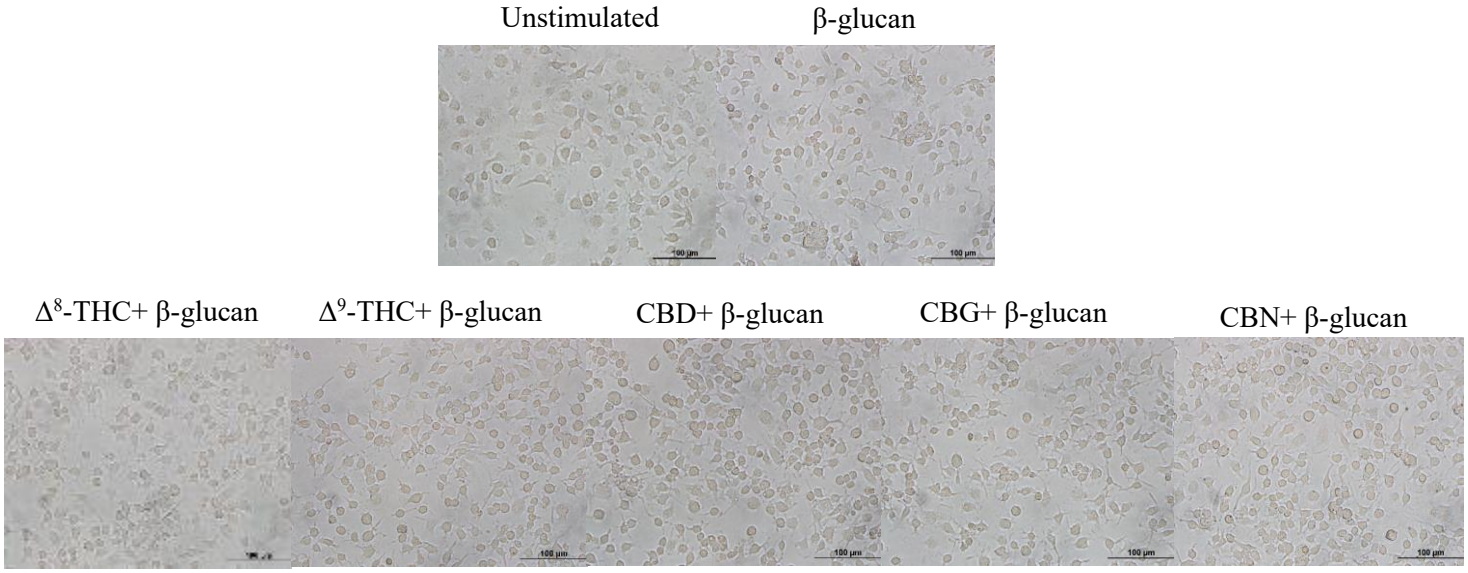


Figure 3 : Morphology of BMDM after 1 hr stimulation with cannabinoid 20 μM in priming time followed by cannabinoid 20 μM and β-glucan (50 μg/mL) compared to unstimulated cells (only incubate using DMEMC Media), and β-glucan stimulated cells. The total magnification was 400x.

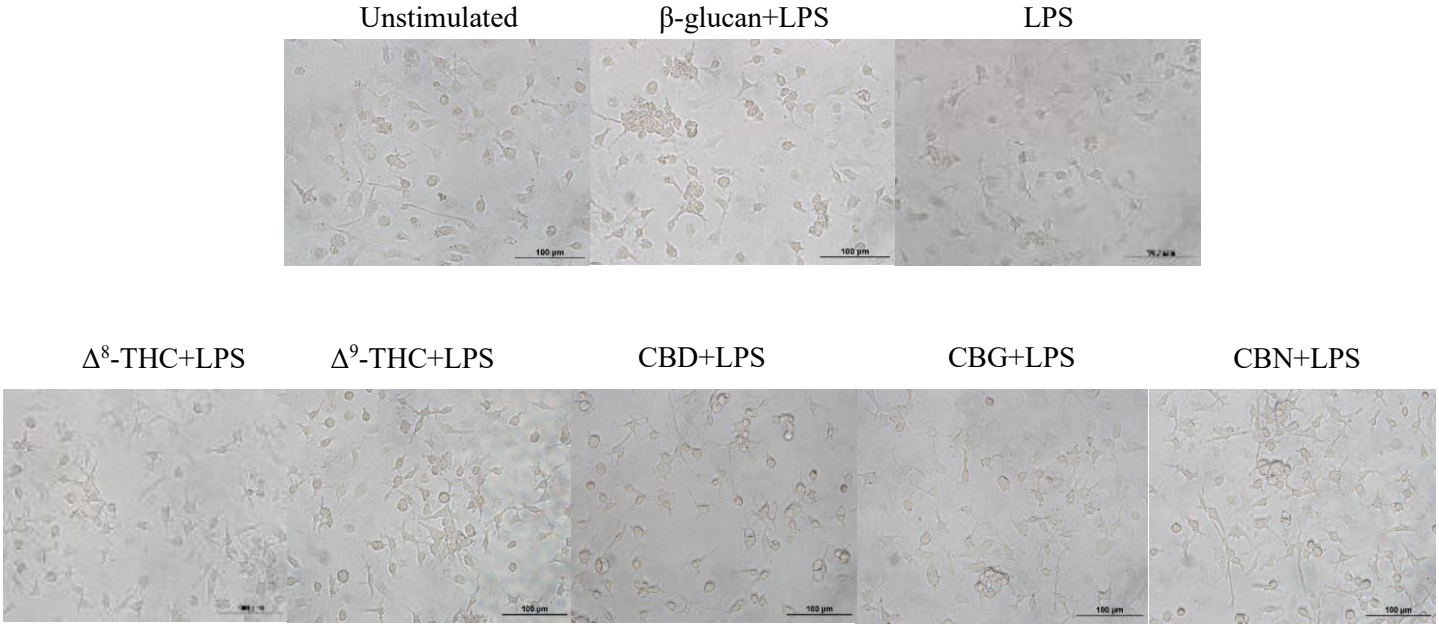


Figure 4: Morphology of BMDM after 1 hr pretreatment by cannabinoids 20 μM followed by 23 hr of cannabinoids 20 μM and LPS (10 ng/mL) stimulation. Unstimulated cells (only incubated using DMEM Media), β-glucan stimulated cells, and LPS-stimulated cells were compared to cannabinoid+LPS treated cells. The total magnification was 400x.

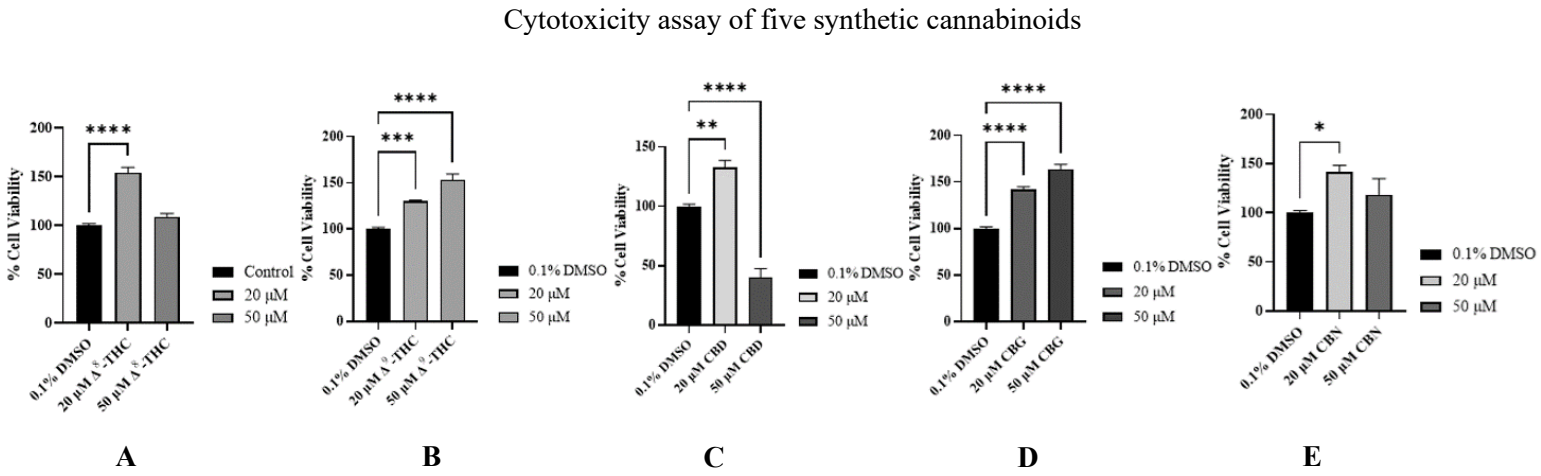


Figure 5 : Cytotoxicity assay result for cannabinoids in 20 µM and 50 µM. A) Δ^8-THC B) Δ^9-THC, C) CBD, D) CBG, E) CBN. This experiment was done triplicates. Cells in 0.1% DMSO in DMEMC media as control and DMEMC media as the blank in the experiment (significance *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

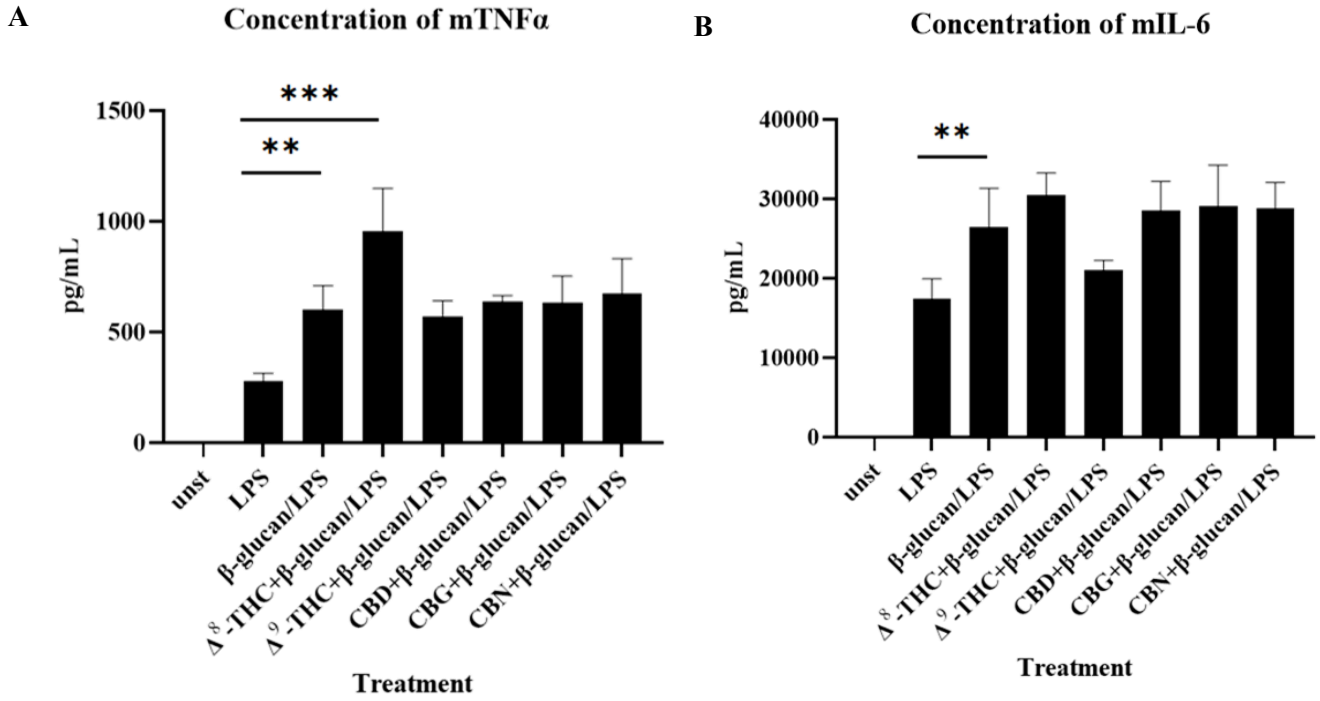


Figure 6: A) mouse TNF-α and B) mouse IL-6 results. This result was obtained after cells were stimulated with cannabinoids 20 µM 1 hr before β-glucan stimulation. Unstimulated cells (Unst) was only incubated in DMEMC media during the experiment. The statistically significant difference was compared to the β-glucan and indicated by **p<0.01, and ***p<0.001. To validate the result, this experiment was done in two independent experiments.

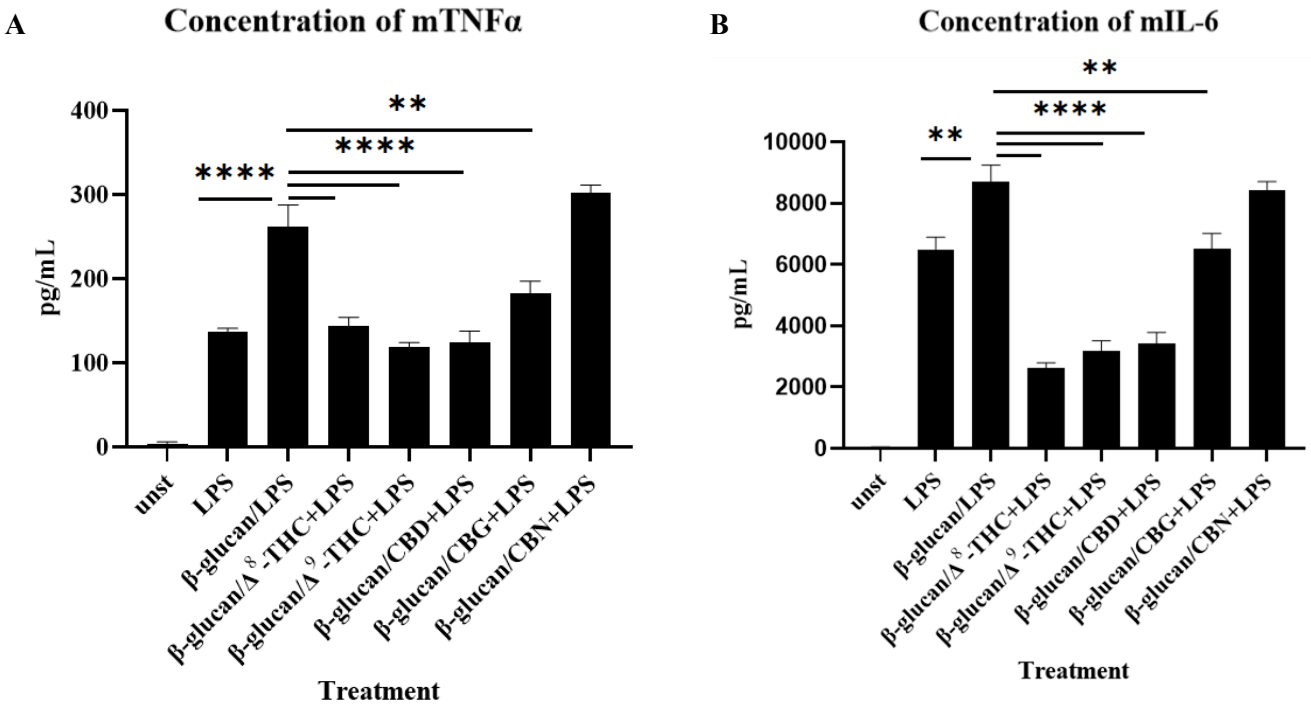


Figure 7 : A) mouse TNF-α and B) mouse IL-6 results when stimulated with cannabinoids 20 µM 1 hr prior to LPS (10 ng/mL) stimulation. Unstimulated cells (Unst) was only incubated in DMEMC media during the experiment. The statistically significant difference was compared to the β-glucan (50 µg/mL) and indicated by **p<0.01, ***p<0.001, ****p<0.0001 respectively. The experiment was done in two independent experiments.

CHITIN EXTRACTION FROM SQUID PEN WASTE THROUGH LACTIC ACID FERMENTATION AND POSTTREATMENT WITH ALKALINE PROTEASE

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ABSTRACT

This study focused on chitin extraction from squid pen waste (SPW) through deproteinization (DP) using a symbiotic co-culture of *Lactobacillus plantarum* and *Streptococcus thermophilus*, which are generally recognized as safe, to enhance protease production. Mature coconut water (MCW) was utilized as the sole low-cost nutrient source. Optimization through response surface methodology yielded the optimal conditions for DP: a sugar content of 4% w/v, an inoculum size of 10% v/w, and a liquid ratio of 11.5 v/w. The highest DP obtained was 62.3±1.35% with a chitin yield of 60.9±1.1%. The liquid fraction after DP contained lactic acid (LA) of 20.26±0.25 g/L, soluble protein of 105±3.6 mg/g-SPW showing DPPH radical scavenging activity of 54.2±2.3 µg-Trolox equivalent (TE)/g-protein. The crude chitin was post-treated by alkaline protease (10 U/mg-SPW) at pH 9 and 40°C for 3 h, resulting in the maximum DP of 98.1±1.1%, chitin yield of 28.2±1.3%, soluble protein of 374.85±2.1 mg/g-SPW with DPPH radical scavenging activity of 25.02±0.28 µg-TE/g-protein. To characterize the bio-extracted chitin, FT-IR, XRD, and SEM analyses were employed. The bio-extracted chitin demonstrated structural characteristics similar to those of commercial β-chitin, preserving chitin structure with a crystallinity index of 75.7% and high degree of acetylation (~100%). This study has shown that the DP of SPW by using symbiotic LA fermentation and alkaline protease post-treatment could efficiently extract chitin and recover valuable co-products.

Keywords: Antioxidants; demineralization; deproteinization; low-cost carbon source.

DEBITTERING OF PROTEIN HYDROLYSATE AND PLASTEIN FROM SALMON FRAME USING MAILLARD REACTION

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ABSTRACT

The study explored the effects of Maillard reaction (MR) on bitterness of hydrolysate and plastein of salmon frame in the presence of glucosamine and ribose at different concentrations. MR products (MRPs) from plastein showed higher bitterness than MRPs from hydrolysate, irrespective of type and concentrations of sugar used ($p < 0.05$). Heating protein hydrolysate (10 %) with 4% glucosamine at 120°C for 120 min yielded MRPs with the least bitterness in comparison to other samples ($p < 0.05$). The lightness and browning intensity of the aforesaid sample were inversely and directly proportional to the treatment time, respectively. It also had the elevated DPPH and ABTS radical scavenging activities, when heating was conducted up to 60 min ($p < 0.05$). It was noteworthy that the bitterness score decreased from 9.37 to 1.44. This was coincidental with the reduction in hydrophobic amino acid contents. In addition, both glucosamine and ribose had no impact on sweetness of resulting MRPs. Therefore, the Maillard reaction could serve as a promising approach to reduce the bitterness of salmon frame protein hydrolysate. As a consequence, MRPs with negligible bitterness and high antioxidant activity could be used widely without limitation caused by bitterness.

Keywords: Bitterness, hydrolysate, plastein, Maillard reaction, antioxidant activity.

PREPARATION AND CHARACTERIZATION OF PROTIEN HYDROLYSATE FROM ULTRASONICATED SHRIMP SHELL PROTIEN ISOLATE

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ABSTRACT

Shrimp shells are the by-products generated from shrimp processing industry, which is widely used for chitosan production. During chitosan production, protein is wasted as an effluent. Those protein could be used as protein isolate or their derivatives such as hydrolysates, peptides etc. Thus, protein hydrolysates from shrimp shell protein isolate (SSPI) were prepared using alcalase and papain at various concentrations in the absence and presence of ultrasonication pretreatment at different amplitudes and times (60 and 70% for 15 and 30 min, respectively). The degree of hydrolysis (DH) resulted to be increased with increase in concentration of enzyme, regardless of enzyme type and ultrasonication pretreatment. DHs of 43 and 31% were attained when 3% papain (UPH) and 2% alcalase (UAH), respectively were used along with pretreatment of ultrasonication at 60% amplitude for 15 min ($p<0.05$). Overall combination of ultrasonication and enzymes in sequential hydrolysis resulted in higher degree of hydrolysis as ultrasound exposed more active sites for the action of enzyme. UAH and UPH showed higher protein solubility (92-98 and 91-98%, respectively) as compared to SSPI (59-88.39%). On the other hand, SSPI had higher emulsifying properties than both hydrolysates. UPH showed decreased foaming capacity (113%) than SSPI (135%) and UAH (165%) ($p<0.05$). UAH had higher ABTS radical scavenging activity, and metal chelating activity whereas higher DPPH radical scavenging activity and ferric reducing antioxidant power were noticed for UPH($p<0.05$). Both hydrolysates were rich in hydrophilic amino acids and volatile compounds of different flavours and had peptides with a wide range of molecular weight. The bitterness score was increased with an increase in concentration of both hydrolysates. Overall, ultrasonication resulted in higher DH for both enzymes. The obtained hydrolysates showed improved physiochemical and functional properties, which could be used as functional ingredient or food additive.

Keywords: Shrimp shell, ultrasonication, protein isolate, protein hydrolysate, volatile compounds, antioxidants

PROPERTIES OF FILM BASED ON CO-PRECIPITATED BAMBARA GROUNDNUT PROTEIN ISOLATE AND FISH SKIN ACID-SOLUBLE COLLAGEN

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ABSTRACT

The present study aimed to investigate the properties of co-precipitated protein (CPP) films made from bambara groundnut protein isolate (BGPI) and fish skin acid-soluble collagen (ASC) at various BGPI/ASC ratios (100:0, 75:25, 50:50, 25:75 and 0:100). All films made from CPPs had similar tensile strength ($p>0.05$). Film from CPP with a 25:75 BGPI/ASC ratio had greater elongation at break and lower water vapor permeability ($p<0.05$), compared to those films from other CPP samples. All films had a bright-yellowish color. Increasing ASC ratio led to increase light transmission of resulting film ($p<0.05$). Fourier-transform infrared spectroscopy (FTIR) analysis revealed that all CPP films had similar patterns and functional groups. In addition, all films were heat sealable, moreover, their seal strength and seal efficiency were similar ($p>0.05$). In conclusion, the co-precipitation of BGPI and ASC could be an effective strategy for enhancing the properties of individual proteins, particularly in terms of their film-forming capabilities.

Keywords: Co-precipitation; Bambara groundnut; Acid soluble collagen; Film; Mechanical properties

POTENTIAL APPLICATION OF ANTIOXIDANT FILM FROM POLY-LACTIC ACID (PLA) FILMS CONTAINING *Alpinia mutica* LEAF EXTRACT AS FOOD PACKAGING

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ABSTRACT

This research was conducted to determine the effect of addition of *Alpinia mutica* leaf extract on antioxidant and physical properties of PLA active film and the effect of PLA active films towards lipid stability of cooked chicken burger stored at 4°C for 16 days compared to control PLA and PLA active films. PLA antioxidant active films were added with antioxidants including BHT, catechin, and *A. mutica* at two concentrations (0.5 and 1.0%) were produced using solvent (chloroform) casting method. Antioxidant properties of the films were determined using total phenolic compound (TPC), DPPH free radical scavenging and ferric reducing antioxidant power (FRAP) assays. Physical characteristics test conducted on PLA active films were thickness, colour, film surface and cross section structure and water vapor permeability rate. Research found that film added with 0.5 and 1.0% *A. mutica* leaves extract had high TPC value ($p<0.05$) compared to control film and comparable to film added with 0.5% BHT. While all of antioxidant active film had higher ($p<0.05$) value of DPPH free radical scavenging activity than control film. Film added with 0.5% *A. mutica* leaves extract possessed comparable FRAP value with film added with 0.5% BHT and higher ($p<0.05$) than control film. Addition of *A. mutica* leaves extract (0.5 and 1.0%) did not affect PLA film thickness. PLA film added with *A. mutica* leaves extract gave different colour ($p<0.05$) compared to other active film. SEM image showed that addition of 1.0% *A. mutica* leaf extract produced weak interface interaction between PLA matrix and the active compounds in the film. Concentration of antioxidant in PLA active film and storage time did not affect peroxide value and malondialdehyde content but affected ($p<0.05$) pH value of packed chicken burger. Overall, the addition of 0.5% *A. mutica* leaves extract was sufficient to increase film antioxidant activity.

Keywords: Active packaging, biodegradable packaging, lipid oxidation, natural antioxidant

SENSORY ACCEPTANCE AND GLYCEMIC INDEX OF SELECTED COMMERCIAL BREAKFAST BAR PRODUCTS

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ABSTRACT

Breakfast is a meal that is often skipped especially among many people due to time constraints. Ready-to-eat food products such as breakfast bars can meet today's demand due to the convenience of eating. Thus, this study was conducted to determine the sensory acceptance, satiety score and glycaemic index (GI) of selected commercial breakfast bar products for students of Universiti Kebangsaan Malaysia. A total of 50 students between the ages of 19-30 participated in survey on their favourite commercial breakfast bars. Three top picked commercial breakfast bars from the survey were selected and evaluated for sensory acceptance were Koko Krunch, Milo and Fitness. An acceptance test was conducted to test the degree of liking on 60 panellists for the attributes of appearance, colour, taste, crispiness, chewiness and overall acceptance. The breakfast bars tested in the satiety and GI which were Koko Krunch and Milo. The GI evaluation was performed on 11 healthy subjects. A total of 25 g of glucose was used as a reference sample in this evaluation. The subject's fingertip blood was taken and the glucose value was read at the intervals of 0, 15, 30, 45, 60, 90, and 120 min after the samples with 25 g available carbohydrate were consumed. The results showed no significant difference ($p>0.05$) were found for all the attribute for the three sensory evaluation samples. For GI and GL (glycaemic load) tests, both samples showed no significant difference ($p>0.05$) as well. Both samples fall into high category GI with values of 79 ± 19.0 (Koko Krunch) and 73 ± 26.9 (Milo) respectively, while Koko Krunch recorded a high GL (20 ± 3.0) and Milo in moderate categories (18 ± 4.1). In conclusion, all samples had similar sensory liking scores and were categorised within the same GI category but caution needs to be taken for diabetics as both bars had high GI.

Keywords: Breakfast bars; Glycaemic index; Sensory acceptance

TEMPERATURE INFLUENCE ON BIOACTIVE COMPOUND EXTRACTION FROM *MORINDA CITRIFOLIA* VIA SUBCRITICAL WATER EXTRACTION

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ABSTRACT

Morinda citrifolia, commonly known as Noni, has garnered substantial interest from the pharmaceutical and food industries due to its extensive medicinal potential. Despite this, exploration into the bioactive compounds of Noni, especially through eco-friendly methods, is limited. Traditional techniques like Soxhlet extraction are time-consuming and involve environmentally harmful solvents. This study investigates the influence of temperature on bioactive compound extraction from *Morinda citrifolia* using subcritical water extraction—a swift, sustainable method dependent solely on water. Examining temperatures of 140, 160, and 180 °C, the research assessed total phenolic content through the Folin-Ciocalteu test and antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric reducing antioxidant power (FRAP) tests. Results indicate a significant ($p<0.05$) positive impact of temperature on bioactive compound recovery, with the highest total phenolic content recorded at 180 °C (164.20 ±5.93 mg GAE/g). Additionally, antioxidant activity peaked at 180 °C for DPPH (96.11 ± 1.77%) and at 160 °C for FRAP (27.16 ± 3.06 mg FAE/g). The study revealed a significant impact of temperature on the recovery of bioactive compounds, emphasizing subcritical water extraction as a green and efficient alternative to conventional methods, as well as promoting more environmentally friendly approaches.

Keywords: Morinda; subcritical water extraction; bioactive compounds; antioxidant; extraction temperature

EVALUATION OF PHYSICOCHEMICAL PROPERTIES OF RED AND GREEN CHILLIES DURING POSTHARVEST STORAGE

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ABSTRACT

Red and green chillies are plants from the same genus, Capsicum and are members of the Solanaceae family. Chillies are usually packed and stored after harvesting for sale and this can result in loss in quality such as nutrient content, and affect the edibility of the product. The objective of this study is to determine the effect of temperature on the physicochemical properties of red and green chillies during postharvest storage at three different temperatures including room temperature (25 °C), cold temperature (5 °C), and freezing temperature (0 °C). The results of the study found that the texture of red and green chillies decreased ($p<0.05$) at 5 °C. The L* values of green chilli increased ($p<0.05$) at 0 ℃. The a* values of red chillies decreased ($p<0.05$) at 25 ℃, whereas green chillies increased ($p<0.05$) at 5 ℃, respectively. The b* values decreased ($p<0.05$) for red chillies at 5 ℃. The pH values of red and green chillies showed an increment ($p<0.05$) for all storage temperatures ranging between 4.32-6.01. The total soluble solid content also decreased ($p<0.05$) at 5 ℃ with values of 20.67 (red) and 16.00 (green) chillies. Total phenolic content decreased ($p<0.05$) significantly at 5 and 0 ℃ for red and green chillies, respectively. In conclusion, the ideal storage condition of 5 ℃ can reduce the qualitative and postharvest losses of both red and green chillies.

Keywords: chilli; physicochemical analysis; phenolic content; antioxidant; postharvest storage

CASHEW LEAF EXTRACT: ANTIFUNGAL ACTIVITY AND APPLICATION FOR EXTENDING SHELF-LIFE OF DRIED SALTED FISH

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ABSTRACT

Cashew leaf extract (CLE) was prepared using 80% ethanol as extracting medium with the aid of ultrasonication or soxhlet extraction method. Extraction by ultrasonication, followed by soxhlet extraction of the residues provided the highest yield (25.72%), total phenolic content (TPC, 402.31 mg GAE/g extract), and total flavonoid content (TFC, 351.24 mg QE/g extract). In contrast, soxhlet extraction alone resulted in lower yield (15.13%), TPC (341.22 mg GAE/g extract), and TFC (241.65 mg QE/g extract). Salt concentration notably decreased TPC and TFC in all CLE samples, plausibly due to compound decomposition in the presence of salt at high concentrations ($p < 0.05$). CLE extracted with soxhlet apparatus exhibited lower minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values, compared to those extracted by other methods. This extract also displayed higher efficacy in inhibiting fungal growth ($p < 0.05$) and reduced spore germination. Nonetheless it had less antifungal potential than potassium sorbate. At 4MIC concentration, CLE hindered mycelium growth and spore germination after 72 h and 10-16 h of treatment, respectively. For challenge test conducted by adding CLE and inoculating fungi on dried salted fish, CLE at 400 ppm was effective in retarding fungal growth over 9 days of storage. Thus, CLE showed the promise as a food additive to prevent fungal proliferation in dried salted fish.

Keywords: Cashew leaf, Soxhlet extraction, Antifungal activity, Dried salted fish

IN SILICO AND IN VITRO STUDIES OF SELECTED FLAVONOIDS ON WOUNDHEALING AND ITS UNDERLYING MECHANISMS VIA WNT/B-CATENIN PATHWAY

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ABSTRACT

This study aims to measure the effectiveness of flavonoids in wound healing through in silico and in vitro studies using the Wnt/β-catenin pathway. The site-specific molecular docking was run under in-silico study for 23 flavonoids that have been demonstrated the wound healing potential via β-catenin and glycogen synthase kinase-3β (GSK-3β) proteins. From in silico screening, only 5 flavonoids were selected for in vitro study. Then, the cell human keratinocytes (HaCaT) were used for an in vitro study of the wound healing ability using cell viability, Cell Counting Kit 8 (CCK8), and scratch assays. The result of molecular docking towards β-catenin protein showed docking score of quercetin (-7.7 kcal/mol), followed by hesperidin (-7.6 kcal/mol), rutin (-7.5 kcal/mol), corylin (-7.2 kcal/mol), morin (-7.0 kcal/mol) and allantoin (-5.8 kcal/mol). GSK-3β protein showed that hesperidin and rutin exhibited the highest docking score at -9.6 and -9.2 kcal/mol respectively, followed by corylin (-8.7kcal/mol), quercetin (-8.6 kcal/mol). During the cell viability investigation, all compounds were not toxic to the HaCaT cells and enhanced cellular proliferation to the cells at doses of 10 to 160 µM with incubation durations of 24, 48, and 72 hours. All compounds showed excellent wound healing rates, with morin being the best flavonoid treatment. Thus, this study reveals the ability of flavonoids to promote the wound healing process of keratinocytes through the Wnt/β-catenin pathway.

Keywords: Wound healing; flavonoids; Wnt/b catenin; in silico; in vitro

ANTIMICROBIAL EFFICACY OF LACTIC ACID BACTERIA FROM ASIAN GREEN MUSSELS AGAINST FOODBORNE PATHOGENS

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ABSTRACT

Lactic acid bacteria (LAB) are a bacterial group that can produce a variety of antimicrobial substances that are widely used as food preservatives. Lactic acid bacteria strongly inhibit many pathogenic microorganisms. This study was carried out to isolate and identify lactic acid bacteria from Asian green mussels (*Perna viridis*) and to determine their antimicrobial activity against foodborne pathogens and spoilage bacteria. Thirty-three isolates of LAB were isolated from the gastrointestinal tract of Asian green mussel and all of them were selected for antibacterial activity screening test. The culture filtrates (CF) of lactic acid bacteria were tested for antibacterial activities against *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Shewanella putrefaciens* using the agar well diffusion method. Based on the primary screening test, the CF of strains MG1, MG2, and MG5 showed the highest activity against *V. parahaemolyticus* and were selected for freeze-drying. The freeze-dried CF of strains MG1, MG2 and MG5 exhibited the most effective against *V. parahaemolyticus*. The minimum inhibitory concentration (MIC) ranged from 4 to 8 mg/mL while the minimum bactericidal concentration (MBC) ranged from 16 to 64 mg/mL. Scanning electron microscopy (SEM) images of *V. parahaemolyticus* cells treated with 4×MIC of freeze-dried CF (MG1, MG2, and MG5) revealed the cell damage with many pores on the cell surface, along with the absence of flagella. The CF of MG2 at the MIC (8 mg/mL) effectively impeded the motility (swimming and swarming) and biofilm formation of *V. parahaemolyticus* with 100% and 98.5%, respectively. The strains MG1 and MG2 were identified as *Liquorilactobacillus nagelii* while the strain MG5 was identified as *Lactococcus garvieae* based on the 16s rRNA gene sequence. Therefore, the LAB isolated from Asian green mussels could be a potential source of antimicrobial agents for foodborne pathogens controlling.

Keywords: Lactic acid bacteria; Antimicrobial activities; Perna virdis; Vibrio parahaemolyticus

ANTIBACTERIAL ACTIVITY OF TURMERIC EXTRACT LOADED CHITOSAN MICROPARTICLES AGAINST *HELICOBACTER PYLORI*

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ABSTRACT

The Gastrointestinal tract (GI), particularly the GI tract, is frequently infected with *Helicobacter pylori*, causing symptoms like abdominal pain and inflammation, leading to serious complications such as peptic ulcer disease and gastric cancer. This study aimed to evaluate the antibacterial activity of turmeric extract loaded chitosan microparticles (or TC microparticles) against *H. pylori*. The TC microparticles were evaluated for anti-*H. pylori* activity. The Minimum Inhibitory Concentrations (MIC) were determined using the agar dilution method. The results demonstrated that TC microparticles inhibited the growth of *H. pylori* ATCC 43504 at MIC of 32 µg/ml. Time-killing curve assay of TC microparticles against *H. pylori* ATCC 43504 is a concentration and time-dependent manner. The antibacterial activity of TC microparticles appears to be pH-dependent and is more effective under pH 3 > 5 > 7. Also, TC microparticles can inhibit the biofilm formation of *H. pylori* ATCC 43504 in a concentration-dependent manner. The mechanisms of action of TC microparticles against *H. pylori* were found to be membrane damage on the *H. pylori* structure and decreasing the expression of virulence genes. This study highlights the potential of TC microparticles as a promising treatment for *H. pylori* due to their potent antibacterial properties.

Keywords: Helicobacter pylori; turmeric; microparticles; chitosan

BIOTECHNOLOGICAL PRODUCTION OF DOCOSAHEXAENOIC ACIDS (DHA) FROM LOCALLY ISOLATED MARINE PROTIST, *Aurantiochytrium* sp. SW1.

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ABSTRACT

Aurantiochytrium sp. SW1 (SW1), a marine thraustochytrid isolated from Malaysia has shown significant potential as a sustainable alternative to fish oil. This is due to its ability to accumulate over 40-50 % (w/w total fatty acids) of docosahexaenoic acid (DHA), an important omega-3 fatty acid that has a significant role in maintaining the normal physiological function of humans. However, the commercial application of DHA from SW1 as well as the majority of other thraustochytrids is hampered due to its moderate volumetric DHA production as well as its high production cost, attributed to the costly cultivation substrates, particularly the refined carbon source. Thus, we have developed several biotechnological approaches to address these pertinent issues. These include screening and optimizing the culture conditions, rewiring the metabolic pathways by the addition of phytohormones and chemical modulator, developing super strain by using atmospheric room temperature plasma (ARTP) techniques, as well as enhancing the large-scale production of DHA through strategic two-stage dissolve oxygen (DO) shift cultivation strategies. Development of these strategies has significantly resulted in an almost 20-fold increase in DHA production as compared to prior to the study. We have also addressed the high cultivation cost issue by employing the extract of rejected fruits as alternative carbon sources, resulting in comparable DHA production to that of when using the refined carbon, but with a much lower cost.

Keywords: Biotechnology; Thraustochytrids; Docosahexaenoic acids (DHA).

OPTIMIZING CO-CULTURES OF OLEAGINOUS YEAST WITH BIOSURFACTANT-PRODUCING BACTERIA FOR LIPID PRODUCTION FROM CRUDE GLYCEROL WASTE

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ABSTRACT

Co-cultures of different microorganisms are considered promising strategies for biovalorizing various industrial wastes. The co-cultures of oleaginous yeast *Yarrowia lipolytica* and biosurfactant-producing bacteria *Bacillus subtilis* were applied in biovalorization of crude glycerol waste from biodiesel plant into lipids. The co-culture gave higher Lipid production (2.7 g/L) than the yeast monoculture (1.8 g/L). Possibly, biosurfactant helps modify hydrophobic substrates and increase cell membrane permeability, causing an increase in substrate entry and the performance of the yeast. The co-culture was then optimized using response surface methodology (RSM) and Box–Behnken design. The optimal conditions for maximum lipid production were: the use of 4% crude glycerol, 0.1% ammonium sulfate and co-inoculum of 10^7 yeast cells/mL with 10^8 bacterial cells/mL. Under these conditions, the maximum lipid production and lipid content obtained were 3.78 g/L and 63.96%, respectively. Furthermore, this yeast also showed a high flocculation efficiency of 64.22% at 30 min. After scaling up in a bioreactor with an optimal aeration rate of 0.5 volume of air per volume of Liquid per minute, the lipid production was increased up to 5.20 g/L. These strategies could be viable options for the cost-effective and efficient production of biodiesel feedstocks from biodiesel industry wastes.

Keywords: Biodiesel; biosurfactant; oleaginous microorganism; palm oil industries

APPLICATION OF SYMBIOTIC BACTERIA-YEAST PROBIOTICS IN PRODUCTION OF GOAT MILK YOGURT

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ABSTRACT

In addition to lactic acid bacteria (LAB), the use of yeast probiotic, *Saccharomyces boulardii*, in yogurt production was attempted to provide an additional probiotic solution for antibiotic-induced diarrhea. Yeast probiotic was added at different levels of 10^5, 10^6, and 10^7 CFU/mL together with 10^6 CFU/mL of two LAB, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) to develop symbiotic bacteria-yeast probiotics (sym-probiotic) goat milk yogurt. The addition of yeast probiotic enhanced LAB growth and also maintained the viability of probiotics above 10^6 CFU/g, meeting the general requirement for probiotics even after 28 days of storage. The physicochemical parameters, such as pH, acidity, syneresis, and texture profiles were similar to the control yogurt without yeast probiotic. In vitro gastrointestinal tests revealed a higher survival rate (>90%) for LAB and yeast probiotics in sym-probiotic goat milk yogurt compared to the control yogurt. Additionally, the analysis of volatile compounds identified a total of 30 compounds in the sym-probiotic goat milk yogurt, whereas 24 compounds were identified in the control yogurt. This study highlights the potential of sym-probiotics to enhance the health benefits and functional values of goat milk.

Keywords: Lactic acid bacteria; symbiosis; yeast probiotic; yogurt fermentation

MICROBIAL AND BIOCHEMICALS DYNAMICS OF KOMBUCHA FERMENTATION INCLUDING ISOLATION AND SCREENING OF BACTERIA AND YEASTS

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ABSTRACT

This study employed metagenomics amplicon sequencing to characterize the microbial composition and dynamics during Kombucha fermentation. *Gluconacetobacter* strains known for organic acid production and potentially beneficial for gut health were found dominant and two yeasts, *Dekkera bruxellensis* and *Zygosaccharomyces bailii* were found increasing then gradually decreasing over 7 days. Total acid and phenol content increased up to 10.21±1.91 g/L and 0.43±0.01 g-gallic acid equivalent/L, respectively. As a result, pH decreased from 3.31 to 2.45, confirming Kombucha's acidic nature. The antioxidant activity also significantly increased to 71.93±2.24%. These findings contribute valuable insights into the intricate interplay of microbial communities and biochemical changes during Kombucha fermentation. However, only acetic acid bacteria (AAB) and yeast could be isolated from the commercial Kombucha sample. Among the AAB isolates, K1A2, LD0A16, and SD0A13 were selected due to their high ability to produce acetic, glucuronic, and gluconic acid, respectively. Among the yeast isolates, SD0Y9, SD0Y10, and PKY1 were selected due to their high ability to produce ethanol, ethyl acetate, and acid-resistant ability, respectively. As LAB could not be isolated from commercial Kombucha, they were separately isolated from fermented fruit and assessed for their probiotic properties. All isolated LAB showed resistance to gastrointestinal (GI) tracts. Among them, B44, P31, and B51 isolates were selected due to their high antimicrobial activity. All screened strains will be identified and further applied as a synthetic microbial community for the production of probiotic Kombucha.

Keywords: Acetic acid bacteria; antioxidant; lactic acid bacteria; metagenomic; microbial diversity

MINERAL NUTRIENT AND HEAVY METAL ACCUMULATION IN RICE GRAINS AND THEIR RELATIONSHIP WITH MORPHO-AGRONOMIC TRAITS

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ABSTRACT

More than 55 varieties of rice have been grown in Malaysia, but none of them are declared to have a high content of mineral nutrients, especially zinc and iron. Furthermore, rice varieties with high nutrient content and yields can be contaminated with heavy metals such as arsenic and cadmium. This study was conducted to evaluate the content of mineral nutrients and heavy metals in polished and unpolished rice with morpho-agronomic characteristics, as well as their relationship with the presence of quantitative trait locus for yield under drought stress (*qDTY*) and submergence tolerance locus (*Sub1*). Five rice genotypes, namely UKM5, UKM112, UKM37, UKM54, and UKMRC9, were tested in the field using a completely randomised design with four replications. The harvested grain was processed and analysed for mineral nutrients and heavy metal content in unpolished and polished rice. Through this study, the content of Zn, Fe, and As in unpolished rice was found to be higher than that in polished rice, while the content of Cd in polished rice is higher. This study found that the UKM112 recorded the best morpho-agronomic characteristics, high mineral nutrient content, and low metal content, especially after polishing. The combination of $qDTY_{3.1}$ and *Sub1* had a positive effect on increasing morpho-agronomic traits and the content of mineral nutrients and heavy metals, while the combination of $qDTY_{12.1}$ and $qDTY_{3.1}$ had the opposite effect. This study also found that mineral nutrients and heavy metals have an effect on increasing grain yield and plant growth. This study can have a significant impact on the problems of malnutrition and rice production in Malaysia. The selected rice genotype can also be used as a donor parent to produce more rice varieties that are high in mineral nutrients, low in heavy metals, and effective for Malaysia.

Keywords: Cadmium; contamination; malnutrition; nutrient; rice

MORPHO-PHYSIOLOGICAL RESPONSES OF RICE (*Oryza sativa* L.) TOWARD SALINITY STRESS

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ABSTRACT

Rice, scientifically known as *Oryza sativa* L., serves as a primary cereal crop for over 90% of the Asian population. Nevertheless, the presence of abiotic stressors, such as salinity, imposes restrictions on global rice production. In this study, nine rice genotypes were subjected to three levels of salinity stress (0 dS/m – T1, control treatment, 8 dS/m – T2, intermediate stress, 15 dS/m – T3, severe stress) during the vegetative stage (VSL). The genotypes included six UKM rice breeding lines (UKMRC2, UKMRC8, UKMPL-5, UKMPL-6, UKMPL-68, and UKMPL-91), a salinity-tolerant check (Nona Bokra), and two salinity-susceptible checks (MR219 and MR297). The aim was to identify rice genotypes that are tolerant at VSL. The UKM breeding lines were developed by incorporating quantitative trait locus for drought tolerant yield (*qDTY*), known as $qDTY_{3.1}$ and $qDTY_{12.1}$, into the mega-variety rice, MR219, using a step-wise marker assisted breeding strategy. The morpho-physiological screening was conducted utilizing a Randomized Complete Block Design (RCBD) with three replications. The results reveal that the UKM breeding line UKMPL-68 had the highest survival rate (SR) of 88.89% in the T2 treatment, surpassing all other genotypes including the salinity tolerant check, Nona Bokra (SR = 66.67%). In T3 treatment, UKM breeding line UKMPL-91 had a highest SR (41.67%) followed by another two lines, UKMPL-6 and UKMPL-68 with SR of 38.89% and 28.89%, respectively. The results also show a positive correlation between SR and all measured traits. To summarize, the incorporation of introgressed *qDTYs* not only enhances rice performance in drought but also increases its ability to withstand salinity stress. Future research should focus on investigating the tolerance mechanisms of the *qDTYs* in response to various abiotic stress conditions and exploring the interactions among them.

Keywords: Abiotic stress; breeding line, quantitative trait locus (QTL), survival rate, rice

EXPLORING THE ANTIOXIDANT CAPACITY OF RICE-DERIVED BIOACTIVE PEPTIDES IN *SACCHAROMYCES CEREVISIAE*

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Abstract

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) play an imperative role in causing oxidative damage. Antioxidants, capable of scavenging free radicals, offer the means to counteract oxidative stress and enhance organismal vitality. In recent investigations, fermented foods are emerging as a promising frontier in research. Subsequently, yielding unique bioactive peptides with free radical-scavenging potential. Notably, Hom Nin rice (*Oryza sativa* L.) contains natural compounds with reported antioxidant activity, addressing the underlying mechanisms of certain diseases. *Saccharomyces cerevisiae*, a unicellular fungus utilized as a model for human studies, offers advantages due to its well-annotated genome and evolutionary conservation of essential biological processes. To assess the antioxidant activity of the derived peptides, DPPH (2,2-diphenyl-1-picrylhydrazyl) assay has been used and it was observed that peptide 18 has the highest free radical scavenging potential. Furthermore, *in vivo,* assessments has involved subjecting *S. cerevisiae* to the concentration of 6 mM hydrogen peroxide to evaluate the ability of the organism to combat oxidative stress and mitigate the effects of free radical-induced damage with the peptide treatments. The results showed that peptide 18 protected *S. cerevisiae*, wild-type strain BY4741 and Δ*tor1*. Contrastingly, peptide 18 was unable to shield the cells from the hydrogen peroxide damage in the Δ*sod2*, indicating it as a likely target for the peptide in enhancing cellular resilience against oxidative stress.This study will integrate both i*n vitro* and *in vivo* approaches to provide a comprehensive understanding of the free radical scavenging of the peptides obtained from the fermentation of rice.

Keywords: Antioxidant; bioactive peptides; fermentation; Hom Nin rice; Saccharomyces cerevisiae

Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) collectively describe free radicals and other highly reactive oxidants. Free radicals are highly reactive molecules that are produced in the body as a result of normal metabolic processes, exposure to environmental toxins, and even during immune system responses (Pham-Huy et al., 2008). These molecules can cause damage to cells and DNA, leading to various health implications such as accelerated aging, inflammation, and increased risk of chronic diseases like cancer, heart disease, and neurodegenerative disorders (Young et al., 2001).

There are various mechanisms, including endogenously produced antioxidants enzymes and those supplied through exogenous sources such as foods, to counteract oxidative stress, with the roles of antioxidants involving neutralizing excess free radicals, protecting cells against their toxic effects, and contributing to disease prevention (Young et al., 2001). Fermented foods are emerging as a promising contributor towards enhancing the overall well-being of an organism. The microbial transformation of raw materials during fermentation produces bioactive compounds, such as probiotics, peptides, and organic acids, which are known for their potential health-promoting effects (Das et al., 2020). Fermentation also generates bioactive metabolites with antioxidant and anti-inflammatory properties, which may counteract oxidative stress and inflammation (Guo et al., 2023). Bioactive peptides are defined as protein fragments that are composed of 2-20 amino acid residues, which possess a variety of beneficial biological properties (Guo et al., 2023).

Rice (*Oryza sativa* L.) is a staple agricultural crop in several Asian countries including Thailand. The scientific importance of rice in the context of antioxidants is noteworthy as they are a rich source of natural antioxidants. Peptides derived from rice can exhibit antioxidant ability and it signifies their potential role in shielding the cells against damage instigated by free radicals. Moreover, it has been substantiated through prior research that the fermentation of unpolished black rice with a defined microbial starter culture resulted in the ability to scavenge free radicals through *in vitro* analysis (Sangkaew and Yompakdee, 2020).

Saccharomyces cerevisiae, commonly known as budding yeast, serves as a unicellular fungus extensively used as a model organism for studying the human system and age-related diseases. Moreover, the well-characterized genome of the yeast makes it a valid model to study complex cellular processes in a simple environment (Janssens et al., 2016). The high degree of conservation makes yeast an extremely reliable biological model to further examine the complex interwoven pathways (Zimmermann et al., 2018). The superoxide dismutase, *SOD1* and *SOD2* genes, are the first line of antioxidant defense against oxygen free radicals (Wang et al., 2018). Antioxidant enzymes are pivotal in modulating cellular redox homeostasis in various organisms. *TOR1* (target of rapamycin) is a pivotal regulator within the nutrient-sensing pathway, holding significant potential to influence the antioxidant process (Calap-Quintana et al., 2015). *TOR1* inhibition has been associated with increased stress resistance, improved mitochondrial function, enhanced autophagy, and reduced pathologies (Kapahi & Zid, 2004). The interaction between these two genes has been illustrated in Figure 1, suggesting an intricate involvement and interaction between *TOR1* and *SOD* to facilitate the antioxidant defense for a prolonged survival of the organism. Further examination of these crucial genes will assist in providing the influence of the tested peptides towards maintenance and repair of the cell against stress.

Therefore, this study aimed to further examine the constituents present in the fermented rice samples, particularly, of the peptides to better understand their pivotal role in modulating pathways associated with antioxidants.

Materials and Methods

1. Preparation of fermented Hom Nin rice samples to screen for bioactive peptides

The preparation of the fermented Hom Nin samples was performed as described according to Sangkaew and Yompakdee (2020). The fermented samples, were purified with the assistance of the C18 HPLC column (3.9 x 150 mm; Waters, USA) and fractionated using OFFGEL fractionator by the laboratory of Dr. Sittiruk Roytrakul, from the Functional Proteomics Technology, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency. Thirteen peptides were derived from the rice samples for further analysis.

2. Screening of the peptides derived from the fermented Hom Nin rice by DPPH radical scavenging activity

The antioxidant activity of the peptide fractions derived from the fermented Hom Nin rice was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The peptide samples at different concentrations along with ascorbic acid (positive control) were incubated with the DPPH solution. Followed by measuring the absorbance of the mixed solution at 515 nm. The antioxidant activity was calculated as follows: DPPH scavenging activity (%) = [(A-B) ÷ A] x 100, whereby 'A' and 'B' are the absorbance of 515 nm of water (as the untreated control) and the sample (Baliyan et al., 2022). All the samples were tested in triplicates.

3. Investigating the protective effects of the selected peptide on hydrogen peroxide-induced oxidative stress

To investigate the free radical scavenging activity of the selected peptide derived from the fermented rice samples, *S. cerevisiae*, wild-type strain BY4741 (*MATa his*3Δ1 *leu*2Δ0 *met*15Δ0 *ura*3Δ0) and isogenic

mutant strains (i.e., *sod2* and *tor1*), were obtained from Yeast Genetic Resource Center, Japan and treated with 6 mM of hydrogen peroxide in the presence of the selected peptide. The survivability of the *S. cerevisiae* strains was monitored, and the selected peptide was tested for its protective effects against hydrogen peroxide. *S. cerevisiae* strains were inoculated in YPD medium, with and without treatment of the selected peptide at 30°C, 200 rpm. After incubation, cell suspension were aliquoted and treated with an appropriate concentration of hydrogen peroxide for 1 hour (Tran & Green, 2019). The qualitative analysis of the viability will be conducted via spot assay. The yeast cell suspension (5 µl) were spotted on YPD agar and after incubation for 2-3 days at 30°C, the colony of the yeast were visualized (Spencer et al., 2014). The positive control for this experimental analysis was quercetin as it is a known lifespan-extending compound (Grünz et al., 2012). All the experiments were independently performed in triplicates.

4. Statistical analysis

All of the experiments were performed in triplicates to verify the obtained results and the data were presented with mean and standard errors. The analysis were performed by using GraphPad Prism (San Diego, USA). The statistical methods will involve the use of one-way analysis of variance (ANOVA) to determine statistically significant differences between the independent test groups with Dunnett's Multiple Comparisons tests.

Results and Discussion

The peptides obtained from the fermented Hom Nin rice samples were given by Ms. Yanika Chontachot, Department of Microbiology, Faculty of Science, Chulalongkorn University, and an investigation into their antioxidant potential was conducted through the assessment of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity (Baliyan et al., 2022). Ascorbic acid, a well-established antioxidant, was employed as a positive control for comparative analysis (Njus et al., 2020). The DPPH free radical scavenging activity of the rice-derived peptides was measured, and the results were compared with those of ascorbic acid to assess the relative antioxidant efficacy as depicted in Figure 2. To validate the antioxidant properties of peptide 18, we conducted additional experiments by observing its DPPH free radical scavenging activity at various concentrations as shown in Figure 3. The findings indicated peptide 18 as a promising candidate, for its antioxidant activity and therefore, it was further examined for its potential activity in this study.

In order to assess the *in vivo* antioxidant potential of peptide 18, hydrogen peroxide stress-induced assay was conducted using *S. cerevisiae* wildtype, as well as in two isogenic mutant strains (*sod2* and *tor1*). The cells were subjected to hydrogen peroxide treatment to induce stress, and the ability of peptide 18 to enhance cell survival under such conditions was evaluated. Quercetin, recognized for its robust antioxidant properties and free radical scavenging abilities, was included in the study as a positive control (Grünz et al., 2012). The results revealed that peptide 18, at the specified concentrations, exhibited a protective effect in wildtype cells compared to untreated cells, as shown in Figure 4. Interestingly, a similar trend was observed in *Δtor1* , suggesting that *TOR1* may not be the direct target of peptide 18, as represented in Figure 5. However, peptide 18 demonstrated the ability to upregulate the stress response in the cell, enabling survival in the presence of hydrogen peroxide. Contrastingly, in the *Δsod2* strain, peptide 18 exhibited an inability to protect the cells subjected to hydrogen peroxide, even after treatment with the peptide, shown in Figure 6. This observation suggested a potential interaction between peptide 18 and the *SOD2* gene, implicating *SOD2* as a plausible target for peptide 18-mediated assistance in cellular resilience against excessive stress. Alternatively, the diminished protective effect could be indicative of the involvement of other underlying pathways that influence the survivability of the cell under oxidative stress conditions.

The activity of *TOR1* and *SOD2* are prime targets to understand the antioxidant defense mechanisms within the cell. This dual modulation suggests a promising target for therapeutic interventions aimed at neutralizing free radicals and mitigating age-related diseases linked to oxidative damage.

Conclusion

This intriguing outcome prompts further exploration to decipher the precise molecular mechanisms by which peptide 18 operates in the context of cellular maintenance and repair against stress. By unraveling the specific pathways and targets involved, a deeper understanding of the potential therapeutic applications of peptide 18 in stress response modulation may be attained, contributing to advancements in cellular health strategies.

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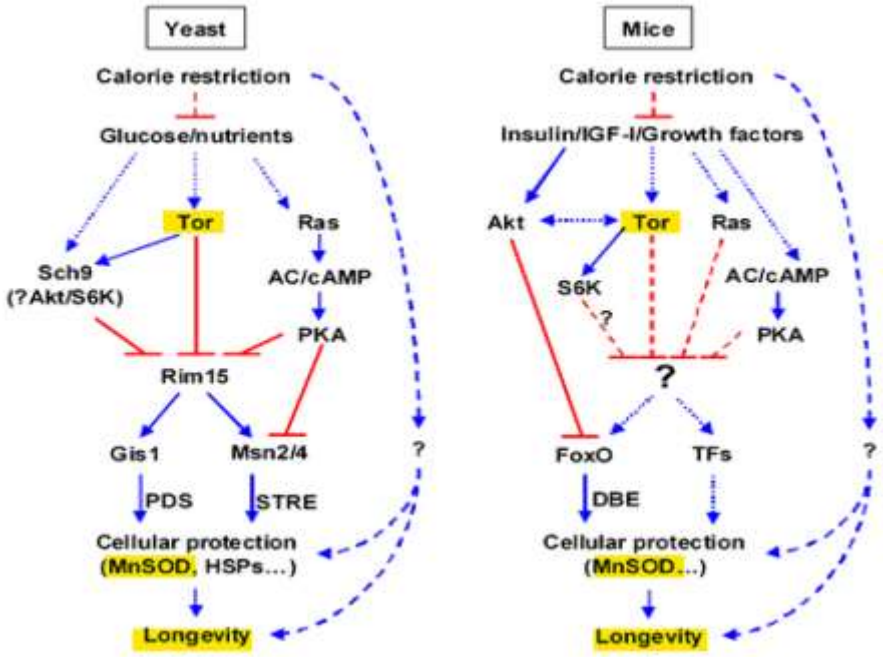

Figure 1: *Tor1* inhibition leads to an upregulation of the antioxidant pathways including *SOD* to assist the cell with prolonged survival (Wang et al., 2018).

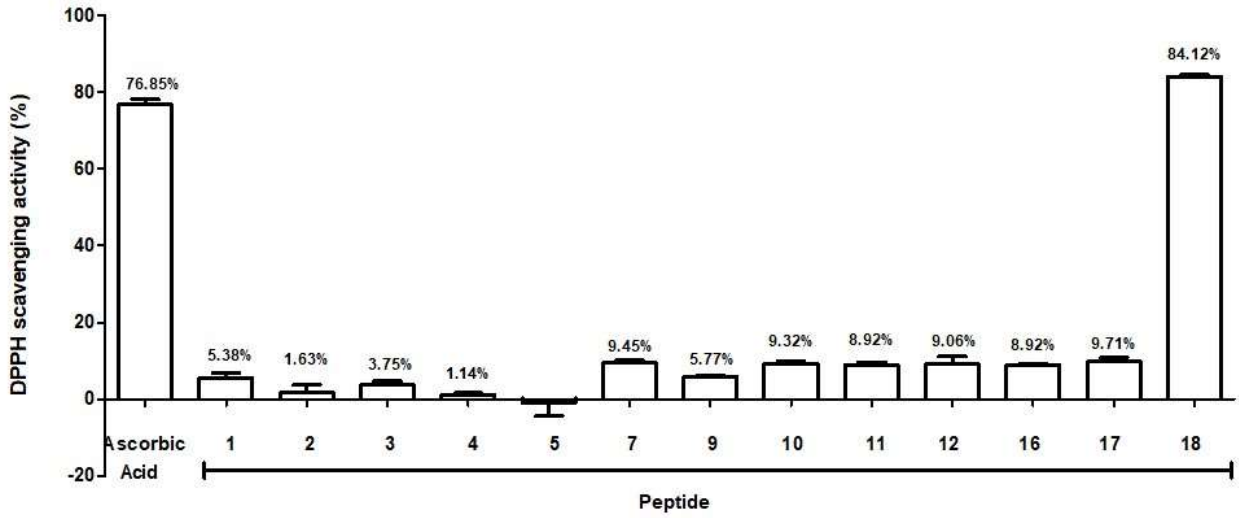

Figure 2: *In vitro* DPPH free radical scavenging activity of peptides obtained from the fermented rice samples. All the experiments were conducted in duplicates. Ascorbic acid (0.06 mg/ml) was used as a positive control. The numbers (1-18) denote the various tested peptides. Each peptide was tested at 10 mg/ml. Peptide 18 was selected as the prime candidate due to it's high radical scavenging activity to evaluate its potential in combating oxidative damage in the cell.

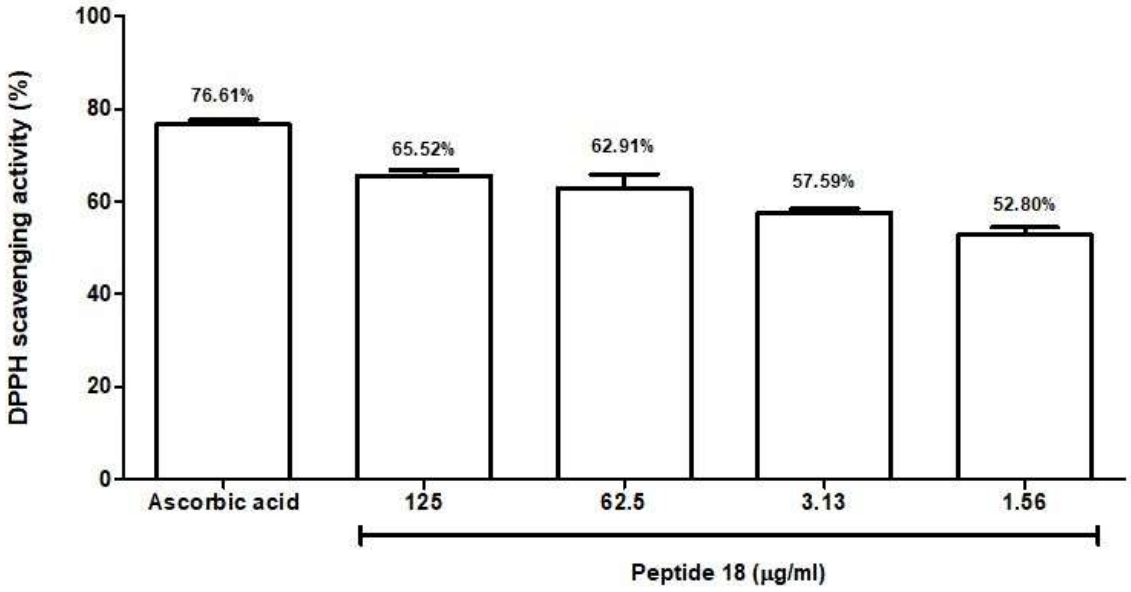

Figure 3: DPPH scavenging activity of the selected peptide 18 at various concentrations. The selected peptide was serially diluted to assess it's ability to scavenge free radicals at different concentrations. All experiments were conducted in triplicates. Ascorbic acid (60 µg/ml) was used as a positive control. The results exhibit peptide 18 as a strong antioxidant agent after assessing its ability to scavenge free radical at diluted concentrations.

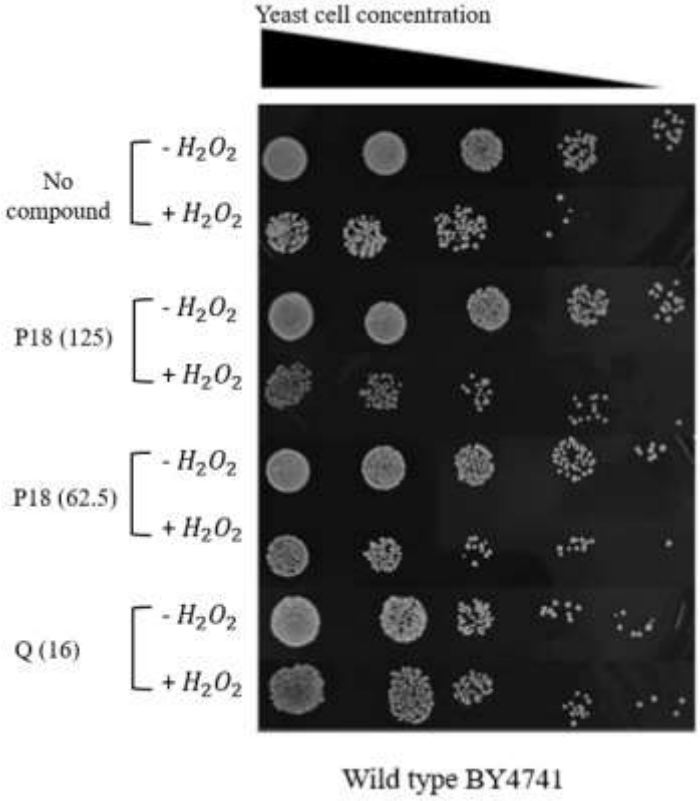

Figure 4: *In vivo* investigating the protective effects of the peptide on hydrogen peroxide-induced oxidative stress in wild type *S. cerevisiae* BY4741. The wildtype yeast cells were pre-treated with peptide 18 (P18) at the selected

concentrations of 125 and 62.5 µg/ml and incubated with 6 mM of hydrogen peroxide (H_2O_2). Quercetin (QE) at 16 µg/ml was used as the positive control. Independent replicates were performed to validate the results.

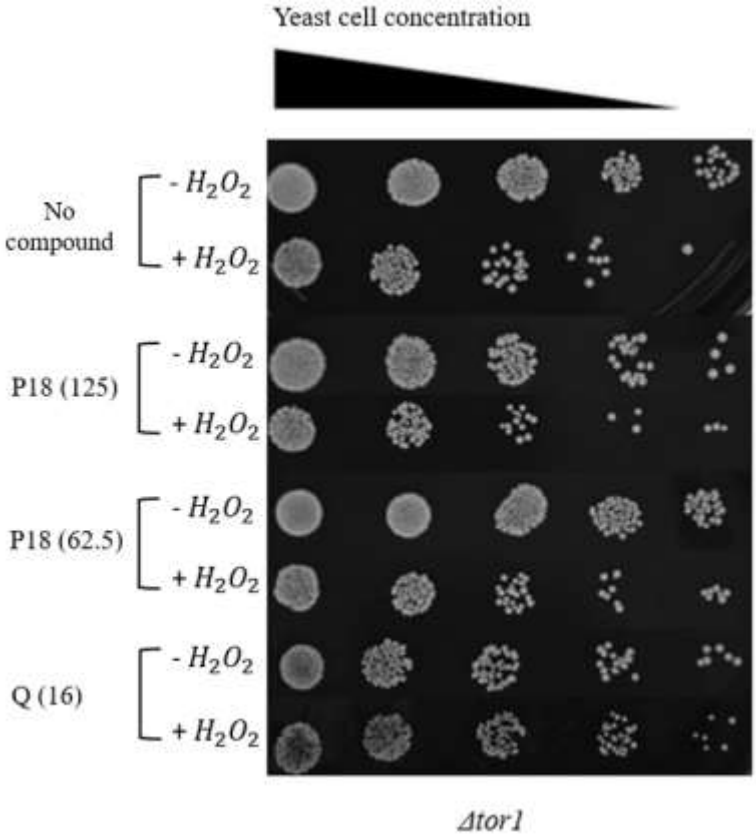

Figure 5: *In vivo* investigating the protective effects of the peptide on hydrogen peroxide-induced oxidative stress in *Δtor1 S. cerevisiae* BY4741. The *Δtor1* yeast cells were pre-treated with peptide 18 (P18) at the selected concentrations of 125 and 62.5 µg/ml and incubated with 6 mM of hydrogen peroxide (H_2O_2). Quercetin (QE) at 16 µg/ml was used as the positive control. Independent replicates were performed to validate the results.

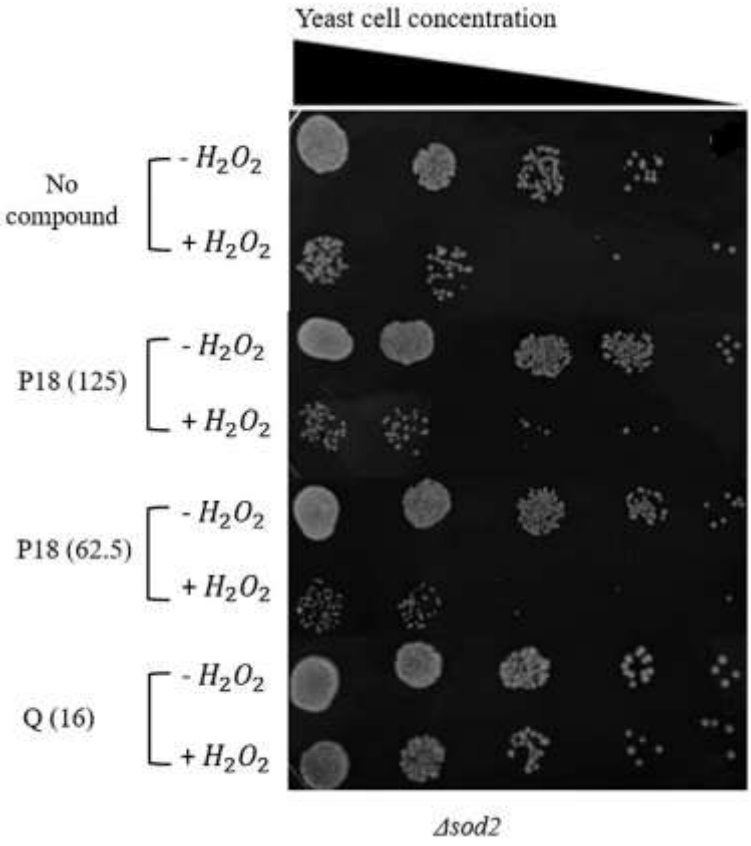

Figure 6: *In vivo* investigating the protective effects of the peptide on hydrogen peroxide-induced oxidative stress in *Δsod2 S. cerevisiae* BY4741. The *Δsod2* yeast cells were pre-treated with peptide 18 (P18) at the selected concentrations of 125 and 62.5 µg/ml and incubated with 6 mM of hydrogen peroxide (H_2O_2). Quercetin (QE) at 16 µg/ml was used as the positive control. Independent replicates were performed to validate the results.

BONE DENSITY, PHYSICAL ACTIVITY AND CALCIUM INTAKE OF INDIAN CHILDREN FROM TWO SCHOOLS OF SELANGOR, MALAYSIA

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ABSTRACT

Bone health (BH) is influenced by lifestyle and diet. Fragile bones can cause osteoporosis, a disease that commonly occurs among the elderly. The disease can be avoided if a person practices optimal physical activity level (PAL) and calcium intake (CI) since childhood. There are many bone mineral density (BMD) studies that have been conducted. However, there are no specific studies on the BH status of Indian children, making their BH status unclear. Hence, this study was conducted to determine the BMD, PAL and CI of Indian children from the school of Selangor, Malaysia, and to examine the relationship between these parameters. This cross-sectional study was conducted among 126 children aged from 9 to 11 years involving measurements of BMD, PAL and CI. BH status was determined by measuring BMD in the calcaneus using quantitative ultrasound. The PAL was determined using the children's PAL questionnaire. Meanwhile, the CI was measured using food frequency questionnaire (FFQ) and 24-hour dietary record (DR). The results showed that the BMD mean was 76.3±12.9dB/MHz. The PAL was at a moderate level (2.65±0.56) and significant difference ($p<0.05$) between gender. The correlation between PAL and BMD was r=-0.180. CI of the subjects was low (FFQ: boys: 694.4±373.4mg, girls=670.9±371.8mg; 24-DR: boys=608.3±307.3mg, girls: 513.4±337.7mg), and not significantly ($p>0.05$) correlated with BMD (FFQ: r=-0.004; 24DR: r=-0.124). In conclusion, Indian children in this study had a moderate risk of osteoporosis and PAL, whilst the CI was low. The PAL had a positive effect on the BMD. Contradictory, CI did not affect BMD.

Keywords: Physical activity; calcium intake; bone density; children

PROTEOLYTIC FERMENTATION OF EDIBLE BIRD'S NEST GLYCOPROTEINS FOR ENHANCED PHYSICOCHEMICAL PROPERTIES AND BIOACTIVITIES

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ABSTRACT

Edible bird's nest (EBN) is made up of glycoproteins with a complex molecular structure that has limited EBN from achieving its full potential for functionality and health benefits. The glycoproteins need to be broken down into peptides to increase the efficacy of EBN. This study was conducted to optimise the proteolytic fermentation parameters of EBN using *Lactobacillus helveticus* DSM 20075 (EBN_{LH}), *Latilactobacillus curvatus* DSM 20019 (EBN_{LC}), and *Latilactobacillus sakei* DSM 20017 (EBN_{LS}), as well as to investigate the physicochemical properties and bioactivities of fermented EBN. The optimisation of proteolytic fermentation was conducted using I-optimal design in response surface methodology (RSM), where three factors were used, including fermentation time (1–12 hours), EBN concentration (1, 3, and 5% w/v), and lactic acid bacteria (LAB) inoculum percentage (5, 10, and 15% v/v). It was found that the optimal EBN fermentation parameters were as follows: fermentation time of 6.31, 4.68, and 2.51 hours, and EBN concentration of 3.11, 2.38, and 3.85% w/v for EBN_{LH}, EBN_{LC}, and EBN_{LS} respectively, with 15% v/v LAB inoculum for all LAB. The fermented EBN revealed enhanced physicochemical properties and bioactivities with a 47.94–52.57% increase in recovery yield, a 111.07–196.93% rise in total peptide content, a 22.39–67.99% elevate in total sugar content, and a nearly double improvement in antioxidative and antihyperglycemic activities compared to the control of double-boiled EBN (EBN_{db}). The 1,1-diphenyl-2-picrylhydrazil radical scavenging (DPPH) activity of fermented EBN (17.15–22.34%) was significantly higher ($p<0.05$) than EBN hydrolysate (EBN_{hydro}) (14.61 ± 0.33%) produced by alcalase hydrolysis. This study provided an economic alternative to increase the nutraceutical value of EBN and broaden the application of EBN in various industries.

Keywords: Bioactivity; edible bird's nest; glycoprotein; proteolytic fermentation

EFFECT OF EXTRACTION CONDITION ON ANTIOXIDANT POTENTIAL, TOTAL PHENOLIC CONTENT AND VOLATILE COMPOUNDS OF *ELETTARIOPSIS SMITHIAE* RHIZOMES

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ABSTRACT

Elettariopsis smithiae is a herbaceous plant from the Zingiberaceae (Ginger) family used in traditional treatments. Samples were dried in a vacuum oven (45 ℃, 0.4 bar) and extracted with distilled water at 1:50 (sample: water) at various temperatures (50, 60 and 70℃) and times (40, 80 and 120 min). Extraction at 60 ºC for 120 min recorded the highest total phenolic content (TPC) of 84.59 mg GAE/g whilst extraction at 60 ºC for 40 min recorded the highest ferric reducing power (FRAP) value of 1176.4 µM FeII/g compared to other samples. Sample extracted at 70 ºC for 80 min recorded the highest percentage of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical removal of 83.37%. However, there was no significant difference ($p > 0.05$) compared to samples extracted at 60 ºC for 80 min. The highest 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) cation radical removal percentage value of 69.59% was also observed at an extraction temperature of 60 ºC for 80 min. *E. smithiae* rhizome extraction at 60 ºC for 80 min was the most optimal extraction condition with the highest antioxidant activities. The effect of temperature treatment and extraction time also contributed to the significant colour change (ΔE) of *E. smithiae* rhizome extract ($p < 0.05$). The compounds eucalyptol, fencil alcohol, borneol, fencil and acetate were the dominant volatile compounds found in control sample (without extraction) and *E. smithiae* rhizome samples extracted from optimum condition. The antioxidant content and the presence of volatile compounds proved that the rhizomes of *E. smithiae* have the potential as a source of natural antioxidants and to be an alternative to synthetic antioxidants.

Keywords: Antioxidant; color; extraction; herbal plant; volatile compounds

EXTENDING THE POST-HARVEST SHELF LIFE OF MANGO FRUITS USING BACTERIAL CELLULOSE CONTAINING ETHYLENE SCAVENGER

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ABSTRACT

Mango (*Mangifera indica* L.) is one of the popular local fruits among Malaysians. Commercially, mangoes harvested in the green-ripe stage, but they ripen quickly and suffer rapid post-harvest deterioration due to ethylene-induced (C_2H_4) overripening. Therefore, ethylene scavengers in mango packaging are essential to help limit ethylene accumulation, extend shelf life and maintain the original quality. Hence, ethylene scavengers inlet were developed by incorporating potassium permanganate ($KMnO_4$), in bacterial cellulose (BC). This study aimed to assess the effectiveness of BC-$KMnO_4$ in controlling ethylene production and its subsequent impact on mango ripening, thereby extending shelf life and maintaining the color of mango. BC produced from MRS broth were impregnated with $KMnO_4$ at different concentrations (0.5, 1 or 1.5% w/v). BC-$KMnO_4$ stored with mangoes in polyethylene bags at room temperature (25°C) for 15 days. Physicochemical analyses such as mango fruit weight loss, color changes, increase in BC weight, and fungal growth were evaluated on days 0, 2, 3, 6, 8, 10, 13 and 15 to evaluate the effectiveness of the ethylene scavenger throughout its storage period. The results found that treatment with BC + 0.5% $KMnO_4$ was the most effective because mangoes had the longest shelf life of 13 days compared to control (6 days), had the lowest fruit weight loss of 6.24%, lowest changes in L* value and black spots. In conclusion, this study successfully proved that the use of KMnO4 absorbed in BC, could potentially extending the shelf life of mangoes.

Keywords: Potassium permanganate, bacterial cellulose, Shelf life, active packaging, local fruits, ethylene scavenger

EFFECT OF INDIGESTBLE CARBOHYDRATES AND PLANT POLYPHENOLS ON GUT MICROBIOTA AND METABOLITE OF HEALTHY INDIVIDUAL

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ABSTRACT

The influence of the gut microbiota on human health has been revealed in recent years. In particular, prebiotics and probiotics have emerged as effective and integrative means of modulating the microbiome. Therefore, this research aims to investigate the effects of indigestible carbohydrates and plant polyphenols on the alteration of gut microbiota and their metabolites in healthy individual. The results from healthy individuals showed that OPP had the highest diversity index of gut microbiota (Chao1 = 445.75) and the highest overall diversity (Shannon = 5.97). The evenness of microbial distribution ranged from 0.84 to 0.94. Regarding the relative abundance of bacteria at the genus level, DFO was found to increase the beneficial gut bacteria Bifidobacterium from 25% to 33%, while BFP increased the pathogenic bacteria Escherichia-Shigella from 5% to 42%. Furthermore, each type of functional food component was found to promote the growth of specific bacteria in the fecal samples of healthy individuals, with the following order: henol (GLP), resistant starch (RS), BFP, isomaltooligosaccharide (IMO) and DFO, respectively. Among the three short chain fatty acids produced by gut microbiota, GLP had the highest impact on the production of acetic acid, propionic acid, and butyric acid ($p \leq 0.05$), with average concentrations of 20.88, 9.66, and 10.14 mM, respectively. As for polyphenol metabolites, in the fecal samples of healthy individuals, OPP stimulated the production of pyrocatechol, while GLP and BFP did not show the presence of metabolites. These findings suggest that DFO has prebiotic properties to promote beneficial bacteria in the human colon.

Keywords: Indigestible carbohydrates; Plant polyphenols; Gut microbiota; Metabolite

UPSCALING THE PRODUCTION OF PROTEIN HYDROLYSATE FROM TUNA VISCERA FOR PETS

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ABSTRACT

Valorization of fish processing byproducts such as viscera from tuna for production of protein hydrolysate (tuna viscera protein hydrolysate: TVPH) is good choice not only environmental concerns but also increase the high value-added product. Our previous process for production of TVPH in laboratory scale had short shelf life and not suitable for industrial use. This research aims to up scaling production and extending the shelf life of TVPH by concentration to ≥30% solid comparison to acidification by pH adjustment to ≤ 4.6. The concentrated TVPH had highly viscous (58% solid) whereas acidified TVPH had less viscous (18% solid) with favorable flavor. The antioxidant of concentrated TVPH (115.25 µg gallic acid equivalent/g sample) was significantly ($p < 0.05$) higher than acidified TVPH (12.80 µg gallic acid equivalent/g sample). The ratio of essential amino acids and total amino acids of the acidified TVPH (0.45:1) was higher than the concentrated TVPH (0.40:1). The shelf life of concentrated TVPH and acidified TVPH were 6 and 4 months, respectively. Then concentrated TVPH and acidified TVPH were added into pet food products and evaluated in cats using the TWO-BOWL palatability test (first choice and eaten amount). The first choice revealed that acidified TVPH (65.70%) had twice higher than concentrated TVPH (34.30%). The eaten amount of concentrated TVPH and acidified TVPH were 1,655.25 g and 1,835.75 g, respectively. The consumption intake ratios were 47.37% and 52.62%, respectively. Thus, TVPH has potential use as functional ingredient in pet food products by increase attractiveness and consumption of cats.

Keywords: Up-scale, tuna viscera protein hydrolysate, acidification, pet food

FORMULAR DEVELOPMENT AND COMMERCIAL PRODUCTION OF FUNCTIONAL DRINK FOR PETS

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ABSTRACT

Presently, pet food industry is growing according to the demands of pet owners around the globe. Thailand is currently the third exporter of pet foods for cats and dogs. Functional drink is a new product for this pet food industry. Pet food with addition of functional ingredient have been shown to exert health benefits to pets. The aim of this study was to develop functional drink for cats by addition of selected nutrients and functional ingredients providing nutritional benefits for cats. Sangyod rice bran oil was compared to other rice bran oil to be a source of antioxidants. The other ingredients used in this were fish oil, vitamins and minerals and inulin (prebiotic). The formulation was designed according to the requirements of the AAFCO (Association of American Feed Control Officials) and the developed pet drink was operated in industrial production using sterilization process to ensure feasible for commercial production. The antioxidants of Sangyod blended Prathum rice bran oil (MRBO) and commercial rice bran oil (CRBO) were 3.05 and 2.27 µg trolox equivalent/g sample, respectively. Gamma-oryzanol in MRBO (18,600 ppm) was twice higher than CRBO (9,000 ppm). Addition of fish extract into pet drink was significant ($p<0.05$) increased palatability of cats. Comparison on palatability between the formula added Sangyod blended Prathum rice bran oil with fish extract (MRBO-F) and commercial rice bran oil with fish extract (CRBO-F) was performed by TWO-BOWL palatability test (first choice and eaten amount) in cats. The first choice by cats indicated CRBO-F (65.53%) was more attractive for consumption than MRBO-F (60.98%).

Keywords: Functional drink, pet food, cat, rice bran oil, commercial

GASTRONOMY INGREDIENTS OF SOUTHERN THAILAND: FERMENTED FISH PASTE AND COCOA CAKE

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ABSTRACT

Indigenous sources of ingredients in Southern Thailand have unique flavors and functions that could be used as ingredients for preparing of gastronomy foods that is representation linked to the culture of a society; the methods, techniques and ingredients of food preparation. *Ka-pi-plaa* is a traditional fermented fish paste that has been widely consumed in Southern Thailand as a condiment. The chemical compositions are reported in two fish pastes from Phatthalung (KP) and Songkhla (KS) province, Southern Thailand. Meanwhile, cocoa cake from Songkhla and its products are known as rich sources of polyphenols such as flavanols. This study showed the results of chemical compositions KP and KS in dry weight basis. The KP was consist of protein (49.62 g/100g), fat (4.22 g/100g), ash (46.82 g/100g), and salt content (19.81 g/100g). Meanwhile, the KS were consist of protein (56.88 g/100g), fat (0.21 g/100g), ash (42.69 g/100g) and salt content (15.26 g/100g). The Protein content of KS was significantly ($p < 0.05$) higher than KP. However, fat content of KP was significantly ($p < 0.05$) higher than KS. All of *Ka-pi-plaa* samples were not detected of carbohydrate. Based on the result, KP and KS are good source of protein. Besides, the pH of KS was higher (6.67 ± 0.05) compared with KP (6.18 ± 0.06). The color of KP was significantly ($p < 0.05$) brighter ($L^* = 50.64 \pm 0.04$) than KS sample ($L^* = 36.76 \pm 0.09$). Furthermore, cocoa cake showed 51.25 g/100g of carbohydrate that was the highest composition compared to protein, ash, fat and moisture content. Meanwhile, total phenolic contents (TPC) of cocoa cake were higher significantly ($p < 0.05$) in original sample (11.26 ± 0.20 mg GAE/g) compared with spray drying (7.99 ± 0.19 mg GAE/g) and freeze-drying treatment (8.83 ± 0.14 mg GAE/g). The result of total flavonoids compound (TFC) showed original sample (255.99 ± 0.01 mg QE/g) was significantly higher ($p < 0.05$) compared with freeze drying (222.70 ± 0.01 mg QE/g) and spray drying (214.11 ± 0.01 mg QE/g). This result indicated that freeze drying and spry drying treatment effected in phenolic and flavonoids compound.

Key words: *Ka-pi-plaa, cocoa cake, gastronomy, peptide, polyphenols*

DEVELOPMENT OF MEAL REPLACEMENT SUPPLEMENT WITH KRATOM EXTRACT (*MITRAGYNA SPECIOSA* KORTH.) FOR WEIGHT CONTROL IN HEALTHY ADULT AND OBESE PATIENT

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ABSTRACT

Kratom (*Mitragyna speciosa* Korth.) has been shown to have beneficial health effects in terms of medicinal properties as rich in mitragynine and polyphenols. However, the consumption as food of kratom extract is still limited due to its bitter taste and medical regulation limitations for food application. The supplement is formulated to be used as a meal replacement by the addition of kratom extract from leaves. It is intended used as a medical food for weight control in people or obese patients if the nutritional composition is safe and beneficial for health. This research aimed to develop kratom extract based on weight management diets for weight control people or obese patients' evaluation on nutritional composition and polyphenol compounds. The development of meal replacement supplements by the addition of kratom extract was studied on nutrition composition microbiology and the content of mitragynine and total phenolics. The energy of meal replacement supplement added kratom extract was 389.25 kcal/100g of powder. The results indicated that the supplement met the requirements of macronutrients providing 23.17g of protein 4.45g of fat and 64.13g of carbohydrate. The supplement was also following the microbial quality. The proportion of mitragynine content and total phenolic content in the supplement was 22.20 mg and 5.57 mg GAE equivalent/g powder respectively. It could be concluded that the developed supplement proved its potential to be used as a meal replacement for healthy adult and obese patients.

Keywords*: Kratom, extract, supplement, weight control, obese*

DEVELOPMENT OF FISH SKIN GELATIN FILM INCORPORATED WITH EPIGALLOCATECHIN GALLATE PRODUCED BY THERMOCOMPRESSION MOLDING PROCESS

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ABSTRACT

The effects of epigallocatechin gallate (EGCG) on properties and characteristics of fish skin gelatin thermoplastic (FG-T) and thermocompression molded film (TCM-F) were investigated. FG-Ts were prepared by mixing fish gelatin powder with distilled water (45% w/w), glycerol (30% w/w), and EGCG at different concentrations (0, 5, 10, 15 and 20% w/w). The prepared FG-Ts were analyzed for moisture content (MC) and thermal property. TCM-Fs based on different FG-Ts were fabricated by thermocompression molding at various compression temperatures (CT) (70 and 100°C) and characterized for thickness, tensile strength (TS), elongation at break (EAB), MC, light transmittance (LT), water vapor permeability (WVP) and antioxidant activities. All FG-Ts had MC and melting temperature in the range of 17.03 – 26.14% and 47.87 – 60.71°C, respectively. The thickness of all TCM-Fs was in the range of 0.150 – 0.235 mm. Higher TS but lower EAB were observed in films with higher concentration of EGCG, compared to that of film without EGCG. The MC, LT, and WVP of films were decreased as level of EGCG was increased, regardless of CT used. Incorporation of EGCG decreased L^*-value along with increased a^*, b^* and ΔE^*-values of resulting films particularly when higher level of EGCG was used. Additionally, the highest DPPH and ABTS radical scavenging activities, ferric reducing antioxidant power and oxygen radical absorbance capacity were found in film containing 20% EGCG, regardless of CT used. Therefore, film containing 10% EGCG prepared with CT at 70°C, which provided satisfactory mechanical properties and antioxidant activities, could be used as a material for food packaging to maintain the quality of products.

Keywords: Fish skin gelatin; epigallocatechin gallate; thermocompression molding; film properties; antioxidant

SENSORY ATTRIBUTES AND ANTIOXIDANT ACTIVITY OF MANGO AND CHIA SEEDS FORTIFIED JELLY

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ABSTRACT

Jelly is a popular gelatinous confectionery usually found low in nutritiona value. Due to this reason, researchers have been formulating jellies wit various functional ingredients and analyzing its positive effects on health and possibility to gain interest in the functional food market. Mango (*Mangifer indica* L.) is an especially common fruit in Thailand, along with superfoo chia (*Salvia hispanica* L.) seeds containing various bioactive compound needed by humans. This study was conducted to fortify jelly formula with 44% (w/w) Nam Dok Mai mango puree and 16% (w/w) hydrated black chia seeds, along with other ingredients such as distilled water, hydrocolloids (konjac and carrageenan powder), citric acid, and stevia, which then furtherly called MCJ (mango-chia jelly). Antioxidant activities of MCJ were measured by DPPH radical scavenging activity and ABTS scavenging activity assay, while sensory attributes were analyzed with 9-point hedonic scale and 5-point scale of purchase intent among 50 untrained panelists. The results were compared with two commercially available jelly samples. ANOVA and Fisher's LSD post hoc test results showed that sensory attributes and antioxidant activity of samples differ significantly ($P < 0.05$). MCJ resulted in the highest value of taste, odor, texture, and overall acceptance attributes compared to commercial samples, with an average of 7.7 to 8 and purchase intent of 1.5. MCJ also had antioxidant activity of 175.68 µg TE/g sample (DPPH) and 331.43 µg TE/g sample (ABTS), highest among others. Positive effects from this research may become consideration for such combinations of functional ingredients to be used in future functional food markets.

Keywords: Jelly; fortification; mango; chia seed; antioxidant activity; sensory

EFFECT OF SOLVENT EXTRACTION ON PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY OF TORCH GINGER FROM SOUTHERN THAILAND

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ABSTRACT

Phytochemicals playing a role as antioxidant can be extracted from plants, such as torch ginger (*Etlingera elatior*). Torch ginger inflorescence is traditionally used for culinary purposes and exhibits polyphenols, such as total phenolics content (TPC) and total flavonoids content (TFC). The aim of this study was to compare torch ginger extract obtained with different solvents including water, 50% and 80% aqueous ethanol. The antioxidant capacity was determined with different reaction mechanisms including oxygen radical absorbance capacity (ORAC), DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals scavenging, and providing single electrons transfer by ferric reducing antioxidant power (FRAP) assay. Among the various solvents, the result showed that 80% aqueous ethanol displayed the highest TPC in Gallic Acid Equivalent (GAE) and TFC in Quercetin Equivalent (QE) with 18.4 ± 0.06 mg GAE/g of fresh weight (FW), and 19.8 ± 0.17 mg QE/g FW, respectively. The results showed that yield of extraction was higher by water extraction of 6.82% (g/FW) followed by 50% and 80% aqueous ethanol extract of 3.8% (g/FW) and 2.5% (g/FW), respectively. However, the 80% aqueous ethanol extract exhibited the highest antioxidant capacity. All antioxidant capacity results were expressed in Trolox Equivalent (TE). The DPPH radical scavenging capacity was 40.6 mM TE/g FW, FRAP value was 69.5 mM TE/g FW, and ORAC assay was 90.1 mM TE/g FW. This study demonstrated that the phytochemical components in torch ginger inflorescence extracted by the 80% aqueous ethanol expressed the highest antioxidant activity and could be a potential source of natural antioxidants for food and nutraceutical applications.

*Keywords**:** Torch ginger inflorescence, antioxidant, phenolics, flavonoids, aqueous ethanol extract.*

QUALITIES AND CHARACTERISTICS OF GREEN MUSSEL TREATED WITH ACID ELECTROLYZED WATER AND SUBJECTED TO HIGH-PRESSURE PROCESSING

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ABSTRACT

The treatments of green mussels (*Perna viridis*) with acid electrolyzed water (AEW) and high-pressure processing (HPP) on microbial, physicochemical, and sensory properties were investigated. The mussels were treated with AEW, packed in nylon/polyethylene bags, and vacuum-sealed with different conditions: 1) sample without AEW treatment, packed in bag and sealed; 2) sample soaked in AEW for 1 h, packed in bag and sealed; 3) sample packed in bag, added AEW in bag (1:10, w/v) and sealed (A-AEW). All bags were subjected to HPP at 0, 100 and 300 MPa for 3 min. Samples were monitored for total viable count (TVC), psychrophilic bacterial count (PBC), *Pseudomonas* spp. count (PC), H_2S producing bacteria (HSPB) Enterobacteria count (EC), *Vibrio* spp. count (VC), shell opening value (S-OP), firmness value (F), toughness value (T), color, weight loss after HPP, cooking loss, total volatile based nitrogen (TVB-N) and protein pattern. The A-AEW subjected to HPP at 300 MPa had low TVC, PBC, and PC, while HSPB, EC, and VC were not detected. The S-OP, F, and T values were 7.7 mm, 4428 g and 11805 g·s, respectively. Overall, color, weight loss, and cooking loss were not different between all samples ($P>0.05$). TVB-N value of HPP-treated samples were lower than those of samples without HPP treatment ($P<0.05$). High integrity of protein pattern was found for samples treated with AEW and HPP. Additionally, the A-AEW with and without HPP treatment at 100 MPa were selected for sensory evaluation in comparison with sample without AEW and HPP treatments. No difference in likeness score of texture between all samples was observed ($P>0.05$). Therefore, the use of AEW together with HPP at 100 MPa could control microorganisms to a certain extent, which also maintained the physicochemical and sensory qualities of mussels at a satisfactory level.

Keywords: Green mussel; high-pressure processing; acid electrolyzed water; quality

DESALINATION OF FERMENTED FISH (BUDU) AND EFFECT ON PROBIOTICS MODULATION

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ABSTRACT

Budu is a traditional fermented fish product that is commonly used in Thai cuisine particularly in southern Thailand that might has a positive impact on health, especially on gut health. However, Budu contains high salt content that possibly induce hypertension, cardiovascular disease, stroke, and kidney disease if high portion is consumed. Desalination was conducted in this study to reduce salt content in Budu. This research aims to produce healthier Budu by salt reduction and evaluation on probiotics modulation by peptides. The samples were original Budu (OB), sterilized Budu (SOB), desalinated Budu (DB), desalinated Budu mixed with fish meat (DBF), and inulin (IN) as positive control. Samples were pretreated by a pilot-scale ultrafiltration (UF) unit (MWCO 300 kDa, membrane area 0.245 m^2) followed by desalination with nanofiltration unit (NF). The optimal conditions for desalination by NF were feed rate 500 L/h, TMP 5 bars, temperature <38°C, membrane MWCO 200 Da, membrane area 1.77 m^2. Salt content in OB was reduced from 25.51% to 7.67% in DB then increased to 14.30% in DBF caused by 3.5% of fish meat was added. Probiotic growth modulation by *L. acidophilus* TISTR2365 was found that Budu sample could increase the number of colonies according to the time increased up to 48 h. Similarly, *B. lactis* BB12 was increased according time up to 72 h. In contrast with Budu sample could not support the growth of pathogenic *E. coli* TISTR073. These findings showed that Budu promoted probiotics and inhibited pathogen growth.

Keywords: Budu, peptide, nanofiltration, desalination, probiotic

MECHANISM OF POLYSACCHARIDES FROM SPLIT GILL MUSHROOM (*SCHIZOPHYLLUM COMMUNE*) IN THE STIMULATION OF GLUCOSE UPTAKE IN MUSCLE CELLS

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ABSTRACT

Diabetes, a chronic health disease that affect millions of people worldwide, is characterized by the inability of the body to regulate blood glucose. Without medication or treatment, a high level of blood glucose could cause damage to tissues and organs. Although several antidiabetic medicines are available, they have been shown to produce some undesirable side effects. To date, much efforts has been put into searching for alternative antidiabetic medicines from functional foods—foods that confer health benefits beyond their nutritional values. Previous research findings have shown an antidiabetic activity of polysaccharides from many mushrooms. However, little is known about the antidiabetic activity of polysaccharides from split gill mushrooms (*Schizophyllum commune*). In this study, we utilized a green and environmental procedure to obtain the polysaccharides. Hot-water extracted polysaccharides were purified with Diethylethanolamine (DEAE) Sepharose Fast-Flow. Three major peaks obtained from the column chromatography were used to investigate the ability to stimulate glucose uptake in muscle cells. Without cellular toxicity, those 3 peak samples showed a dose-dependent stimulation of glucose uptake in muscle cells. Interestingly, we found that the 3rd peak sample which contains the lowest polysaccharide content but the highest protein concentrations was the most effective in stimulating the cells. These findings suggest an antidiabetic activity of protein-polysaccharide complexes and warrant future mechanistic investigation.

Keywords: Diabetes; split gill; polysaccharides; glucose uptake; antidiabetic activity

METABOLOMICS APPROACH TO ASSESS METABOLITE PROFILE OF MODIFIED TEMPE AND EVALUATION OF ITS ANTIOXIDANT ACTIVITIES

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ABSTRACT

Tempe, a fermented soybean product has garnered attention for its nutritional value and various health benefits. Traditionally, its production relies on the fermentation process of soybean with single inoculation of the fungus *Rhizopus oligosporus*. However, advancements on the modifications of the traditional tempe recipe have been made in effort to enhance its health properties. One notable approach involves the utilization of mixed inoculation of microbes during tempe fermentation. In this study, soybean (*Glycine max*) was subjected to different fermentation stages using mixed culture of *R. oligosporus* and the yeast *Saccharomyces cerevisiae* (modified tempe). LCMS/MS analysis was employed to identify the metabolites present in modified tempe and metabolite annotation was conducted using GNPS and SIRIUS computational tools. The findings from multivariate analysis using SIMCA 14.1 revealed distinct separation between tempe fermented during initial stages (12 and 24-hours) and tempe subjected to longer fermentation (36, 48, 60, and 72-hours), suggesting that the metabolites present in modified tempe were different across various fermentation stages. Additionally, antioxidant assays were performed to assess the antioxidant properties of modified tempe. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) results showed significant increase of antioxidant activities for tempe fermented at later stages of 60 and 72-hours ($p < 0.05$). In general, the findings offer valuable information about the metabolites profile of modified tempe at various fermentation stages and its corresponding impact on the antioxidant activities.

Keywords: Tempe; soybean; fermentation; metabolite; antioxidant

EFFECT OF *CLITORIA TERNATEA* FLOWER SUPPLEMENTATION ON THE PHENOLIC METABOLITE PROFILE AND ANTIOXIDANT ACTIVITY OF TEMPE

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ABSTRACT

Tempe is a nutritionally rich plant-based food made by solid-state fermentation of *Glycine max* (soybean) with *Rhizopus oligosporus* fungus which offers various health benefits primarily attributed to soy isoflavones. However, there is limited knowledge regarding the antioxidant properties of tempe when supplemented with phenolic rich-flower, *Clitoria ternatea* (butterfly pea). This study aims to investigate the phenolics profile and antioxidant properties of tempe supplemented with *C. ternatea* flower. The tempe extracts were analyzed using tandem high-resolution mass spectrometry (HPLC-HRMS/MS) followed by computational metabolomics including SIRIUS *in silico* annotation and GNPS feature-based molecular networking for metabolite annotation. The results revealed that *C. ternatea* supplementation introduced additional metabolites such as quercetin-3-neohesperoside, kaempferol-3-neohesperidoside and kaempferol 3-(2"-rhamnosylrutinoside) which are absent in conventional tempe extract. Furthermore, *C. ternatea* supplementation not only enhanced the overall diversity of phenolic compounds, but also increased the relative abundance of several phenolics, including kaempferol glycosides, coumestrol, naringenin, and luteolin. Subsequently, free radical scavenging ability (ABTS) of conventional and *C. ternatea*-infused tempe extracts across different fermentation periods (48, 72, and 96 hours) was conducted to measure their antioxidant capacity. The results indicate that *C. ternatea*-infused tempe exhibits enhanced antioxidant activity compared to conventional tempe. The findings of this study have the potential to innovate the tempe fermentation process by enriching its bioactive phenolic metabolite content and positioning it as a promising healthy dietary option for the future.

Keywords: metabolomics; phenolics; antioxidant; Clitoria ternatea; tempe

COMPARATIVE ANALYSIS OF METABOLITE RELEASE FROM CONVENTIONAL AND ORGANIC TEMPE DURING *IN VITRO* DIGESTION, EXAMINING THEIR ANTIOXIDANT PROPERTIES

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ABSTRACT

Tempe, a nutrient-rich soybean food rich in polyphenols including isoflavones, is valued for its health benefits, notably its antioxidants. However, concerns about glyphosate residues in crops have led to increased demand for organic soy products, including tempe. This study aimed to investigate metabolomic profiles of tempe and the bioactive potentials after *in vitro* simulated gastrointestinal digestion. Conventional soybean (CS), conventional tempe (CT), conventional tempe digesta (CTD), organic soybean (OS), organic tempe (OT) and organic tempe digesta (OTD) were analyse for various assays. The results showed that there was a significant decrease in total phenolic content from tempe extracts (CT, OT) to tempe digesta (CTD, OTD). Organic tempe digesta had higher total phenolic content (CTD = 22.55 ± 0.73 µg GAE/g, OTD = 41.36 ± 2.52 µg GAE/g) than conventional tempe digesta. Tempe digesta, particularly in organic samples, showed higher antioxidant contents compared to tempe and soybean in antioxidant assays such as DPPH and FRAP. Annotation using GNPS and the SIRIUS database revealed 34 metabolites with VIP > 1.5, log2(FC) > 1, and $p < 0.05$. From this set, 26 metabolites correlated positively with antioxidant activity, DPPH and FRAP. Molecular networking allows for the visualization of 12 major isoflavones. Overall, the results showed that tempe digesta has different metabolic profiles and bioactive potentials.

Keywords: Metabolomics; tempe; in vitro digestion; antioxidant

IDENTIFICATION OF RICE (*Oryza sativa* L.) GENOTYPES WITH HIGH ADAPTATION UNDER LESS INPUT SYSTEM

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ABSTRACT

The development of crop types that are less reliant on the extensive use of synthetic fertilisers is necessary to ensure the long-term viability of agriculture. Therefore, development of high-yielding rice varieties that can adapt to less-input systems seem to provide a long-term solution to address this issue. The objective is to carry out preliminary experiment and evaluations to identify best-performed varieties with adaptation to minimal inputs. A total of 80 rice genotypes were screened in less-input system (LISA) using alpha lattice design with three replications. Evaluation was made by collecting 13 morpho-physiological and agronomical traits. Management of LISA is based on the System of Rice Intensification approach where alternate wetting and drying (AWD) techniques were applied accompanied with appropriate natural fertilisation practices. Significant differences were found in all traits. A significant positive correlation was also observed between total grain weight (TGW) and plant height, number of tiller (NT), number of panicle (NP), panicle length (PL), spikelet fertility (SF), and spikelet sterility (SS) ($0.18 \leq r \leq 0.62$). Based on clustering analysis, all studied genotypes were divided into three clusters. Cluster I, II, and III consist of 15, 57 and 8 genotypes, respectively. The highest mean values of TGW (22.29 g) and its component including NT (11.05), NP (8.17) and SF in (150.32) were observed in Cluster III, while Cluster I have the highest mean value of other morpho-physiological traits. Mahsuri Mutan, Padi Kutip, Awal, and Tongkat Ali were the best genotypes that show high productivity and good traits under LISA. These four selected rice genotypes can be used as parental lines for the development of LISA- adapted variety.

Keywords: Low input system; sustainable; traditional rice; total grain yield

EVALUATING THE PERFORMANCE OF MALAYSIAN RICE GENOTYPES UNDER CONVENTIONAL AGRICULTURE SYSTEM

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ABSTRACT

Malaysia's rice production industry has been very slow, with less than 60 rice varieties as of 2021, compared to other rice-producing countries such as Indonesia (183), Vietnam (96), and Thailand (82). This project was aimed at screening fifty-eight Malaysian traditional, pyramidal, and modern rice genotypes to identify the best-performing varieties that could potentially serve as parental lines for population growth under the conventional agricultural system (CAS) guided by the Rice Check, Department of Agriculture (DoA). Eight traits, including days to 50% flowering (DTF), panicle number (PN), plant height (PH), panicle length (PL), and grain yield (GY), were evaluated for each genotype. All traits exhibited significant differences through ANOVA, and their consistency was confirmed by coefficients of variation (CV) and correlations (r) among genotypes. PN, PL, and PH were observed to have a highly significant positive correlation to GY ($0.50 \leq r \leq 0.39$). Based on clustering analysis, all genotypes were divided into two clusters, consisting of 33 genotypes for Cluster I and 25 genotypes for Cluster II. The highest mean values for GY (5780.64 g), PN (9), and PL (23.21 cm) were observed in Cluster I. Pongsu Seribu, Huma Kuning Lenggong, and Towuti—all three were traditional rice genotypes that emerged as varieties with the potential for high yield and favorable traits under conventional agricultural systems, making them suitable parent candidates for mixing with modern early-matured high-yielding varieties. These varieties also exhibited higher and more consistent PN compared to other genotypes. Therefore, these genotypes are recommended for an advanced breeding program, focusing on producing crossed populations and further evaluation for improved trait adaptation and yield stability.

Keywords: Conventional agricultural system; Oryza sativa; high yield; plant breeding

PRIORITISING FUNCTIONALLY SIGNIFICANT STRESS RESISTANCE MUTATIONS IN CROPS USING PROTEIN STRUCTURES

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ABSTRACT

Crops encounter significant challenges due to environmental stresses, such as pathogens, salinity, and drought. Genome-wide association studies (GWAS) tend to identify a large number of genetic mutations responsible for stress resistance. This study leverages the crop 3D protein models from AlphaFold Protein Structure Database to explore the impacts of stress-resistant missense mutations on plant protein structures, aiming to prioritize potentially functional mutations for plant molecular geneticists. The protein structures of rice, maize, and soybeans were dissected into structural domains using our in-house CATH-Assign protocol. Domains with low predicted local distance difference test (pLDDT) scores, high disorder, poor packing, or non-globular characteristics were filtered out. Stress-resistant mutations (covering both abiotic and biotic stresses) from the GWAS atlas were then assessed based on amino acid chemical properties, MutPred2 mutation pathogenicity, and proximity to functional sites. Out of 197 stress resistance mutations, 95 could be mapped to structural domains, with 86 classified as non-pathogenic. This aligns with expectations, as these mutations often represent favourable gain-of-function changes crucial for plant adaptation. Notably, 70 mutations were in close proximity to a functional site (e.g. ligand-binding, post-translation modification, and protein interfaces). To further understand the impact of mutations on specific protein interaction partner, AlphaFold2-Multimer was employed to model the 3D structures of protein complexes, shedding light on changes in binding affinity. This study marks the first time high-quality AlphaFold models have been employed to unravel pathogenicity and functional sites associated with stress-resistant mutations in plants. Our future plans include expanding the study to explore diverse crop traits and develop strategies to enhance plant resistance.

Keywords: Protein structures; Stress modulating mutations; Functional impacts; Plants; Bioinformatics

COLD-ACTIVE *GLACIOZYMA ANTARCTICA* ARGINASE CRYSTAL STRUCTURE REVEALS FLEXIBILITY AT THE ACTIVE SITE

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ABSTRACT

Glaciozyma antarctica PI12 is a psychrophilic yeast isolated from Antarctica. Structural genomic initiative on this yeast has allowed characterization of an arginase (LAN_05_105, GaArg) using biochemical and biophysical approaches. Arginase is a metal-dependent metalloenzyme catalysing the conversion of L-arginine to L-ornithine and urea. Crystallization of the purified apo arginase was able to produce a hexagonal crystal in R3 space group. X-ray diffraction data was collected and the crystal structure was solved at 2.35 Å resolution by molecular replacement. GaArg is folded in a αβα sandwich fold, and it is similar to the human arginase II structure (PDB ID 4hze) with RMSD 1.09 Å/244 Cα atoms. The GaArg crystal structure contains several disordered regions with missing electron density particularly at the active site compared to other arginase homologs indicating that GaArg has a higher conformational flexibility. Comparison with other homologous structures showed that these regions are putative loops. Overall, GaArg is an

arginase that adopts higher number of flexible substructures formed by small and uncharged amino acids that allows it to catalyse optimal activity at cold temperatures. The relationship between the structure and function of GaArg provides insights into the potentiality of new applications in various biotechnology and pharmaceutical industries.

Keywords: Psychrophilic catalyst; enzyme assay; L-arginine; structural flexibility; recombinant enzyme

ELUCIDATING POTENTIAL GENES IN GLUCOSINOLATE BIOSYNTHESIS USING NETWORK BIOLOGY

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ABSTRACT

Glucosinolates (GSLs) represent sulfur and nitrogen-containing secondary metabolites prevalent in *Brassicaceae* plants, including *Arabidopsis thaliana* and various cruciferous vegetables. These compounds play pivotal roles in plant defence mechanisms and exhibit established anticancer properties in humans. Despite significant advancements in understanding GSL biosynthesis, transport, hormone pathways, and regulatory mechanisms in the model plant *A. thaliana*, a comprehensive elucidation of the GSL mechanism remains challenging. In this study, we implemented a 'guilt-by-association' (GBA) approach to predict genes encoding proteins potentially involved in GSL mechanisms. Employing a co-expression network constructed with 113 known GSL genes sourced from the Sulfur Containing Compound Database (SuCComBase - https://plantscc.org/), we queried co-expression databases such as ATTED, GeneMANIA, Search Tool for Retrieval of Interacting Genes/Proteins (STRING), and AraNet. The resultant network, comprising 752 genes and 9,121 edges, underwent clustering analysis using the DPClusO algorithm to identify GSL network clusters. Evaluation through pathway enrichment analysis revealed the application of graph clustering analysis to unveil potential genes not captured through sequence-based searches. In this research, we propose ARIA-interacting double AP2-domain protein (ADAP) transcription factor (TF) as a putative negative regulator in aliphatic GSL biosynthesis, substantiated by the observed over-expression of downstream aliphatic GSL genes; UDP-glycosyltransferase 74C1 (*UGT74C1*) and isopropylmalate isomerase small subunit 1 (*IPMI1*) in the ADAP plant-specific EAR-motif repressor domain (SRDX) line. The over-expression of the *ADAP* gene in the ADAP-SRDX line suggests a regulatory role of the TF in negatively affecting the expression of *UGT74C1* and *IPMI1* through a

putative feedback mechanism in *A. thaliana*. This bioinformatics pipeline showcases the potential to unravel intricate biological mechanisms and contribute to the forefront of crop improvement of GSL-containing plants.

Keywords: glucosinolate; Arabidopsis thaliana; co-expression; network; graph clustering.

ANTIBACTERIAL AND ANTI-BIOFILM ACTIVITIES OF MICRO-PARTICLES CONTAINING TURMERIC EXTRACT AND CHITOSAN AGAINST GASTROINTESTINAL PATHOGENS

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ABSTRACT

Health problems associated with gastrointestinal infections are prevalent throughout the world. This research aims to investigate the antibacterial and anti-biofilm activities of micro-particles containing turmeric extract and chitosan (TC microparticles) on Gram-positive and Gram-negative enteric pathogens. A total of 6 bacteria, including *Staphylococcus aureus* ATCC25923, *Listeria monocytogenes* 11-2, *L. monocytogenes* PR1, *Bacillus cereus* PSU3874, *Vibrio parahaemolyticus* ATCC17802, and *V. cholerae* PSU6072 were tested in this study. The time kill assay was conducted using TC microparticles at a concentration ranged between 2MIC to 1/8MIC (4,096 - 128 ug/ml). Results revealed that TC microparticles inhibited the growth of all bacteria by concentration and time-dependent killing. Furthermore, biofilm inhibition assays were performed using TC microparticles at MIC and sub-MICs on a 96-well cell culture plate, using 3 representative high-biofilm forming bacteria, including *S. aureus* ATCC25923, *B. cereus* PSU3874, and *V. cholerae* PSU6072. It was found that TC microparticles at 1/2MIC significantly inhibited ($p<0.05$) biofilm formation of *S. aureus* ATCC25923 with a 27.57 ± 9.93% biofilm inhibition rate. Therefore, this study sheds light on the potential of TC microparticles for treating bacterial enteric infections.

Keywords: Antibacterial; biofilm; Curcuma longa; enteric infection; turmeric

Effect of Sugarcane *(Saccharum officinarum* L.*)* Molasses on Vasodilation in Rat Thoracic Aorta

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Abstract

Sugarcane molasses (SM) is a viscous syrup produced during sugar processing. Its main composition consists of carbohydrates, followed by moisture, ash, and bioactive compounds. SM has the potential to be utilized to provide health benefits. This study aims to determine the effect of SM on vasodilation in the rat thoracic aorta using an isolated tissue model (*ex vivo*). A healthy male Wistar rat was isolated, and the tracing was recorded using Power Lab. The result of phenylephrine (PE) induced maximal contraction at 10^{-4} mol/L in the tissue bath, and PE to induce vasoconstriction. The result of acetylcholine (ACh) as a positive control was presented. The linea vasodilation of the rat thoracic aorta at concentrations of 10^{-5} and 10^{-4} mol/L of thoracic aorta vasodilation was significantly different ($p < 0.05$) compared with the control group. In addition, SM can reduce PE-induced vasoconstriction with 25.22±1.46% of vasodilation at a concentration of 10 mg/ml. In the future, sugarcane molasses (SM) may be studied in *vivo* for its potential anti-hypertensive effects, and it could also be used in the creation of nutritionally alternative foods.

Keywords: Sugarcane molasses, vasodilation, vasocontraction, rat thoracic aorta

Introduction

Saccharum officinarum L. commonly known as sugarcane molasses (SM), is a dark brown liquid with viscous texture and a caramel-like aroma. SM is a by-product that is obtained during the crystallization process of sugar production (Zhang et al., 2020). In the previous studies have identified various chemical components in crude SM, including phenolic compounds such as gallic acid, ferulic acid, rutin, quercetin, and reducing sugars.

Sugarcane molasses (SM) has been found to have an effect on DNA oxidative damage. This effect is closely linked to the antioxidant capacity of the SM fractions that were studied (Guimaraes et al., 2007). Several studies, including those on oxidative stress in HUVECs, have shown that rutin can enhance the production of nitric oxide (NO) (Ugusman et al., 2014). Additionally, antioxidants are known to enhance the biological effects of NO by protecting against cell or tissue damage induced by reactive oxygen species (ROS) through oxidation (Ignarro et al., 2006). Researchers have observed strong and positive correlations between the ABTS radical scavenging activity and the polyphenol levels in SM. Previous reports have indicated that SM plays a role in reducing the generation of reactive nitrogen species (RNS) (Wang et al., 2011). Various biological pathways associated with RNS involve multiple radicals and are connected to NO. These compounds have diverse biological functions, including antioxidant properties, anti-inflammatory effects, inhibition of angiotensin-converting enzyme (ACE), and potential impacts on cardiovascular diseases (Liu et al., 2023). In a pharmacological study of phytochemicals in sugarcane, various compounds such as phenolic acids, flavonoids, and different glycosides found in sugarcane products were investigated (Jia et al., 2019).

Currently, traditional medicinal treatments using medicinal plants are being used to treat various noncommunicable diseases, including diabetes mellitus, cardiovascular disease, and inflammation (Liyanagamage et al., 2020). SM has gained popularity in human diets and is used to

treat numerous diseases such as arthritis, ulcers, dermatitis, hair damage, eczema, and hypertension (Rahiman and Pool, 2010). Oxidative stress is known to contribute to endothelial dysfunction, which can play a role in the initiation and progression of cardiovascular diseases. Antioxidants are recognized for their ability to enhance the biological effects of NO, leading to vasodilation in vascular smooth muscle. Previous research has shown that SM can increase nitric oxide production in HUVEC cells, potentially contributing to its anti-hypertensive effects. Therefore, this study aims to investigate whether bioactive compounds from SM can enhance vasodilation in the thoracic aorta when faced with PE-induced vasoconstriction in tissue bath. Utilizing SM may be a valuable approach to reducing the risk of cardiovascular diseases, highlighting the potential benefits of incorporating SM into diets for improved health outcomes.

Materials and Methods

1. Materials

Sugarcane molasse, phenylephrine hydrochloride (Sigma, China), acetylcholine (sigma, USA), Krebs-Henseleit (KH) solution were prepared using potassium chloride (KCl) (KEMUS, Australia), magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) (KEMUS, Australia), potassium hydrogen phosphate (KH_2PO_4) (KEMUS, Australia), Sodium hydrogen carbonate ($NaHCO_3$) (QReC, New Zealand), calcium chloride ($CaCl_2$)) (KEMUS, Australia), D-glucose ($C_6H_{12}O_6$) (VWR Chemicals, Belgium), sodium chloride (NaCl)) (KEMUS, Australia) , and ascorbic acid (POCH SA, Poland).

2. Methods

2.1 Sample preparation

SM was obtained from a factory in Thailand. 1 g of SM was diluted with distilled water, and the final stock solution had a concentration of 1,000 mg/ml. SM were kept in the freezer (-20 °C) until analyzed.

2.2 Animal preparation

Healthy male Wistar rats weighing between 250 and 300 g were obtained from Nomura Siam International Ltd., Bangkok, Thailand. All the animals were kept in cages under controlled conditions, with a temperature of 23–25 °C, 50–55% relative humidity, and 12-hour light/dark cycles. They were provided with commercial animal feed (S.W.T., Thailand) and had free access to RO water. All rats were handled and managed in accordance with the approval of the Prince of Songkhla University Animal Ethics Committee (Project License Number Ref. 32/2021).

2.3 Tissue isolation

Following the experimental protocol, the mice were anesthetized with thiopental sodium (120 mg/kg, i.p.) and incisions were made along the linear alba of the anterior abdominal wall to access the thoracic cavity (adapted from Benter et al., 2006). The thoracic tissues were carefully isolated and transferred to a petri dish containing an oxygenated Krebs-Henseleit (KH) solution and carbogen gas (95% O_2, 5% CO_2) throughout the procedure. The thoracic aorta is approximately 2–3 cm long. Cut the thoracic aorta to a size of 5 mm each to prepare the aortic ring (mechanical disruption) by inserting a 0.6 mm stainless steel rod into the blood vessel. It was placed in an organ bath containing KH solution at 37 °C to allow carbogen gas to pass over time. The hanging vessels are attached to two L-shaped hooks: the lower one attached to the base of the organ bath, and the top hook connected to the force transducer. This is connected to the bridge amplifier shown in Figure 1. The date was recorded as the result by Power Lab (adapted from Jespersen et al., 2015).

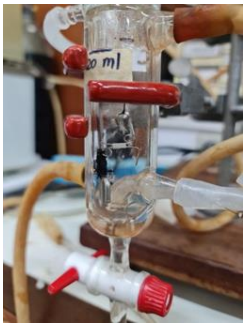

Figure 1. Thoracic tissue in tissue bath under controlled conditions

2.4 Vasodilation of isolated thoracic aorta

The vasoconstrictor response to phenylephrine was observed at concentrations ranging from 10^{-9} to 100 mol/L and 10^{-7} to 1 mol/L, respectively. After preparing the aortic ring, it has to be under resting tension before starting the experiment. To begin, added 200 µl of the stock solution of phenylephrine (PE) to the final concentration in the organ bath 10^{-9} to 10^{-2} mol/L with cumulative concentrations. The concentration of phenylephrine (PE) was chosen for the next vasodilation study due to its ability to induce a 70 percent contraction of the aortic ring.

$$\% \text{ Increase from resting tension} = \frac{(T_{PE} - T_C)}{T_C} \times 100$$

T_{PE} = Tension of PE
T_C = Tension of rest

The vasodilator response to acetylcholine (ACh) at concentrations ranging from 10^{-10} to 10^{-2} mol/L after the aortic ring caused precontraction by PE, which induced tension above. To begin the experiment, add a droplet with 200 µl of Ach, which will result in a final cumulative concentration in the organ bath 10^{-12} to 10^{-4} mol/L cumulative concentration. The concentration of ACh that causes vasodilation is 80%. After suspending blood vessels and allowing them to adjust to a resting tension of 1 g, expected for 30 minutes for the constriction of blood vessels to stabilize at the baseline. PE-induced maximal contraction of the aortic ring was initiated, followed by the addition of droplets of stock solution of SM at 6 concentrations to be concentrated in organ bath 10^{-3} to 10^{3} mg/ml, respectively, cumulatively in the organ bath. The tension was recorded throughout the experiment every 5 minutes. Vasodilation percent was calculated by the equation below:

$$\%\text{Vasodilation} = \frac{(T_{PE} - T_C) - (T_{VSX} - T_C)}{(T_{PE} - T_C)} \times 100$$

T_{PE} = Tension of PE
T_C = Tension of rest
T_{VSX} = Tension of vasorelaxant (ACh, and SM)

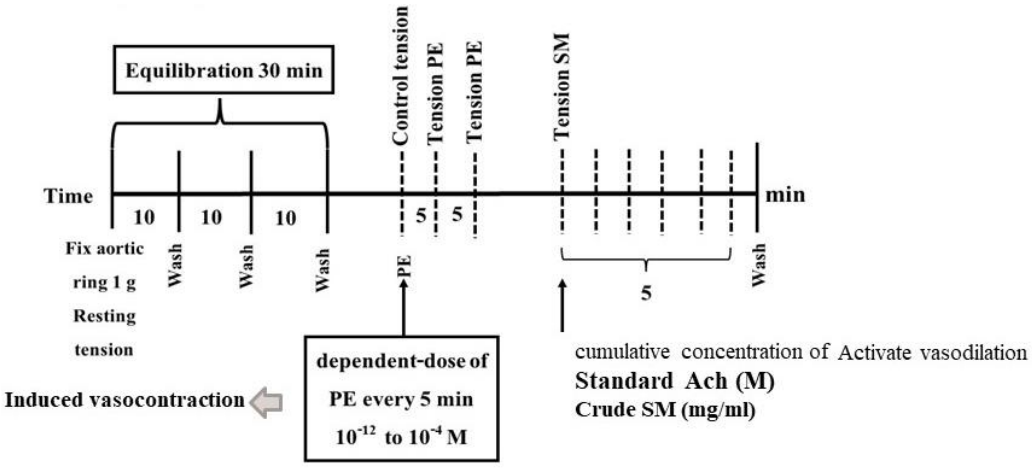

Figure 2. Chart of vasodilation of isolated thoracic aorta

2.5 Statistical analysis

The data presence with mean and standard mean error was calculated for each treatment and compared between samples using analysis of variance (ANOVA) one-way with Turkey (HSD).

Results and Discussion

This research investigated the effects of SM on vasodilation in the rat thoracic aorta, indicating a potential decrease in the risk of hypertension or cardiovascular diseases (CVD).

Table 1 shows the consequences of phenylephrine (PE) vasoconstriction in an isolated thoracic aorta. Resulting from the cumulative contraction response curve to PE, it led to 4.75 ±1.90, 14.52±2.58, 30.68±5.42, 55.42±2.57, 61.44±3.33, 68.97±4.41, 72.96±4.34, 80.43±2.71, and 90.24±2.88% of vasoconstriction at the concentration of 10^{-10} to 10^{-2} mol/L, respectively, at contractions of 10^{-5} to 10^{-3} and 10^{-2} mol/L of the PE effect. The vasoconstriction was not significantly different ($p \geq 0.05$), and the concentration of PE to have more than 70% induced vasoconstriction at the concentration of 10^{-5} to 10^{-2} M was not significant ($p \geq 0.05$). It was found that PE caused NO release from aortic rings and abolished the increase in the Ca^{2+} mechanism (Zhao, J. and Majewski, H., 2008). PE-induced vasoconstriction is caused by the vascular smooth muscle adrenergic receptor being activated, which mobilizes both intracellular and extracellular Ca^{2+} pools (Salahdeen, H.M., and Murtala, B.A., 2012). Figure 3 shows that dropping vasorelaxants occurred through the cumulative application of increasing concentrations of PE, ACh, and crude SM.

Table 1. Percentage of thoracic aorta vasoconstriction induced cumulative phenylephrine concentration

Log [Concentration of PE] (mol/L)	Maximal contraction (%)
-10	4.75±1.90
-9	14.52±2.58
-8	30.68 ± 4.52
-7	55.42 ± 2.57
-6	61.44 ± 3.33
-5	68.97 ± 4.41
-4	72.96 ± 4.34
-3	80.43 ± 2.71
-2	90.24 ± 2.88

Data was presented by average ± S.E.M (n = 5)

Resulting from ACh-induced vasodilation of the rat aortic ring, the response showed vasodilation at concentrations of 10^{-12} to 10^{-4} mol/L through cumulative concentration. The vasodilation response curve displayed percentages of vasodilation at 21.75±5.49, 37.68±6.12, 50.79±6.83, 63.43±8.77, 75.77±9.08, 91.23±8.20, 103.42±7.51, 108.05±8.20 and 108.39±4.01% vasodilation, respectively. Furthermore, when the vasodilation responses of the aortic ring were activated to 70% by PE at a concentration of 10^{-5} mol/L. The maximal vasodilation response created by ACh at 10^{-6} mol/L was 91.23±8.20% vasodilation, and the rat aortic ring response to increased vasodilation at the concentration of 10^{-12} to 10^{-2} mol/L of ACh was significantly different ($p < 0.05$) as shown in Figure 4 (A). Resulting in crude SM at concentrations of 10^{-5} to 10^{0} mg/ml, there was an increase in vasodilation on the rat aortic ring with 7.74±0.86, 9.64±0.91, 16.89±1.25, 19.83±1.87, 17.20±3.96, 25.22±1.46, and 21.62±0.88% vasodilation, respectively. SM was shown to decrease vasocontraction and significantly inhibit PE-induced vasoconstriction in a K+ solution in Figure 4 (B). Crude SM was shown to reduce vasoconstriction and significantly inhibit PE-induced vasoconstriction in a K^+ solution. The results of this investigation showed that crude SM. Potentially, by influencing a Ca^{2+} dependent mechanism, it had a vasorelaxant effect on isolated rat thoracic aorta. When it was activated with PE in a Ca^{2+}-free K-H solution (Devi et al., 2012). PE also found that it caused NO release from the thoracic aorta. It has been shown that the receptor-operated Ca^{2+} channels (ROCC) and voltage-dependent calcium channels (VDCC) of SM include flavonoids that prevent vasoconstriction induced by the influx of extracellular Ca^{2+} (Gan et al., 2016). They have a variety of mechanisms, some of which are dependent on the endothelium and some of which are independent (Akhlaghi and Bandy, 2009). In addition, polysaccharides present in SM serve as a source of antioxidants that inhibit the free radical chain reaction and decrease the production of free radicals that generate NO (Mu et al., 2021). They are involved in regulating carbohydrate metabolism, inhibiting certain enzymes, and preventing diseases such as neurological and cardiovascular disorders (Ji et al., 2019). Vasodilation plays an important role in the treatment of hypertension as it impacts blood pressure in the cardiovascular system.

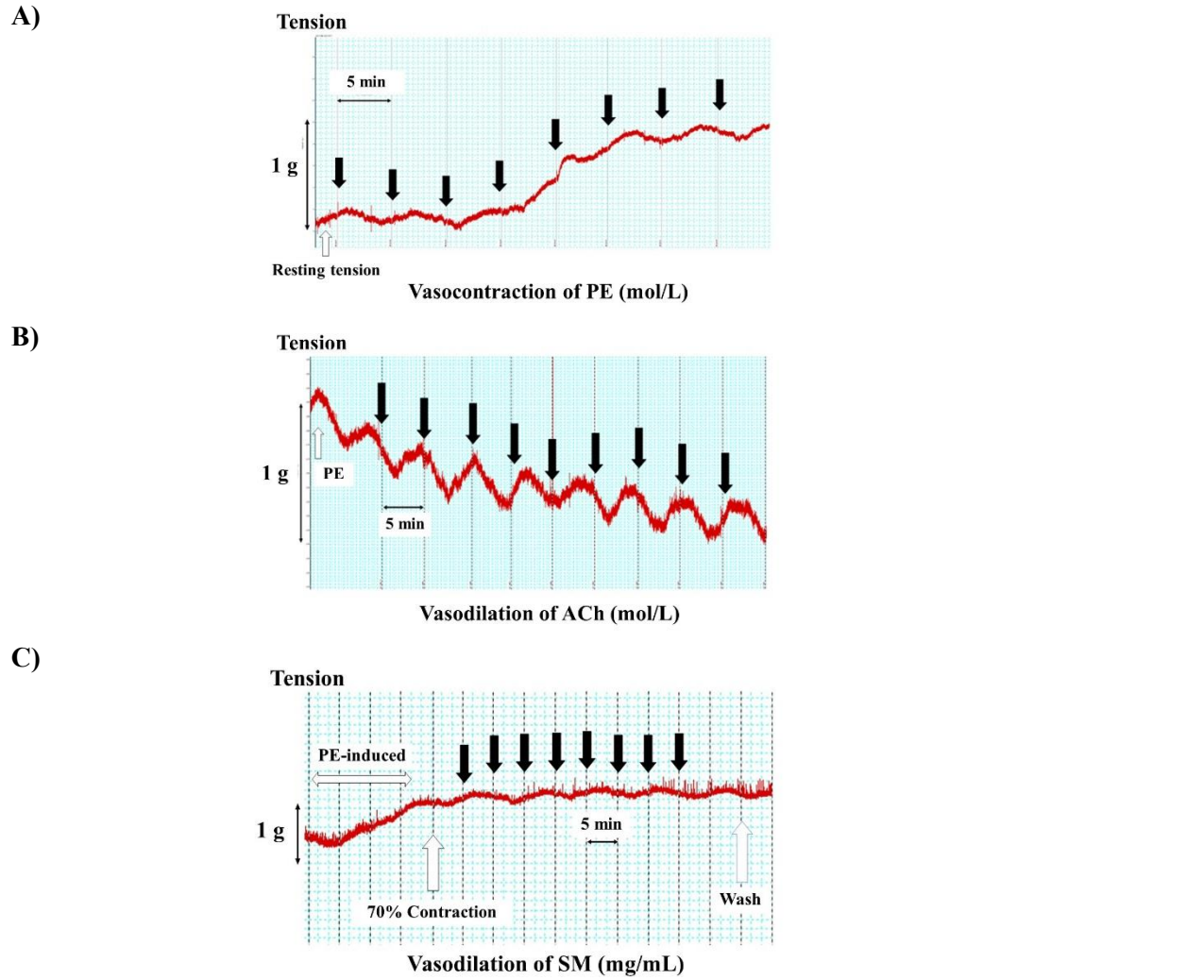

Figure 3. Representative Power Lab tracing of vasodilation study on rat thoracic aorta induced by phenylephrine by *ex vivo* assay. (A)Tracing of PE on thoracic aorta contraction, (B) Tracing of ACh on thoracic aorta vasodilation, and (C) Trancing of SM on thoracic aorta by *ex vivo* assay.

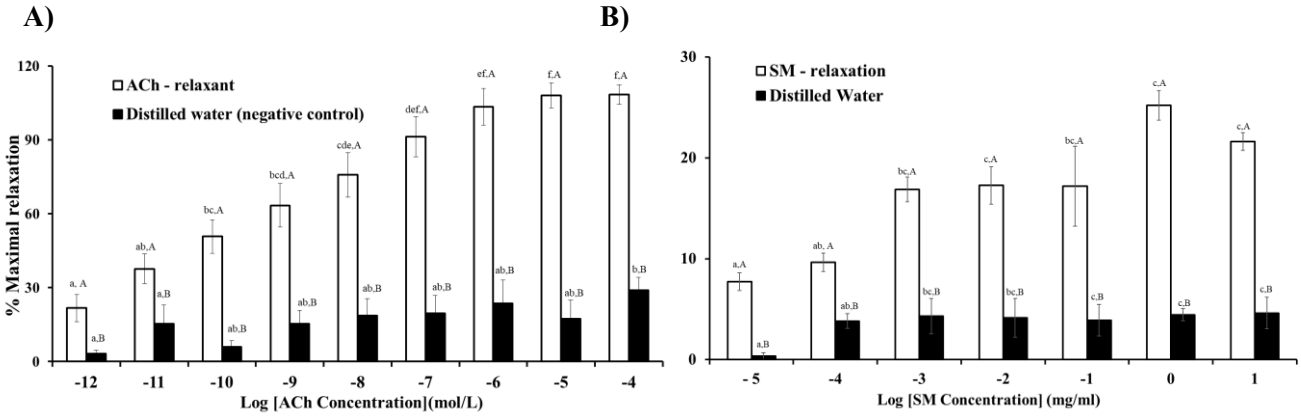

Figure 4. Thoracic aorta on vasodilation study contraction induced by PE (A) Thoracic aorta on vasodilation of ACh and (B) Thoracic aorta on vasodilation of SM (n = 5), with *ex vivo* assay.

Conclusion

Investigation of SM vasodilation on PE induction at a concentration of 10-4 mol/L resulted in the highest maximal contraction. SM on vasodilation decreased vasoconstriction, resulting in a 25.22±1.46% vasodilation at a concentration of 10 mg/ml. Thus, it can be concluded that SM exhibits pharmacological properties that increase vasodilation of thoracic tissue as demonstrated by *ex vivo* assay. With this valuable information, the investigator proceeded to develop a functional product using SM for the next study.

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ANTIBACTERIAL EFFECT OF BLACK SOLDIER FLY LARVAE EXTRACTS AGAINST *V. CHOLERAE* AND *V. PARAHAEMOLYTICUS*

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ABSTRACT

Currently, antibiotics remain a common tool in shrimp farming to control bacterial infections, given their prevalence in aquaculture settings, thereby posing a significant risk of antibiotic resistance development. *Vibrio cholerae* and *V. parahaemolyticus*, both members of the *Vibrio* species, are recognized as significant pathogens of shrimp which can be transmitted to humans through the consumption of contaminated seafood. Black soldier fly larvae (*Hermetia illucens*) have been reported to be a potential source of fatty acids and antimicrobial peptides (AMPs) which have the ability to act as antimicrobial agents against many pathogens. This study aimed to investigate the antibacterial effect of black soldier fly larvae extracted by 0.01% acetic acid or ethanol on growth of *V. cholerae* and *V. parahaemolyticus*. The result showed that the ethanolic extract exhibited a tendency to be more effective against growth of *V. cholerae* and *V. parahaemolyticus* than acetic acid extract in a concentration-dependent manner. These findings suggest the potential of black soldier fly larvae extracts, particularly the ethanolic extract, as natural antimicrobial agents against *Vibrio* species. Further research is warranted to elucidate the underlying mechanisms and explore their application in combating bacterial infections in aquaculture and food safety.

Keywords: Antibacterial activity, black soldier fly, Hermetia illucens, Vibrio cholerae, Vibrio parahaemolyticus

BIODEGRADATION OF PHENOLIC COMPOUNDS IN PALM OIL MILL EFFLUENT BY WHITE ROT FUNGI

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ABSTRACT

Palm oil mill effluent (POME) generally contains high organic content and phenolic compounds. Phenolic compounds exhibit inhibitory effects on microorganisms and are not easily degraded. In this study, two white rot fungi, namely *Trametes hirsuta* AK04 and *Lentinus squarrosulus* LL12, were tested separately (monoculture) and jointly (mixed culture) for their capability to degrade phenolic compounds in POME. These strains were well compatible with a slightly higher growth rate when grown together on potato dextrose agar plates. The fungal strains AK04 and LL12 separately degraded 58.0 % and 36.8 % of phenolic compounds in POME, respectively, while a mixed culture of both fungi could remove 33.81 % of 863 mg/L of phenolic compounds after 8 days of incubation at 30 °C and 120 rpm shaking. The activities of ligninolytic enzymes, namely manganese peroxidases (MnP) and lignin peroxidases (LiP), were detected and found to be correlated with the reduction of phenolic compounds in POME while incubating with strains AK04 and LL12, respectively. In contrast, the production of laccase (Lac)was very low compared to the aforementioned two enzymes. Although a mixed culture showed less performance in the degradation of phenolics than strain AK04 alone, higher decolorization efficiency was obtained using them together. This result is associated with LiP production. In addition, about 17.52-18.71 % of phenolic compounds were removed by the heat-killed fungal cells, indicating that the removal of these compounds was attributed to enzymatic processes (biodegradation) and biosorption onto the fungal mycelia.

Keywords: Phenolic compounds; Ligninolytic enzymes; Palm oil mill effluent

were present at diagnosis (13-17 mutations per patient) and 49 variants present at relapse (12-20 mutations per patient). There were 35 new variants that were detected at relapse but not present at diagnosis. Six potential driver mutations (*Proto-oncogene c-KIT (KIT), Cell division cycle 73 (CDC73), Hepatocyte Nuclear Factor 1 Homeobox A (HNF1A), RNA-binding motif 10 (RBM10), Zinc Finger MYM-Type Containing 4 (ZMYM4)* and *ETS variant 6 (ETV6)*) were identified in predicting relapse, with recurrent mutations in the ETV6 gene. This study has uncovered the mutational landscape of relapse in our local childhood AML patients and have identified mutations in key genes involved in the pathogenesis of myeloid leukemia.

Keywords: Acute myeloid leukemia; relapsed AML; children; whole genome sequencing; mutation; *ETV6*

EXPLORING THE ANTIOXIDANT CAPACITY OF RICE-DERIVED BIOACTIVE PEPTIDES IN *SACCHAROMYCES CEREVISIAE*

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Abstract

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) play an imperative role in causing oxidative damage. Antioxidants, capable of scavenging free radicals, offer the means to counteract oxidative stress and enhance organismal vitality. In recent investigations, fermented foods are emerging as a promising frontier in research. Subsequently, yielding unique bioactive peptides with free radical-scavenging potential. Notably, Hom Nin rice (*Oryza sativa* L.) contains natural compounds with reported antioxidant activity, addressing the underlying mechanisms of certain diseases. *Saccharomyces cerevisiae*, a unicellular fungus utilized as a model for human studies, offers advantages due to its well-annotated genome and evolutionary conservation of essential biological processes. To assess the antioxidant activity of the derived peptides, DPPH (2,2-diphenyl-1-picrylhydrazyl) assay has been used and it was observed that peptide 18 has the highest free radical scavenging potential. Furthermore, *in vivo,* assessments has involved subjecting *S. cerevisiae* to the concentration of 6 mM hydrogen peroxide to evaluate the ability of the organism to combat oxidative stress and mitigate the effects of free radical-induced damage with the peptide treatments. The results showed that peptide 18 protected *S. cerevisiae*, wild-type strain BY4741 and Δ*tor1*. Contrastingly, peptide 18 was unable to shield the cells from the hydrogen peroxide damage in the Δ*sod2*, indicating it as a likely target for the peptide in enhancing cellular resilience against oxidative stress.This study will integrate both i*n vitro* and *in vivo* approaches to provide a comprehensive understanding of the free radical scavenging of the peptides obtained from the fermentation of rice.

Keywords: Antioxidant; bioactive peptides; fermentation; Hom Nin rice; Saccharomyces cerevisiae

Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) collectively describe free radicals and other highly reactive oxidants. Free radicals are highly reactive molecules that are produced in the body as a result of normal metabolic processes, exposure to environmental toxins, and even during immune system responses (Pham-Huy et al., 2008). These molecules can cause damage to cells and DNA, leading to various health implications such as accelerated aging, inflammation, and increased risk of chronic diseases like cancer, heart disease, and neurodegenerative disorders (Young et al., 2001).

There are various mechanisms, including endogenously produced antioxidants enzymes and those supplied through exogenous sources such as foods, to counteract oxidative stress, with the roles of antioxidants involving neutralizing excess free radicals, protecting cells against their toxic effects, and contributing to disease prevention (Young et al., 2001). Fermented foods are emerging as a promising contributor towards enhancing the overall well-being of an organism. The microbial transformation of raw materials during fermentation produces bioactive compounds, such as probiotics, peptides, and organic acids, which are known for their potential health-promoting effects (Das et al., 2020). Fermentation also generates bioactive metabolites with antioxidant and anti-inflammatory properties, which may counteract oxidative stress and inflammation (Guo et al., 2023). Bioactive peptides are defined as protein fragments that are composed of 2-20 amino acid residues, which possess a variety of beneficial biological properties (Guo et al., 2023).

Rice (*Oryza sativa* L.) is a staple agricultural crop in several Asian countries including Thailand. The scientific importance of rice in the context of antioxidants is noteworthy as they are a rich source of natural antioxidants. Peptides derived from rice can exhibit antioxidant ability and it signifies their potential role in shielding the cells against damage instigated by free radicals. Moreover, it has been substantiated through prior research that the fermentation of unpolished black rice with a defined microbial starter culture resulted in the ability to scavenge free radicals through *in vitro* analysis (Sangkaew and Yompakdee, 2020).

Saccharomyces cerevisiae, commonly known as budding yeast, serves as a unicellular fungus extensively used as a model organism for studying the human system and age-related diseases. Moreover, the well-characterized genome of the yeast makes it a valid model to study complex cellular processes in a simple environment (Janssens et al., 2016). The high degree of conservation makes yeast an extremely reliable biological model to further examine the complex interwoven pathways (Zimmermann et al., 2018). The superoxide dismutase, *SOD1* and *SOD2* genes, are the first line of antioxidant defense against oxygen free radicals (Wang et al., 2018). Antioxidant enzymes are pivotal in modulating cellular redox homeostasis in various organisms. *TOR1* (target of rapamycin) is a pivotal regulator within the nutrient-sensing pathway, holding significant potential to influence the antioxidant process (Calap-Quintana et al., 2015). *TOR1* inhibition has been associated with increased stress resistance, improved mitochondrial function, enhanced autophagy, and reduced pathologies (Kapahi & Zid, 2004). The interaction between these two genes has been illustrated in Figure 1, suggesting an intricate involvement and interaction between *TOR1* and *SOD* to facilitate the antioxidant defense for a prolonged survival of the organism. Further examination of these crucial genes will assist in providing the influence of the tested peptides towards maintenance and repair of the cell against stress.

Therefore, this study aimed to further examine the constituents present in the fermented rice samples, particularly, of the peptides to better understand their pivotal role in modulating pathways associated with antioxidants.

Materials and Methods

1. Preparation of fermented Hom Nin rice samples to screen for bioactive peptides

The preparation of the fermented Hom Nin samples was performed as described according to Sangkaew and Yompakdee (2020). The fermented samples, were purified with the assistance of the C18 HPLC column (3.9 x 150 mm; Waters, USA) and fractionated using OFFGEL fractionator by the laboratory of Dr. Sittiruk Roytrakul, from the Functional Proteomics Technology, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency. Thirteen peptides were derived from the rice samples for further analysis.

2. Screening of the peptides derived from the fermented Hom Nin rice by DPPH radical scavenging activity

The antioxidant activity of the peptide fractions derived from the fermented Hom Nin rice was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The peptide samples at different concentrations along with ascorbic acid (positive control) were incubated with the DPPH solution. Followed by measuring the absorbance of the mixed solution at 515 nm. The antioxidant activity was calculated as follows: DPPH scavenging activity (%) = [(A-B) ÷ A] x 100, whereby 'A' and 'B' are the absorbance of 515 nm of water (as the untreated control) and the sample (Baliyan et al., 2022). All the samples were tested in triplicates.

3. Investigating the protective effects of the selected peptide on hydrogen peroxide-induced oxidative stress

To investigate the free radical scavenging activity of the selected peptide derived from the fermented rice samples, *S. cerevisiae*, wild-type strain BY4741 (*MATa his*3Δ1 *leu*2Δ0 *met*15Δ0 *ura*3Δ0) and isogenic

mutant strains (i.e., *sod2* and *tor1*), were obtained from Yeast Genetic Resource Center, Japan and treated with 6 mM of hydrogen peroxide in the presence of the selected peptide. The survivability of the *S. cerevisiae* strains was monitored, and the selected peptide was tested for its protective effects against hydrogen peroxide. *S. cerevisiae* strains were inoculated in YPD medium, with and without treatment of the selected peptide at 30°C, 200 rpm. After incubation, cell suspension were aliquoted and treated with an appropriate concentration of hydrogen peroxide for 1 hour (Tran & Green, 2019). The qualitative analysis of the viability will be conducted via spot assay. The yeast cell suspension (5 µl) were spotted on YPD agar and after incubation for 2-3 days at 30°C, the colony of the yeast were visualized (Spencer et al., 2014). The positive control for this experimental analysis was quercetin as it is a known lifespan-extending compound (Grünz et al., 2012). All the experiments were independently performed in triplicates.

4. Statistical analysis

All of the experiments were performed in triplicates to verify the obtained results and the data were presented with mean and standard errors. The analysis were performed by using GraphPad Prism (San Diego, USA). The statistical methods will involve the use of one-way analysis of variance (ANOVA) to determine statistically significant differences between the independent test groups with Dunnett's Multiple Comparisons tests.

Results and Discussion

The peptides obtained from the fermented Hom Nin rice samples were given by Ms. Yanika Chontachot, Department of Microbiology, Faculty of Science, Chulalongkorn University, and an investigation into their antioxidant potential was conducted through the assessment of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity (Baliyan et al., 2022). Ascorbic acid, a well-established antioxidant, was employed as a positive control for comparative analysis (Njus et al., 2020). The DPPH free radical scavenging activity of the rice-derived peptides was measured, and the results were compared with those of ascorbic acid to assess the relative antioxidant efficacy as depicted in Figure 2. To validate the antioxidant properties of peptide 18, we conducted additional experiments by observing its DPPH free radical scavenging activity at various concentrations as shown in Figure 3. The findings indicated peptide 18 as a promising candidate, for its antioxidant activity and therefore, it was further examined for its potential activity in this study.

In order to assess the *in vivo* antioxidant potential of peptide 18, hydrogen peroxide stress-induced assay was conducted using *S. cerevisiae* wildtype, as well as in two isogenic mutant strains (*sod2* and *tor1*). The cells were subjected to hydrogen peroxide treatment to induce stress, and the ability of peptide 18 to enhance cell survival under such conditions was evaluated. Quercetin, recognized for its robust antioxidant properties and free radical scavenging abilities, was included in the study as a positive control (Grünz et al., 2012). The results revealed that peptide 18, at the specified concentrations, exhibited a protective effect in wildtype cells compared to untreated cells, as shown in Figure 4. Interestingly, a similar trend was observed in *Δtor1* , suggesting that *TOR1* may not be the direct target of peptide 18, as represented in Figure 5. However, peptide 18 demonstrated the ability to upregulate the stress response in the cell, enabling survival in the presence of hydrogen peroxide. Contrastingly, in the *Δsod2* strain, peptide 18 exhibited an inability to protect the cells subjected to hydrogen peroxide, even after treatment with the peptide, shown in Figure 6. This observation suggested a potential interaction between peptide 18 and the *SOD2* gene, implicating *SOD2* as a plausible target for peptide 18-mediated assistance in cellular resilience against excessive stress. Alternatively, the diminished protective effect could be indicative of the involvement of other underlying pathways that influence the survivability of the cell under oxidative stress conditions.

The activity of *TOR1* and *SOD2* are prime targets to understand the antioxidant defense mechanisms within the cell. This dual modulation suggests a promising target for therapeutic interventions aimed at neutralizing free radicals and mitigating age-related diseases linked to oxidative damage.

Conclusion

This intriguing outcome prompts further exploration to decipher the precise molecular mechanisms by which peptide 18 operates in the context of cellular maintenance and repair against stress. By unraveling the specific pathways and targets involved, a deeper understanding of the potential therapeutic applications of peptide 18 in stress response modulation may be attained, contributing to advancements in cellular health strategies.

Acknowledgements

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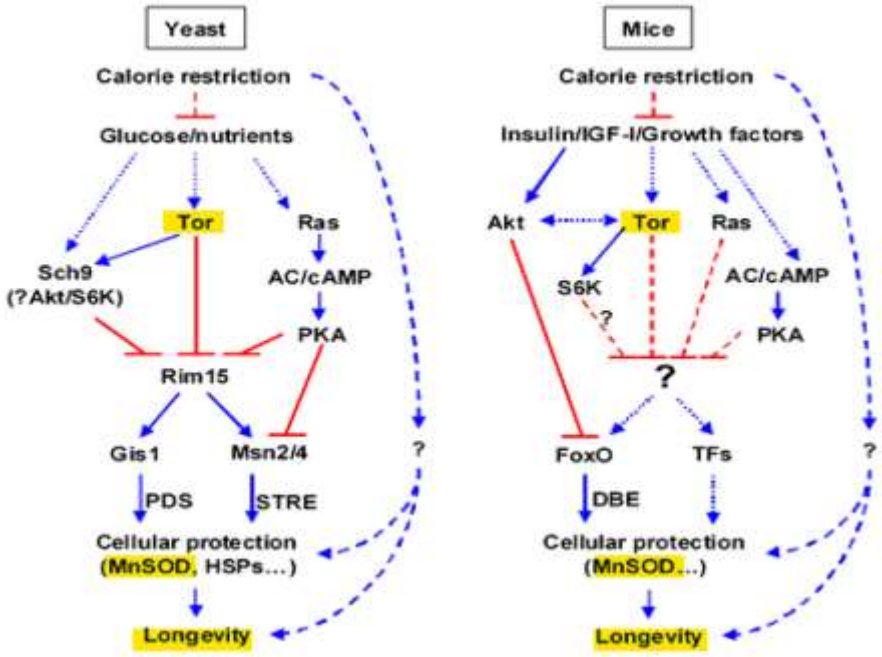

Figure 1: *Tor1* inhibition leads to an upregulation of the antioxidant pathways including *SOD* to assist the cell with prolonged survival (Wang et al., 2018).

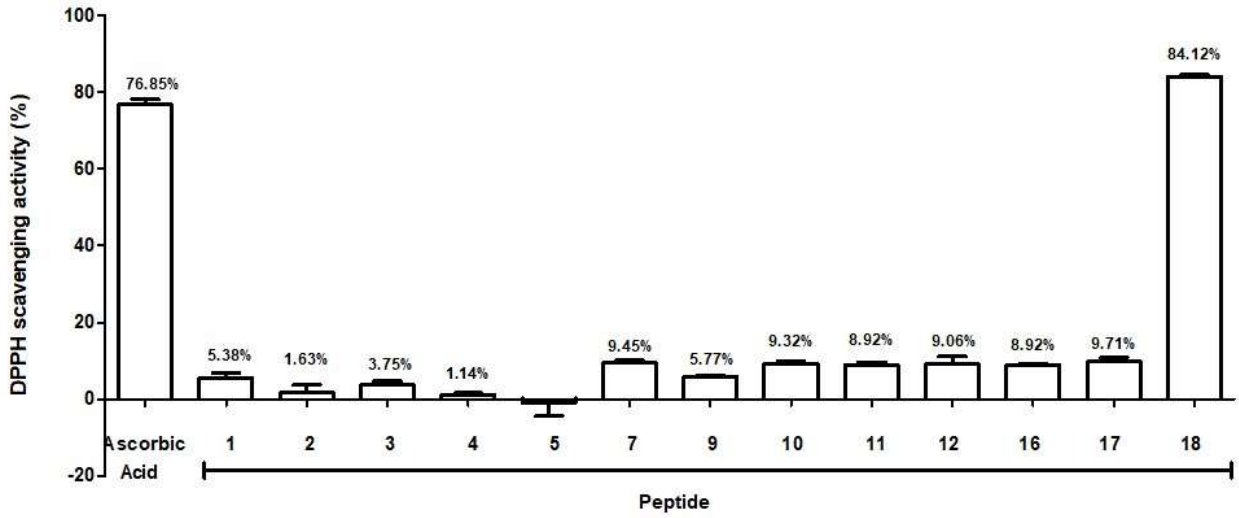

Figure 2: *In vitro* DPPH free radical scavenging activity of peptides obtained from the fermented rice samples. All the experiments were conducted in duplicates. Ascorbic acid (0.06 mg/ml) was used as a positive control. The numbers (1-18) denote the various tested peptides. Each peptide was tested at 10 mg/ml. Peptide 18 was selected as the prime candidate due to it's high radical scavenging activity to evaluate its potential in combating oxidative damage in the cell.

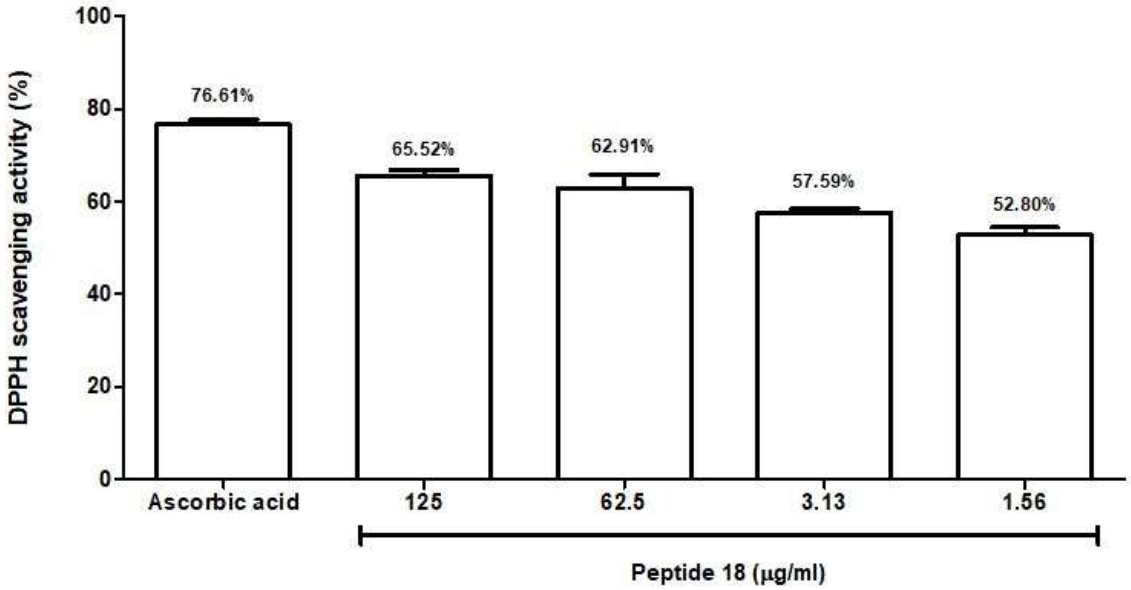

Figure 3: DPPH scavenging activity of the selected peptide 18 at various concentrations. The selected peptide was serially diluted to assess it's ability to scavenge free radicals at different concentrations. All experiments were conducted in triplicates. Ascorbic acid (60 µg/ml) was used as a positive control. The results exhibit peptide 18 as a strong antioxidant agent after assessing its ability to scavenge free radical at diluted concentrations.

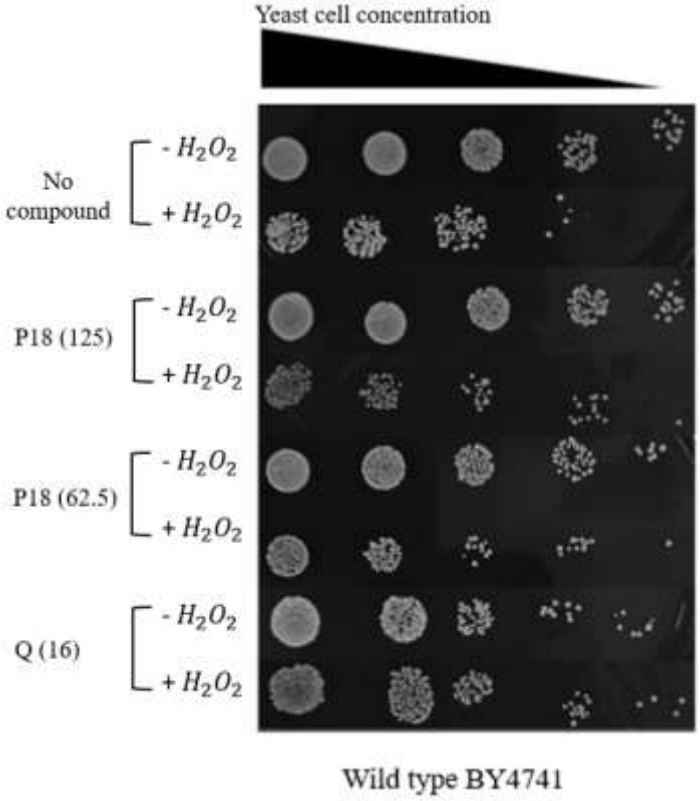

Figure 4: *In vivo* investigating the protective effects of the peptide on hydrogen peroxide-induced oxidative stress in wild type *S. cerevisiae* BY4741. The wildtype yeast cells were pre-treated with peptide 18 (P18) at the selected

concentrations of 125 and 62.5 µg/ml and incubated with 6 mM of hydrogen peroxide (H_2O_2). Quercetin (QE) at 16 µg/ml was used as the positive control. Independent replicates were performed to validate the results.

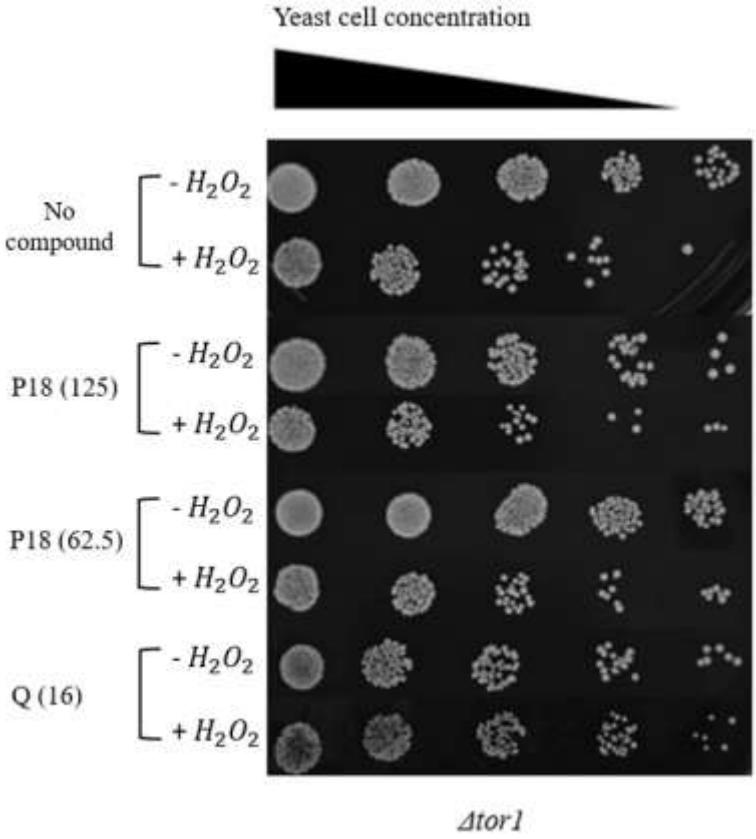

Figure 5: *In vivo* investigating the protective effects of the peptide on hydrogen peroxide-induced oxidative stress in *Δtor1 S. cerevisiae* BY4741. The *Δtor1* yeast cells were pre-treated with peptide 18 (P18) at the selected concentrations of 125 and 62.5 µg/ml and incubated with 6 mM of hydrogen peroxide (H_2O_2). Quercetin (QE) at 16 µg/ml was used as the positive control. Independent replicates were performed to validate the results.

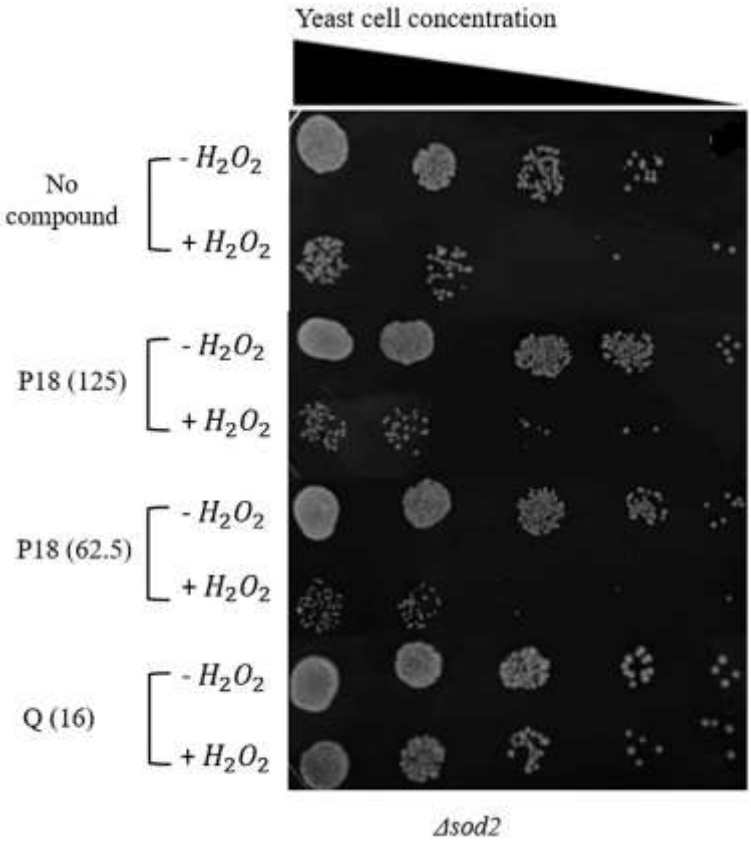

Figure 6: *In vivo* investigating the protective effects of the peptide on hydrogen peroxide-induced oxidative stress in *Δsod2 S. cerevisiae* BY4741. The *Δsod2* yeast cells were pre-treated with peptide 18 (P18) at the selected concentrations of 125 and 62.5 µg/ml and incubated with 6 mM of hydrogen peroxide (H_2O_2). Quercetin (QE) at 16 µg/ml was used as the positive control. Independent replicates were performed to validate the results.

EFFECT OF SYNTHETIC CANNABINOIDS ON B-GLUCAN-INDUCED TRAINED IMMUNITY IN MACROPHAGES

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Abstract

Innate immunity is well known as the first line defense against pathogens. This immunity is characterized by a fast response, limited specificity, and not have memory for future insults. However, there is evidence that innate immunity such as monocytes, macrophages, and NK cells, also has memory phenotypes, one of them is called trained immunity. Trained immunity relies on metabolic reprogramming and epigenetic modification to regulate inflammatory-related gene expression. Maladaptive of it can be the cause of diseases, which makes it a novel target therapy. Nowadays, numerous biological compounds can affect the innate immune response. Cannabinoids are known to have anti-inflammatory effects, but in some studies, they were reported to induce pro-inflammatory response. Cannabinoids have been reported to decrease the paw inflammation induced by zymosan, which can be used to train macrophages. Thus, in this study, we investigated the effect of cannabinoids on trained immunity. We found that Δ^8-tetrahydrocannabinol (Δ^8-THC), Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD), cannabigerol (CBG), and cannabinol (CBN) were not toxic to bone marrow-derived macrophages (BMDMs) at concentration of 20 μM. Moreover, pretreatment with Δ^8-THC (20 μM) followed by stimulation with β-glucan (50 μg/mL) in the presence of Δ^8-THC induced more TNF-α production, compared to β-glucan (50 μg/mL) stimulation alone. On the other hand, Δ^8-THC, Δ^9-THC, and CBD at concentration of 20 μM significantly reduced both TNF-α and IL-6 production in β-glucan-trained macrophages when added before LPS stimulation. These results indicated the differential impact of cannabinoids on trained immunity which may have long-lasting effects on innate immune memory.

Keyword : Cannabinoids, ELISA, Macrophages, Trained Immunity

Introduction

Immunity in vertebrates is characterized as adaptive and innate immunity. Adaptive immunity is characterized by the role of T cells and B cells, which exhibit specificity in pathogen recognition and possess the capacity to retain memory for subsequent encounters. In contrast, innate immunity is widely recognized as the first line of defense that lacks specificity and the ability to develop memory against future invasions (Netea et al., 2011). However, recent evidence found that innate immunity also has memory after exposure to certain pathogens or stimuli.

In humans, the finding of innate immune memory was initiated by the non-specific protection from BCG vaccine in children in West Africa. BCG vaccination reduced overall mortality caused by infections other than tuberculosis (Garly et al., 2003). Another evidence was the immune response of human monocytes ex vivo elevated when stimulated with bacterial and fungal pathogens after BCG vaccination (Kleinnijenhuis et al., 2012). This memory that showed an increased state of activation in innate immune response after primary stimulation is called trained immunity (Netea et al., 2011). Train immunity has been known to be affected by epigenetic reprogramming such as DNA methylation, noncoding RNA, and histone modification (Benjaskulluecha et al., 2022). The metabolism in innate immune cells also shifted in trained immunity from oxidative phosphorylation into glycolysis through activation of the Akt/mTOR pathway (Chen et al., 2023).

Trained immunity can be found in innate immune cells, including macrophages. Compared to the naïve macrophages, the trained macrophages give a faster response (Zubair et al., 2021). Until 2021,

numerous stimuli can train immunity, such as β-glucan, BCG vaccine, oxidized low-density lipoprotein (ox-LDL), lipopolysaccharides (LPS), aldosterone, High Mobility Group Box 1 (HMGB1), lysophosphatidylcholines (LPC), fungal chitin, cytomegalovirus (CMV), western diet (WD), and uric acid (Drummer et al., 2021). This knowledge is beneficial because trained immunity is found to be involved in the pathogenesis of some diseases, such as cardiovascular diseases, autoimmune diseases, and atherosclerosis.

Because there is still a growing need of knowledge in innate immune memory, this research will contribute to investigating the effect of synthetic compounds from the plant, *Cannabis sativa*, which has gained interest for medical use. This plant may have the potential to be applied in the medical field because the study observed that cannabinoids have an immune-modulating effect that involves the endocannabinoid system (Yekhtin et al., 2022). Regardless of the cannabinoid potential, variations of the cannabinoid composition in plant extract can have different effects. The study performed by Namdar et al., 2017 showed that different locations of inflorescence samples, the polarity of solvents, and the separating methods affected the composition of the yielded compounds. Because of the variation in extract composition, the synthetic method to produce the cannabinoids will help to obtain a certain amount of compounds and also produce some derivatives that may have a better effect on human health. This research used five synthetic cannabinoids, which are Δ^8- tetrahydrocannabinol (Δ^8-THC), Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD), cannabigerol (CBG), and cannabinol (CBN). The effect of those synthetic compounds was observed in mouse bone marrow-derived macrophages (BMDM) whether they can repress or enhance the trained immunity. The result of this research is expected to provide knowledge on the effect of the synthesized cannabinoids in innate immune memory which can further be a candidate of target treatment in innate immune memory-associated diseases.

Materials and Methods

1. Cannabinoids

The five cannabinoids included in this study were chemically synthesized, and their identity and purity were confirmed by Nuclear Magnetic Resonance (NMR). They were prepared as stock solutions in DMSO. The details about molecular weight are Δ^8-Tetrahidrocannabinol (Δ^8-THC, 314.5 g/mol), Δ^9-Tetrahidrocannabinol (Δ^9-THC, 314.5 g/mol), Cannabidiol (CBD, 314.5 g/mol), Cannabigerol (CBG, 316.49 g/mol), and Cannabinol (CBN, 310.44 g/mol).

2. Generation of Bone Marrow Derived Macrophages (BMDM)

DMEM (Dulbecco' Modified Eagle Medium) Complete Media, which consists of DMEM High Glucose (Cytiva, Canada) media with 1% Sodium Pyruvate, 1% HEPES, and 1% Penicillin-Streptomycin and 10% Fetal Bovine Serum (Gibco, USA) were used for this experiment. Bone Marrow-Derived Macrophage Media (BMDM Media), which contained DMEM complete media (DMEMC) supplemented with 20% L929 cells supernatant containing M-CSF and 5% horse serum. C57Bl/6 mice (8-10 weeks old) were purchased from Nomura Siam International (Bangkok, Thailand). The mice were sacrificed using isoflurane inhalation and cervical dislocation. The femur, tibia, and humerus were dissected and the bone marrow was flushed and strained using 70 μM cell strainer in Serum Free Media + 1% Penicillin/Streptomycin and centrifuged. The bone marrow cells were resuspended in BMDM media and differentiated on the Hyclon™ plate for 7 days. After 7 days, the cells were harvested and used for the experiment.

3. Cytotoxicity test of compounds

Cytotoxicity test was performed using MTT assay (Thiazolyl blue tetrazolium bromide, Alfa Aesar). For observing the cytotoxicity of synthetic compounds, 2 x 10^4 BMDM cells were cultured in 96-well plate for 20 hr, then incubated using the compounds at concentrations of 20 μM and 50 μM. The detail scheme and calculation are shown in Figure 2A and B respectively.

4. β-glucan-induced Trained Immunity in Macrophages

To induce β-glucan trained immunity in macrophages, BMDM were cultured in DMEMC overnight

and primed with 50 µg/mL Pachymann BG (Megazyme, USA). After 24 h of priming, the medium was replaced with fresh DMEMC, and the cells were rested for 48 h. The resting step was followed by *Escherichia coli* LPS (L2880, Sigma Aldrich, USA) (10 ng/mL) stimulation before collection of the supernatant.

5. Investigating the synthetic cannabinoid effect on β-glucan-trained macrophages

To investigate the effect of synthetic cannabinoids on β-glucan-trained macrophages, the 2 x 10^5 BMDM were cultured in a 48-well plate overnight. There were two timelines for observing the effect of synthetic cannabinoids, which are shown in Figure 1A and 1B. After the LPS stimulation, the supernatant was collected and subjected to ELISA (Biolegend, USA) to examine the cytokine level for TNF-α and IL-6. ELISA was performed based on the manufacturer's suggestion.

6. Statistical analysis

All of the experiments were performed in duplicates except the cytotoxicity test (triplicates), and the data were presented with SEM. Statistical analysis was performed by using GraphPad Prism (San Diego, USA). One-way analysis of variance (ANOVA) was chosen to determine statistically significant differences between the results ($p<0.05$ considered significant).

Results and Discussion

Before investigating the effect of synthetic cannabinoids, the cytotoxicity assay was performed using compounds with the concentrations of 20 µM and 50 µM and 0.1% DMSO in DMEM media as the control. As shown in Figure 5, all cannabinoids were not toxic at both concentrations, except for the CBD which had some cytotoxicity at 50 µM. It was consistent with the other studies which found that CBD had a cytotoxic effect at higher concentrations, starting from 40 µM in various immune cells from PBMC (Jindaphun et al., 2024). However, other studies reported various cytotoxicity of CBD in different cell types, but immune cells were found to be more sensitive to cannabidiol. The cytotoxicity of CBD varies depending on several factors such as the cell type, the dose, CB receptor presentation, and time of exposure (Yeisley et al., 2021). Thus, for further experiment, the concentration at 20 µM of each cannabinoid was used.

To investigate the effect of synthetic cannabinoids, the experiment was conducted in two different conditions to see whether different exposures could have different effect or not. In the first scheme (Figure 1A), the morphology of the cells was more rounded, had more pseudopodia compared to the cell without stimulation, and showed an increasing number of cells after 24 hr of incubation (Figure 3). It may be caused by the activation of dectin-1 (β-glucan receptor) which activates the mTOR pathway that plays role in cell growth (Wang and Levine, 2010). Furthermore, the treatment of cannabinoids also affected cytokine production. It can be seen in Figure 6 that Δ^8-THC can increase TNF-α in β-glucan trained macrophages, but it did not affect the IL-6 level. The increase of TNF-α may be caused by the downstream effect of mTORC1 activation by the signaling pathway of the CB1/CB2 receptor through activation of PI3K phosphatidylinositol-3 kinase (McCoy K. L., 2016), which then enhances the pro-inflammatory gene expression.

The other stimulation scheme, which is shown in Figure 1B, revealed the different effect on β-glucan induced trained macrophages. In terms of cell morphology, cannabinoid-treated cells have more pseudopodia compared to unstimulated cells, β-glucan-stimulated cells, and LPS-stimulated cells (Figure 4). Interestingly, the effect of Δ^8-THC was different if it was given 1 hr before LPS stimulation compared to 1 hr before β-glucan treatment. The result showed that it lowered the cytokine production of both TNF-α and IL-6, as shown in Figure 7A and 7B. Δ^9-THC and CBD also had a suppressing effect on both TNF-α and IL-6 production (Figure 7A and 7B). The effect of CBD was determined by which receptor is activated by it. A study reported that CBD can lower TNF-α and IL-6 cytokine production mediated by CB2 receptor and Adenosine 2A receptors (Anil et al., 2022). These differences may be caused by different activated signaling pathways in macrophages immune response that should be studied further.

Conclusion

All synthetic cannabinoids showed no toxic effect in BMDM at concentration of 20 μM. The synthetic cannabinoids have different effect on the cytokine production in β-glucan-induced trained immunity in macrophages either enhancing or dampening effect.

Acknowledgements

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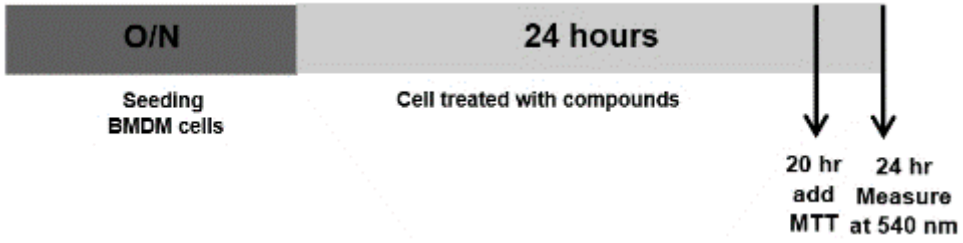
A

B

Figure 1: This timeline shows the experimental procedure to examine the effect of synthetic cannabinoids. A) In the priming step: cannabinoid (20 μM) was given 1 hour ahead in the priming step, then the media changed into cannabinoid 20 μM and β-glucan (50 μg/mL) for 23 hr,. After that, cells start to rest in freshly changed DMEMC for 48 hours. Finally, LPS stimulation was 10 ng/mL for 24 hr, and the supernatant was harvested. B) In the stimulation step: Firstly, the cells were only stimulated with β-glucan (50 μg/mL) for 24 hr, then the media changed into DMEMC followed by 48 hr resting. Next, the synthetic cannabinoids (20 μM) were added 1 hr before LPS stimulation. In the end, the media was changed into cannabinoids 20 μM and LPS (10 ng/mL) for 23 hr before the supernatant was harvested.

A



B

$$\% \text{ Cell Viability} = \frac{(\text{Absorbance at 540 nm of samples}) - (\text{Absorbance of Blank at 540 nm})}{(\text{Absorbance at 540 nm of control}) - (\text{Absorbance of Blank at 540 nm})} \times 100$$

Figure 2 : A) The timeline for MTT Assay. B) Calculation of cytotoxicity test. Control was the cells with 0.1% DMSO in DMEMC media and DMEMC media only was used as the blank. The viability ≥80% is considered as non-toxic to the cells.

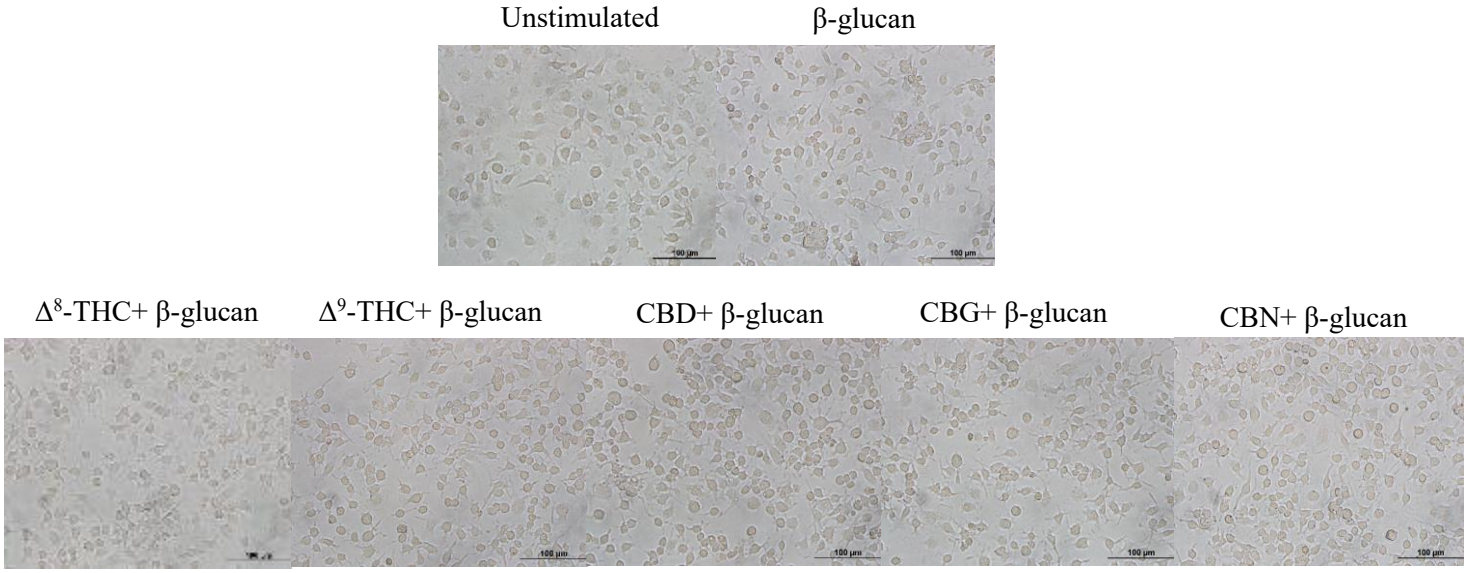


Figure 3 : Morphology of BMDM after 1 hr stimulation with cannabinoid 20 μM in priming time followed by cannabinoid 20 μM and β-glucan (50 μg/mL) compared to unstimulated cells (only incubate using DMEMC Media), and β-glucan stimulated cells. The total magnification was 400x.

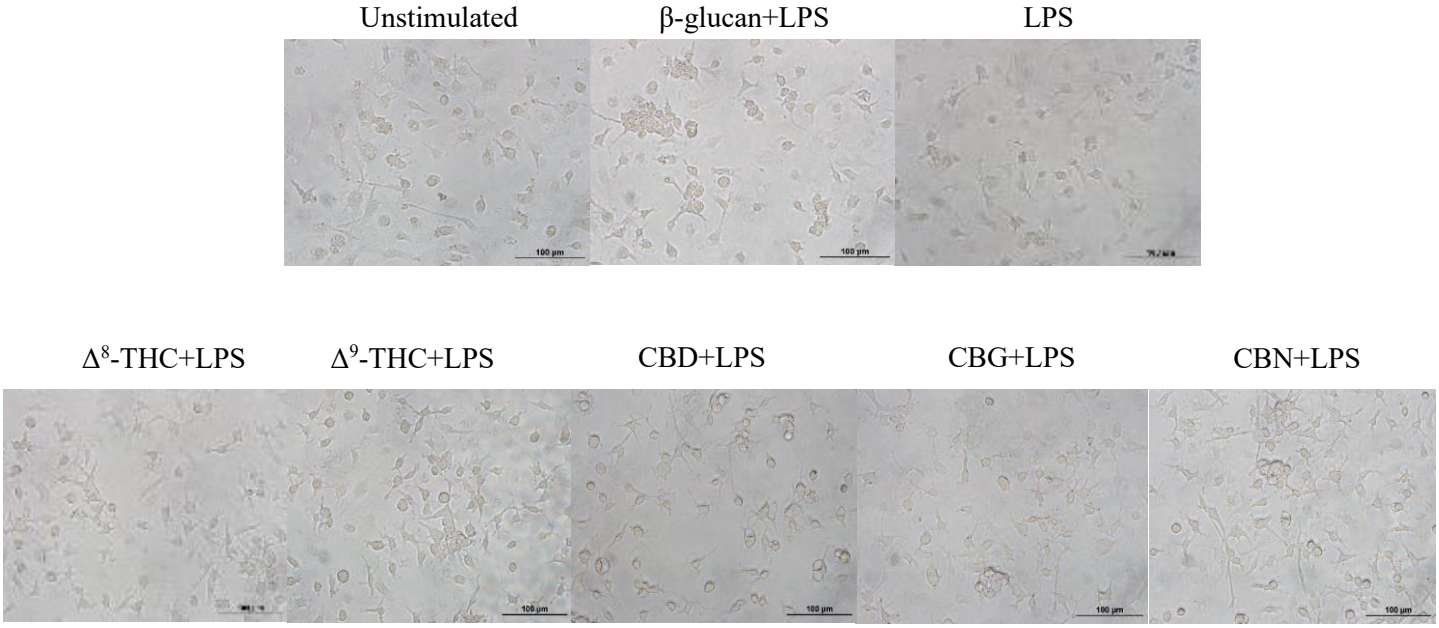


Figure 4: Morphology of BMDM after 1 hr pretreatment by cannabinoids 20 μM followed by 23 hr of cannabinoids 20 μM and LPS (10 ng/mL) stimulation. Unstimulated cells (only incubated using DMEM Media), β-glucan stimulated cells, and LPS-stimulated cells were compared to cannabinoid+LPS treated cells. The total magnification was 400x.

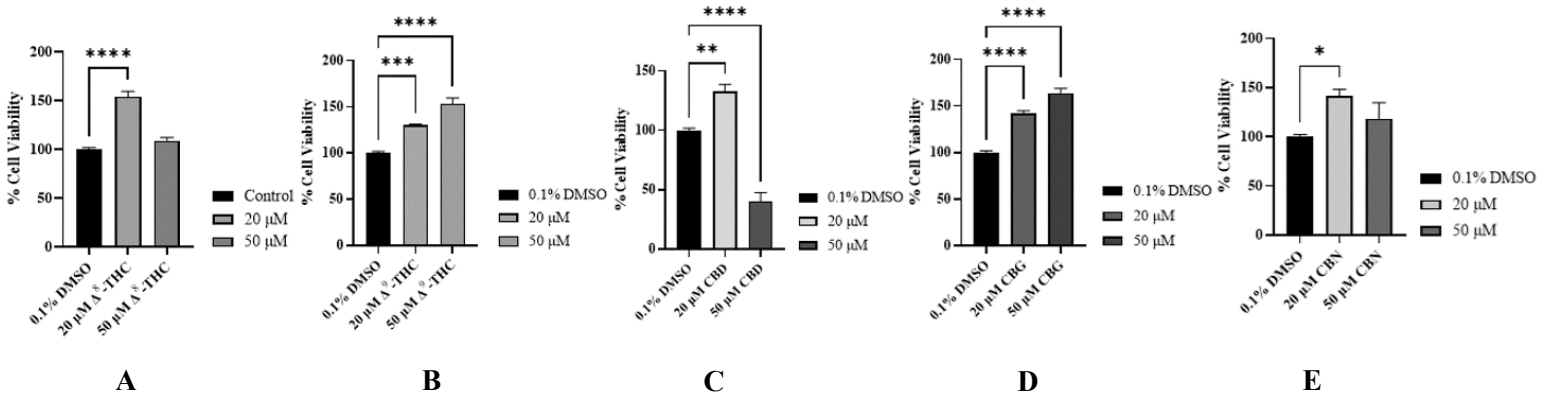


Figure 5 : Cytotoxicity assay result for cannabinoids in 20 µM and 50 µM. A) Δ^8-THC B) Δ^9-THC, C) CBD, D) CBG, E) CBN. This experiment was done triplicates. Cells in 0.1% DMSO in DMEMC media as control and DMEMC media as the blank in the experiment (significance *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

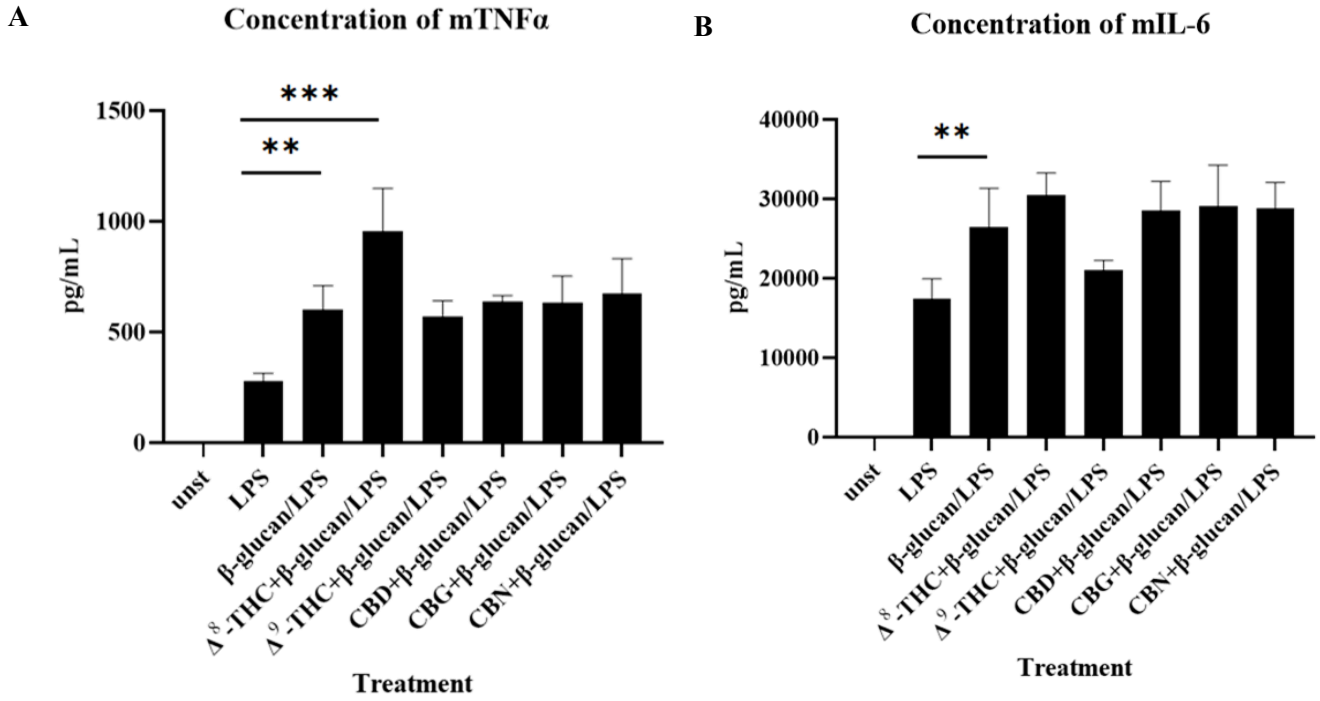


Figure 6: A) mouse TNF-α and B) mouse IL-6 results. This result was obtained after cells were stimulated with cannabinoids 20 µM 1 hr before β-glucan stimulation. Unstimulated cells (Unst) was only incubated in DMEMC media during the experiment. The statistically significant difference was compared to the β-glucan and indicated by **p<0.01, and ***p<0.001. To validate the result, this experiment was done in two independent experiments.

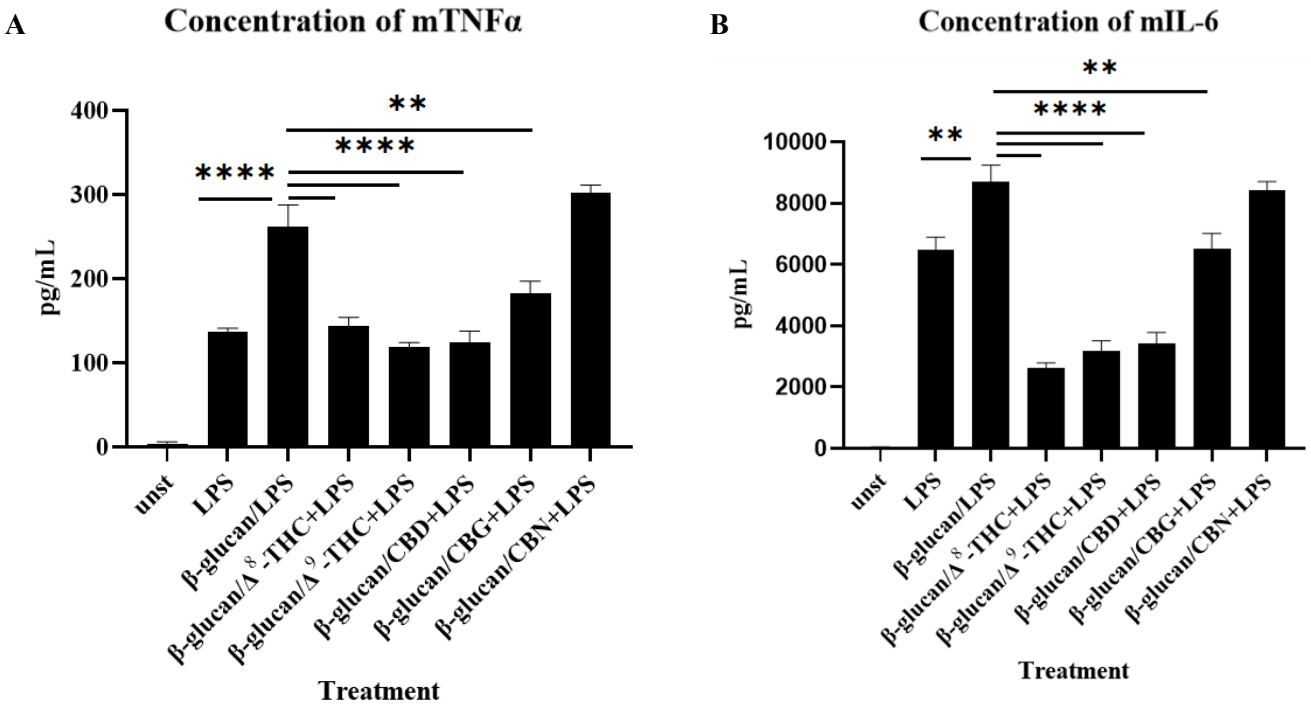


Figure 7 : A) mouse TNF-α and B) mouse IL-6 results when stimulated with cannabinoids 20 µM 1 hr prior to LPS (10 ng/mL) stimulation. Unstimulated cells (Unst) was only incubated in DMEMC media during the experiment. The statistically significant difference was compared to the β-glucan (50 µg/mL) and indicated by **p<0.01, ***p<0.001, ****p<0.0001 respectively. The experiment was done in two independent experiments.

SCREENING OF SOME PROBIOTIC PROPERTIES OF YEASTS ISOLATED FROM THAI TRADITIONAL KOMBUCHA SAMPLES

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ABSTRACT

Kombucha, a beverage often regarded as a healthful drink. The kombucha fermented tea originates from China, where it has been traditionally prepared by fermenting green or black tea with a symbiotic culture of bacteria and yeast (SCOBY). This study aims to screen yeast strains present in Thai kombucha products for their probiotic properties. Fourteen samples of commercially available ready-to-drink kombucha were randomly collected for analysis. Various physical characteristics, including color, pH level, and concentrations of acetic and lactic acids were assessed. Results indicated that the majority of kombucha samples exhibited a clear yellow color, with pH values between 2.74 and 3.54, acetic and lactic acid concentrations ranging from 0.42% to 0.89%, and 0.36% to 1.33%, respectively. Yeast isolation from the samples was performed on YMA medium using the spread plate method, yielding a total of 86 yeast isolates. All isolates were examined for their probiotic properties, namely their ability to survive in acidic conditions (pH 2.0-5.0), resistance to bile salts at a concentration of 0.3%, and red blood cell hydrolysis. It was found that only 11 isolates were resistant to acids, bile salts, and did not hydrolyze red blood cells. Morphological examination revealed predominantly round or oval-shaped cells. The strains were identified using the MALDI-TOF MS technique as *Brettanomyces bruxellensis, Candida parapsilosis, Hanseniaspora opuntiae, Pichia kudriavezii, Rhodotorula mucilaginosa, Saccharomyces cerevisiae, and Schizosaccharomyces pombe*. These findings contribute to the understanding of the probiotic potential of yeast strains in kombucha products, furthering research in the field of functional foods and beverages.

Keywords: Kombucha, Yeast, Probiotic property, Fermented drink, MALDI-TOF MS

ANTIMICROBIAL POTENTIAL OF ENDOPHYTIC FUNGAL EXTRACTS FROM VEGETABLES AND MEDICINAL HERBS AGAINST FOODBORNE PATHOGENS

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ABSTRACT

Food preservatives have been used to combat food-borne pathogenic organisms. Chemical preservatives can adversely affect human health. Natural food preservatives may provide new and safe way to protect the food fro bacterial contamination. In this study, antimicrobial effects of nine endophyti fungi isolated from eight vegetables and medicinal plants, including *Capsicum frutescens, Elaeagnus latifolia, Lantana camara, Mansonia gagei, Oroxylu indicum, Orthosiphon aristatus, Talinum paniculatum,* and *Terminalia bellirica* were investigated against selected pathogenic foodborne bacteria. The antibacterial activities of water crude extracts were tested against *Bacillu cereus* PSU414, *Escherichia coli* ATCC25922, and *Staphylococcus aureu* ATCC29213. Antimicrobial screening test was conducted by agar well diffusion assay. All endophytic fungal isolates exhibited inhibitory zones against all examined bacteria, with an inhibition zone ranging from 20 mm. to 41 mm. Minimal inhibitory concentrations (MICs) were determined using a broth microdilution method. The endophytic fungal extracts exhibited MIC ranging from 32 µg/mL to 128 µg/mL. The endophytic fungi from *Lantana camara* showed the highest antimicrobial potency against *S. aureus* with MIC and minimum bactericidal concentration (MBC) values of 64 and 512 µg/mL, respectively. The active endophytes have been identified by morphological analysis. The endophytic fungi from *Lantana camara* was identified as

Fusarium sp. Time-kill assay was performed to elucidate the bactericidal kinetics of endophytic fungal extracts against *S. aureus*. The extract shows antibiofilm activity against *S. aureus* by crystal violet assay. Our results suggested that the extract derived from *Fusarium* sp. from *Lantana camara* shows promise against foodborne bacteria.

Keywords: antibacterial activity; endophytic fungi; foodborne pathogens; medicinal plants

EFFECTS OF MYCORRHIZAL FUNGI ON THE GROWTH OF *DURIO ZIBETHINUS* SEEDLINGS

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ABSTRACT

Phlebopus portentosus, often known as the king bolete mushroom, was collected near Sritrang reservoir at Prince of Songkla University and cultivated on potato dextrose agar (PDA). The macroscopic and microscopic morphological study results exhibited black spores, dark gold with septal mycelium, and basidiospores. Subsequently, the mycelium grown on PDA was transferred to a sterile millet for mass production and used as an inoculum. According to previous reports, the king bolete mushroom is said to have the ability to promote plant growth. Then, the one-year-old durian seedlings were inoculated with millet covered with above mentioned mushroom mycelium, and the growth was monitored for three months. The experiments were performed as follows: Experiments A, B, C, and D were only *Phlebopus portentous* was inoculated; *Phlebopus portentosus* plus plant supplements were inoculated; without *Phlebopus portentosus*, plant supplements and insecticides were used; and the latter was controlled, respectively. The results examined under a microscope found that experiments A and B exhibited the colonization of *Phlebopus portentosus* in the root system. Regarding the physical characteristics of durian seedlings, experiment A showed the most extensively branched taproot system compared to the rest of the experiments. Additionally, experiments A and D showcased the yellowing of leaves. Therefore, this study indicates the establishment of a symbiotic relationship between mycorrhizas and durian plants.

Keywords: mycorrhizal fungi; durian; mycorrhizal Symbiosis

ISOLATION AND CHARACTERIZATION OF DOMINANT ACETIC ACID BACTERIA AND YEAST ISOLATED FROM KOMBUCHA SAMPLES

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Abstract

Kombucha, a beverage produced through fermenting tea and sugar, has gained popularity due to its refreshing taste and health benefits. However, the microbial composition of the products at the time of consumption is unknown. Understanding the microbial characteristics of Kombucha cultures allows manufacturers to better control them, promoting the production of safe, consistently high-quality products. Therefore, this research aimed to isolate and identify acetic acid bacteria (AAB) and yeast as dominant microorganisms in six kombucha samples from Thailand, comprising two commercial and four locally sourced samples. The samples were determined for total soluble solids (TSS, °Brix) and pH values. The results indicated a pH range of 2.00-3.00 and TSS ranging from 5 to 15 °Brix, depending on the type of Kombucha. Acetic acid bacteria and yeast were isolated from the kombucha samples using CARR medium and yeast extract glucose chloramphenicol (YGC) media, respectively. Phenotypic and taxonomic identification of AAB and yeast were achieved through morphological and MALDI-TOF MS analyses. The AAB isolate species was identified as *Acetobacter indonesiensis*. The yeast isolates were classified as *Pichia kudriavzevii*, *Schizosaccharomyces pombe*, *Brettanomyces bruxellensis*, and *Meyerozyma guilliermondii*.

Keywords: *Kombucha*; *Acetic acid bacteria*; *Yeast*

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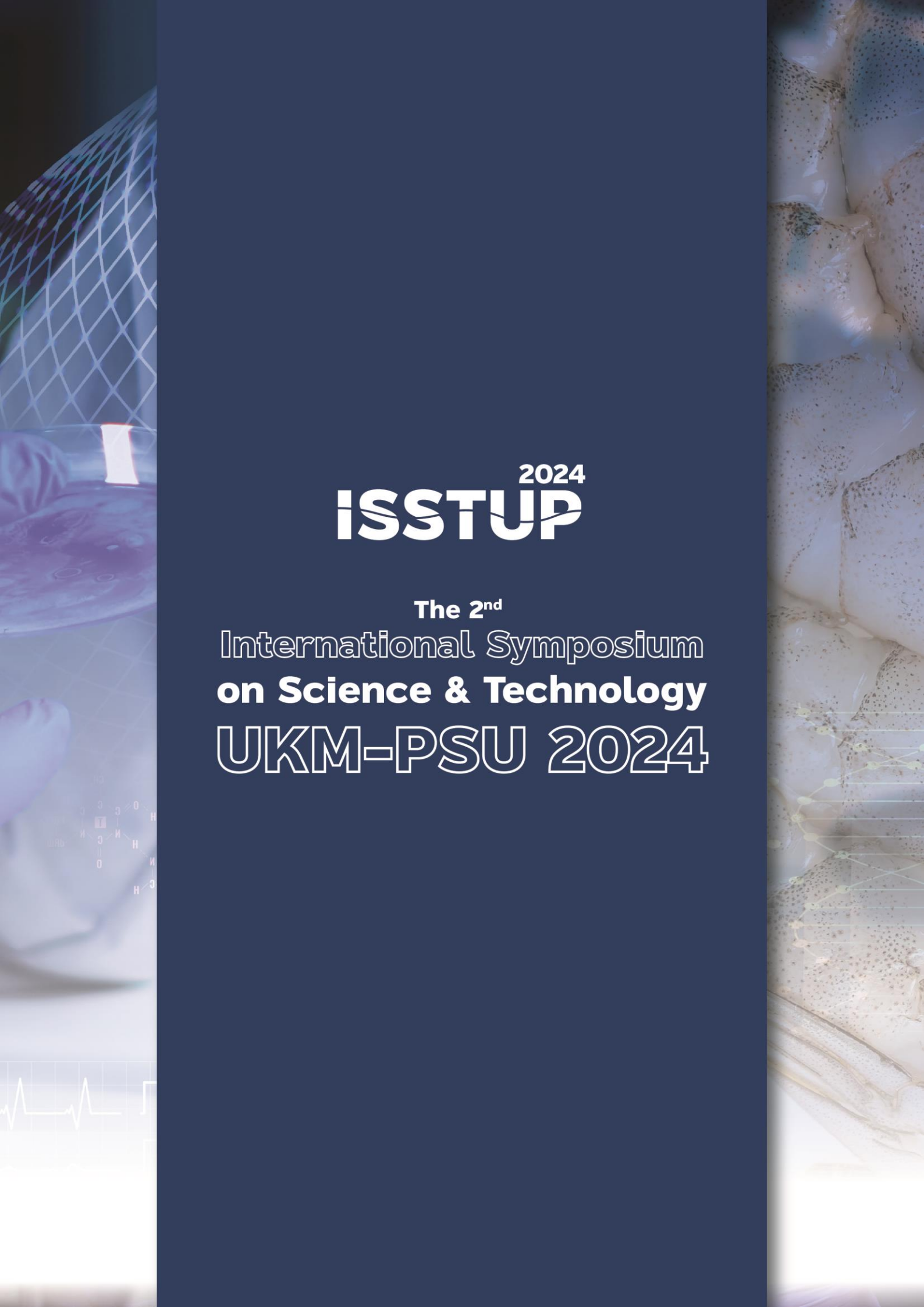

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