REPORT BOOK

The 6th International Conference of Indonesian Society for Lactic Acid Bacteria and Gut Microbiota

FRIDAY / 13 AUGUST 2021

6TH-IC-ISLAB-GM

ORGANIZED BY

Indonesian Society of Lactic Acid Bacteria and Gut Microbiota

Center for Food and Nutrition Studies Universitas Gadjah Mada

Department of Food and Agricultural Product Technology Universitas Gadjah Mada

Center of Excellence in Science and Technology

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PROGRAM BOOK

The 6th International Conerence of Indonesian society for lactic acid bacteria and gut micriobiota (6th- IC-ISLAB-GM)

August, 13 th 2021

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Indonesian Society of Lactic Acid Bacteria and Gut Microbiota

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INTRODUCTION

The International Conference of International Society Lactic Acid Bacteria and Gut Microbiota (IC-ISLAB) is one of the event facilitated by Indonesian Society for Lactic Acid Bacteria and Gut Microbiota (ISLAB-GM). This conference is held every 2 years. In 2021, the 6th International Conference of Lactic Acid Bacteria and Gut Microbiota wa held virtually due to pandemic Covid-19.

It is the aim of this conference to gather the advanced research information and application technologies of Lactic Acid Bacteria, Probiotic, and Gut Microbiota. The conference would serve as a platform for researchers from academia and industry in Asia to obtain and exchange information on scientific progress and applications.

PARTICIPANTS

- Indonesian Society for Lactic Acid Bacteria and Gut Microbiota (ISLAB-GM)
- Asian Federation of Societies for Lactic Acid Bacteria (AFSLAB)
- Member of other scientific organization
- Researchers and profesional
- Industry partners

PREFACE

Head of Department of Food and Agricultural Product Technology
Universitas Gadjah Mada
By Tyas Utami

I would like to warmly welcome all of you to the 6th International Conference of Indonesian Society for Lactic Acid Bacteria and Gut Microbiota (6th IC ISLAB-GM), which is held on August 13, 2021, at Webinar via Zoom Meeting Application.

It is very pleasure for the Department of Food and Agricultural Product Technology, Universitas Gadjah Mada to host the 6th International Conference of Indonesian Society for Lactic Acid Bacteria and Gut Microbiota.

Lactic acid bacteria, probiotic and gut microbiota have received tremendous attention from scientists around the world. Many researchers have isolated, selected and characterized lactic acid bacteria and probiotic strains from various sources and studied their role on food qualities and human health. Department of Food and Agricultural Product Technology take a role to provide support for our academics: undergraduate and postgraduate student with supervision from respective lecturers to do research in this field. Exploration of the function and the application of indigenous strain of lactic acid bacteria continuously be carried out in collaboration with various parties. I strongly believe that our works are creating wide impacts for the society.

I hope that this international meeting activity can provide a great opportunity to share our works, extend network among students, scientists, and industries, and also improve the education value for nation and worldwide. It is important that research activities and community outreach should be running in accordance with science development which can strengthen the competence of higher education institutions.

Finally, I would like to express my gratitude toward all speakers, presenters, guest, industry professionals, participants, and other parties for the support to the conference. Have a wonderful time in the conference.

Tyas Utami

PREFACE

Head of Center for Food and Nutrition Studies and
University Center of Excellence for Integrated Research and Application for Probiotic Industry, Universitas Gadjah Mada
By Endang S Rahayu

Ladies and Gentlemen, invited speakers, presenters, and participants of the 6th International Conference of Indonesian Society for Lactic Acid Bacteria and Gut Microbiota. I would like to warmly welcome all of you to the 6th International Conference of Indonesian Society for Lactic Acid Bacteria and Gut Microbiota (6th IC-ISLAB-GM) which is held on August 13, 2021, at Webinar via Zoom Meeting Application.

Nowadays, scientists have more concern for lactic acid bacteria field. One of the interesting research topics of lactic acid bacteria is associated with probiotics and gut microbiota. Many researchers have conducted research related with this topic for many years. Selected indigenous probiotic strains have been promoted for industrial use. Probiotic is believed to be important in supporting intestinal health, together with the indigenous gut microbiota. Lactic acid bacteria, probiotics, and gut microbiota have scientifically correlated each other.

Center for Food and Nutrition Studies anticipate this interest by providing facilities for research experiment related to food and nutrition, one of them is collecting indigenous LAB culture into Food and Nutrition Culture Collection (FNCC) since 1995. With University Center of Excellence for Probiotics Research, we aid with providing big data resource system for archive the important information of the research. One of our aims is to be able to conduct downstream research related to food and nutrition, especially in probiotic field, for advancement in research, education, and community service.

I would like to appreciate all the invited speakers, presenters, participants, and other parties for their works in the field of lactic acid bacteria, and especially for their time and efforts to participate in this conference. I wish that the conference will be a great opportunity to bring together prominent scientists in Asia, along with students and industries and to explore possibilities to work together in the future.

I wish you good luck for the conference and have a wonderful time in the conference.

Endang S. Rahayu

PREFACE

Chairwoman of International Society of Lactic Acid Bacteria and Gut Microbiota
By Endang S Rahayu

It is a great pleasure for the Indonesian Society for Lactic Acid Bacteria-Gut Microbiota (ISLAB-GM) to host the 6th International Conference of ISLAB-GM. We're delighted to offer the most hospitable welcome we can to all the speakers, presenters, and all participants.

The 6th IC ISLAB-GM is held on August 13, 2021, at Webinar via Zoom Meeting Application. The main aim of this conference is to bring together the advanced research and application technologies of Lactic Acid Bacteria, Gut Microbiota, and Probiotic. The objectives of the meeting are to disseminate the recent research achievements in lactic acid bacteria, to explore the role, benefit, and novel function of lactic acid bacteria; and to strengthen the network among the national and international universities, research institutes, government agencies, and industries in exploring the role and application of lactic acid bacteria and gut microbiota.

The participants of this conference come from universities, research institute, government agencies and industries from Indonesia and overseas. We have invited expert speakers from Japan, Philippines, Malaysia, and Indonesia. There are two main sessions for expert speakers and three sessions for oral presentation, also best poster selection in the conference. We wish that the 6th ISLAB to be an opportunity not only for sharing ideas and experiences but also for establishing and extending friendship among scientists and strengthening your personal and professional networks.

I would like to appreciate all the speakers, presenters, and participants for the tremendous efforts and time spent for the conference. I would like to express my sincere gratitude to ISLAB-GM members, the Department of Food and Agricultural Product Technology, and the members of organizing team for their contribution and helps. Without them we would not have been able to organize this great meeting.

We wish the conference will have significant contribution in the field of Lactic Acid Bacteria, Gut Microbiota, and Probiotic at worldwide level. I wish all of you lots of success during the conference and have a pleasant day in the conference.

Endang S Rahayu

PROGRAM 6TH IC-ISLAB-GM

August 13th, 2021		
08.00 – 08.30	Registration	
08.30 – 08.45	Opening	
08.45 – 08.55	Welcome Remarks from Prof. Dr. Ir. Endang S Rahayu, MS as chairman of ISLAB-GM	
Main Session 1 **(Moderator: Dian Anggraini Suroto, S.TP., M.P., M.Eng, PhD)**		
08.55 – 09.25	Prof. Francis B. Elegado	Utilization Of Linamarase-Producing Lactic Acid Bacteria And Yeasts For Cassava (*Manihot Esculenta Crantz*) Sourdough Fermentation
09.25 – 09.55	Prof. Dr. Hooi Ling Foo	Nutritional Products Developed by Lactic Acid Bacteria
09.55 – 10.25	Prof. Jiro Nakayama	Gut Microbiome and Metabolome of Asians Associated with Diets and Health
10.25 – 10.35	Poster Session	
Main Session 2 **(Moderator: Dian Anggraini Suroto, S.TP., M.P., M.Eng, PhD)**		
10.35 – 10.55	Dr. rer. nat. Agus Wijaya	Safety Aspects of Probiotic Bacteria: Current Status
10.55 – 11.15	Dr. Ir. Tyas Utami	Lactic Acid Bacteria, Fermented Food and Its Health Benefit

11.15 – 11.30	Discussion
11.30 – 12.45	Lunch Break and Poster Session
12.45 – 14.15	**Oral Session 1** **(Moderator: Prof. Dr. Ir. Titiek F. Djaafar, MP)** 1. Raja B.D. Sormin 2. Rosario Trijuliamos Manalu 3. Usman Pato 4. Hendry Noer Fadllillah 5. Siti Lutfiah Anggraeni 6. Ni Luh Putu Manis Widiyanti
14.15 – 15.45	**Oral Session 2** **(Moderator: Dr. Tri Marwati)** 1. Roti Jati Kusuma 2. Yuliana 3. Iskandar Azmy Harahap 4. Dimas Rahadian Aji Muhammad 5. Laksmi Hartajanie 6. Yoga Dwi Jatmiko

15.45 – 16.00	Break and Poster Session
16.00 – 17.00	**Oral Session 3** **(Moderator: I Nengah Sujaya, PhD)** 1. Yan Ramona 2. Isti Handayani 3. Delima Citra Dewi Gunawan 4. Yoyok Budi Pramono
17.00 – 17.10	Best Poster Announcement
17.10 – 17.15	Closing

ORAL SESSION PROGRAMME

Time	Note	Speaker	Title
Oral Session 1 Moderator : Prof. Dr. Ir. Titiek F. Djaafar, MP			
12.45-13.00	OR1	Raja B.D. Sormin	The Presence Of Lactic Acid Bacteria (Lab) On 'Inasua' Originated From Teon, Nila And Serua, Central Maluku District.
13.00-13.15	OR2	Rosario Trijuliamos Manalu	Isolation And Characterization of Lactic Acid Bacteria In Fermentation of Brown Rice As An Antibacterial
13.15-13.30	OR3	Usman Pato	Antimicrobial activity of bacteriocin from *Pediococcus pentosaceus* strain 2397 and its application as a natural preservative in fish meatballs
13.30-13.45	OR4	Hendry Noer Fadlillah	Effect of Soybean Germination on The Growth of *Lactobacillus kefiri* YK4 and *Lactobacillus rhamnosus* BD2
13.45-14.00	OR5	Siti Lutfiah Anggraeni	Effect of Fermentation Using *Lactococcus Lactis, Lactobacillus Bulgaricus* And *Lactobacillus Paracasei* on Edamame Protein
14.00-14.15	OR6	Ni Luh Putu Manik Widiyanti	Various volumes of palm sugar in making yogurt toward characteristics of yogurt (pre-organoleptics test)
Oral Session 2 Moderator: Dr. Tri Marwati			
14.15-14.30	OR7	Rio Jati Kusuma	Types of Acidifier Impact Microbiome, Nutrient Composition, Antioxidant and Anti-Diabetic Activity of Soy Tempeh
14.30-14.45	OR8	Yuliana	The Possible Role of Probiotics in Alleviating Inflammation during the COVID-19 infection

14.45-15.00	OR9	Iskandar Azmy Harahap	"Can probiotics be a promising therapeutic agent in bone health?" A gut-bone axis perspective between calcium bioavailability and microbiota modulation
15.00-15.15	OR10	Dimas Rahadian Aji Muhammad	Nanoencapsulation of probiotics: An updated review
15.15-15.30	OR11	Laksmi Hartajanie	The effect of probiotics number in fermented bitter melon (*Momordica charantia*) juice in decreasing blood sugar and lipid profiles
15.30-15.45	OR12	Yoga Dwi Jatmiko	Isolation and Characterization of Yeast from Fermented Sumbawa Mare's Milk with Probiotic Properties
Oral Session 3 **Moderator : I Nengah Sujaya, PhD**			
16.00-16.15	OR13	Yan Ramona	Functional effects of probiotic candidates, isolated from local sources (Indonesia), on human health
16.15-16.30	OR14	Isti Handayani	Antimicrobial Activity and Colour Properties of Annatto Extract Using *Bacillus subtillis* as A Pathogen
16.30-16.45	OR15	Delima CItra Dewi Gunawan	Effect Of Synbiotics Powder (*L. Plantarum Dad-13*) and Frukto-Oligosaccharide on Defecation Pattern and Stool Acidity in Stunting Children in Yogyakarta
16.45-17.00	OR16	Yoyok Budi Pramono	Critical Control Point Analysis of Rendang Cooking in Padang Restaurants for Food Safety and Maintenance of Consumer's Gut Microbiota Balance

LIST OF POSTER

No	Name	Title	Kode
1	Sekar Layung Prabandari	The Influence of Different Temperature of Extraction Annatto Seeds (*Bixa orellana* L) as Natural Colourant and Antibacterial *Staphylococcus aureus*	PO1
2	Sopi Nur Paidah	Effect of Annatto Seed Extraction Temperature (*Bixa orellana* L) as Antimicrobial *Escherichia coli*	PO2
3	Merkuria Karyantina	Angiotensin I Converting Enzyme Inhibitory Activity of Jambal Roti With *Lactobacillus Plantarum* 307 Application	PO3
4	Rikhsan Kurniatuhadi	Growth pattern of *Bacillus sp*. (NrLtF5) isolated from the gastrointestinal tract of the nypa worm (*Namalycatis rhodochorde*) at different pH and salinity values	PO4
5	Martha Christina	Influence Fermentation of Chocolate Tempeh on Antioxidant Activity and Blood Sugar Reduction In Vivo	PO5
6	Angelia Wattimury	In Silico Analysis of Antibiotic Resistance Genes in *Lactobacillus plantarum* T3	PO6
7	Riyan andila	Physicochemical Properties and Volatile Compound of Yoghurt Powder Encapsulated by Spray Drying Method	PO7
8	Eunike Marganingrum A. S	Cold Stress Response Gene of *Lactobacillus plantarum* Mut-3 and *Lactobacillus plantarum* Mut-7 supports their ability to survive in freezing condition	PO8
9	Ria Dewiyanti	Viability and Volatile Compound of *Lactobacillus plantarum* Dad-13 and *Lactobacillus plantarum* Kita-3 as Starter Cultures in Probiotic Cream Cheese	PO9

10	Ellyzsabeth Lambertus	Effect of Purple Sweet potato (*Ipomoea batatas* L) Paste Concentration on Lactic Acid Bacteria Growth in Soygurt	PO10
11	Ignatius Srianta (Tim with PO11)	Effect of Purple Sweet potato (*Ipomoea batatas L*) Paste Concentration on Lactic Acid Bacteria Growth in Soygurt	PO11
12	dr. Ni Nengah Dwi Fatmawati, S.Ked., SpMK(K), PhD	*Weissella confusa* F213 Modulated Tumor Necrosis Factor-a in DSS-induced Colitis Rats	PO12
13	Ninik Rustanti, STP, MSi	Comparison of Food Habits and Gut Microbiota in Type 2 Diabetes and Healthy Women in Yogyakarta Indonesia	PO13
14	Monika Olvi	Effectiveness of Jelly Candy Formulation Nipah Leaves (*Nypa fruticans*) Fermented by *Lactobacillus plantarum* To Lower Blood Triglycerides Levels In Vivo	PO14
15	Rafli Zulfa Kamil	Gut microbiota profile between normal and moderate malnutrition children in Yogyakarta, Indonesia	PO15
16	Dian Kumala Ratna	Indigenous Lactic Acid Bacteria From Halloumi Cheese As A Probiotics Candidate Of Indonesian Origin	PO16
17	Imaduddin Priambudi	Effect of Sucrose and Skim Milk Addition on the Acid Production during black soybean milk fermentation by *L. plantarum* Dad-13	PO17
18	Ir. Ira Nugerahani, M.Si. (Tim with PO11)	Effect of Purple Sweet potato (*Ipomoea batatas* L) Paste Concentration on Lactic Acid Bacteria Growth in Soygurt	PO18
19	Ni Wayan Nursini	Screening and Effect of MSG on the Increase of -Aminobutyric Acid (GABA) Lactobacillus plantarum Fermented Food Isolates	PO19
20	Miftakhussolikhah	Inhibitor alpha glucosidasion activity of fermented milk from *lactobacillus plantarum* DAD 13 in various fermentation time	PO20

21	Ngatirah	Viability, acid, and bile tolerance of spray-dried synbiotic microcapsules using various encapsulating materials from Iles-iles (*Amorphophallus oncophyllus*) and a protective agent	PO21
22	Ilzamha Hadijah Rusdan, S.TP.,M.Sc	Mother's Knowledge of Probiotic's Benefit for Their Children in Java Indonesia	PO22
23	Miifta Gatya	Isolation and Identification of Lactic Acid Bacteria from Halloumi Cheese Produced by SME Mazaraat Artisan Cheese Yogyakarta	PO23
24	Aprilia Nur Khasanah	Consumption of Probiotics and Gut Health of Students of Faculty of Agricultural Technology Universitas Gadjah Mada During Pandemic Covid-19	PO24
25	Joshua Christmas Natanael Luwidharto	Effect of Spirulina platensis Addition on Physicochemical and Microbiological Properties of Fermented Milk by Indonesian Probiotics Bacteria	PO25
26	Arum Darmastuti	Adhesion Properties of *Lactobacillus Plantarum* Dad-13 And *Lactobacillus Plantarum* Mut-7 On Rat Intestine	PO26
27	Baiq Rani Dewi Wulandani	Inclusion of Lebui Nut (*Cajanus. sp*) Extract to Increase the Functional Value of Goat Milk Yogurt."	PO27
28	I Nengah Sujaya	Weissella confusa F213 is a Promising Probiotic Strain	PO28
29	Taslia Rizky Putri	Antiadherence And Antimicrobial Activity Crude Extracts Bioactive Peptides Traditional Dayak Fermented Food Pekasam Black Tilapia (*Oreochromis niloticus*)	PO29
30	Ratu Safitri	Catfish Waste (*Pangasius sp.*) Fermentation by Probiotics for Essential Amino Acid and Fatty Acid Production	PO30

31	Afifah Khairunnisa Baasir	The Existence of Bacteriophage in Lactic Acid Fermentation: How Far They Can Fail The Fermentation?	PO31
32	Agussalim Matti	Exploration Of Lactic Acid Bacteria from Chao Products	PO32
33	Asmawati	The Fortification of Purple Sweet Potato (*Ipomea batatas Blackie*) on The Quality of Soyghurt	PO33
34	Endang S. Rahayu	Current Taxonomic Name of Indigenous Probiotic Strains	PO34
35	Aulia Pasha Adiyani	Study of Characteristic and shelf-life of Cream Cheese using Lactobacillus plantarum Dad-13 as Starter	PO35

ABSTRACTS OF INVITED SPEAKERS

Utilization of Linamarase-Producing Lactic Acid Bacteria And Yeasts For Cassava (*Manihot Esculenta* Crantz) Sourdough Fermentation

Francisco Elegado, Johanna Bangoy, Hazel Alena Tan and Margarita Mercado
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ABSTRACT

Cassava (*Manihot esculenta* Crantz) is a tropical root crop considered as staple food to millions of people worldwide. However, one of the drawbacks of this root crop is the presence of cyanogenic compound that can be degraded during fermentation. On the other hand, a hydrolytic enzyme called linamarase can be used to hydrolyze this cyanogenic compound and this can be produced by lactic acid bacteria (LAB) and yeasts. LAB and yeasts were screened for their ability to produce linamarase which can then be utilized for cassava sourdough fermentation. A total of 126 LAB and 113 yeast isolates from existing collection of cultures and from naturally-fermenting cassava sourdough, were inoculated in a screening medium to determine their ability to degrade cyanide. Three isolates (*Lb. plantarum* BS, *L. mesenteroides* SLI5 and *S. cerevisiae* 2030) were identified to have promising linamarase activities, which ranged from 48.0 to 51.67 U/mL. These isolates were applied as starter cultures in cassava sourdough to determine their ability to degrade hydrogen cyanide (HCN) initially present in cassava flour. Results showed that after 6 hours of fermentation, more HCN were liberated significantly (from 1.12±0.02 to 1.59±0.06 mg/kg). With the application of the starter cultures, the shelf-life of the different products (sourdough bread loaf, sourdough baguette and sourdough pizza) lasted for more than four months in the refrigerator at 5°C. Results of the proximate composition analysis also showed that sourdough breads with 30% of cassava flour has significantly more fiber content (0.92±0.09 %) compared to sourdough bread made only with wheat flour (0.62±0.03), although it contained less protein. Results of the sensory evaluation for the cassava sourdough bread showed that it did not differ significantly from the wheat flour sourdough bread in terms of the eight attributes (brown color, crumb color, fermented odor, firmness, crispiness of the crust, chewiness of the crumb, sour taste and fermented flavor). Moreover, results of this study also showed that sourdough bread fermented using the developed starter had significantly higher antioxidant activity (0.342 %) because it has more total phenolics (as gallic acid) compared to the unfermented bread (0.320 %).

Keywords: Cassava sourdough bread, Lactic acid bacteria starter, Yeast starter, Linamarase

Nutritional Products Developed by Lactic Acid Bacteria

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ABSTRACT

Lactic acid bacteria (LAB) are among the first bacteria discovered in 1875. However, they have been involved in food fermentation for centuries. Thus, LAB was associated with the well-known term “Probiotics” in 1960s. Since then, the promising health prospects of LAB and fermented products mediated by LAB have been extensively reported. As a result, LAB are now extensively exhibited to promote various health benefits, such as gut, skin, liver, lung and brain health. Subsequently, a few “-biotics” terms that are closely related to probiotics have been suggested, for instances ‘Prebiotic” and “Synbiotics”. However, probiotic LAB's beneficial effects generally rely on cell viability, exhibiting various challenges in the applications. Nevertheless, these remarkable bacteria also produce a variety of unique bioactive metabolites. Hence, the term “Postbiotic” and “Paraprobiotic” were suggested around the 2010s to address the functionalities of bioactive metabolites produced by the LAB and the mixture of bioactive metabolites and non-viable probiotic cells. In comparison, we have noted the pronounced potential applications of LAB isolated from Malaysian foods in addition to the beneficial effects of postbiotic metabolites, cell-less futuristic probiotics considerably. Therefore, potential applications of LAB isolated from Malaysian foods for nutritional products development will be discussed in my presentation.

Gut Microbiome and Metabolome of Asians Associated with Diets and Health

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ABSTRACT

Asia differs substantially among and within its regions populated by diverse ethnic groups, which maintain their own respective cultures and dietary habits. On the other hand, Oriental and Western cultures are now merging at many sites in Asia and affecting our life style, especially daily diets. To inquire into diversity in gut microbiota of Asian people, which must respond to daily diets and link to host health, Asian Microbiome Project (AMP) was established in 2009. AMP aims to build a basal microbiome database of Asians covering the entire region and all age groups and gain an insight into the link between life style and gut microbiota. By sharing information and knowledge gained through this project, we hope to promote health of Asian people.

To overview microbiome variation in Asian population, PCA analysis was performed using family or genus level compositional data of stool samples collected from Asian people. As a result, enterotype-like clusters were observed, which are defined by *Bacteroides, Prevotella*, and *Ruminococcaceae/Lachnospiraceae* genera, respectively. Japanese are mostly involved in *Bacteroides*-type, while Indonesian and Mongolian are mostly involved in *Prevotella* type. Interestingly, Thai samples are mainly involved in *Ruminococcaceae/Lachnospiaceae*-type. However, urban samples in each country tend to be more classified in *Bacteroides*-type. Notably, samples from type-2 diabetes patients are more localized in *Bacteroides*-type. It appears that *Prevotella*-type is tolerate to metabolic disorder, while *Bacteroides*-type is sensitive to metabolic disorder.

Metabolomic profile also reflects the status of gut microbiota. Propionate enriched in *Prevotella*-type microbiota and butyrate enriched in *Ruminococcaceae/Lachnospiraceae*-type microbiota may benefit host health. The loss of production of these SCFAs in the gut microbial community may sensitize inflammatory diseases as well as metabolic diseases. Dysbiosis which represents the status of dysfunction of gut microbial community is observed in some samples with substantially reduced production of SCFAs and also bile acid metabolism. Bile acid metabolism is also one of main function of gut microbiota but the products are complicatedly involved in health in human body. It is known that conjugated bile acids, such as tauro ursodeoxy cholic acid (TUDCA), funcions as antagonist of farnesoid X receptor involved in the feedback control of bile acid biosynthesis as well as glucose metabolism. The deconjugation of TUDCA occurs by *Bacteroides fragilis* and the loss of TUDCA is known to associate with impairment of glucose metabolism. 7α-dehydroxylation of primary bile acids, which is performed in the gut commensal bacteria member, results in hydrophobic bile acid molecules that are generally more cytotoxic and carcinogenic than primary bile acids. However, the 7α-dehydroxylation substantially occurs in active gut microbial community, while unhealthy subjects suffering from gut dysbiosis tends to lose this function and resulted in high ratio of primary bile acids, that often correlated with obesity

In conclusion, different functionality in different enterotype and main metabolic activity in each microbial community, which may be affected by daily diets, are strongly associated with health and disease.

Safety Aspects of Probiotic Bacteria: Current Status

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ABSTRACT

Lactic acid bacteria are bacterial group which are most applied as probiotics. As probiotic, they should show technological and functional characteristics, and, at the same time, they do not posses virulence properties. This undesirable characteristic includes horizontal gene transfer, antibiotic resistance, virulence traits, deleterious metabolic activities, gastrointestinal side effects, as well as excessive immune stimulation in succeptible individuals. The current status of safety aspects of probiotic bacteria will be discussed and a future outlook will be provided.

Keywords: Probiotic, Safety aspects, Virulence traits

Lactic Acid Bacteria, Fermented Food and Its Health Benefit

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ABSTRACT

Most of researches in Indonesia are related to the roles of lactic acid bacteria in fermented food, as bio-preservative and as probiotic. Many lactic acid bacteria have been isolated from Indonesia fermented foods. They are evaluated their ability to be used as starter cultures, and their functional properties and physiological properties important to be considered as probiotic. Indigenous lactic acid bacteria strains are promising for improving the process, quality and safety of Indonesian fermented foods. They also have some beneficial effects such as productions of antimicrobial agents, ACE inhibitor, improving antioxidant activity, and as probiotic. Some of lactic acid bacteria that were isolated from Indonesian fermented foods show their properties as probiotic candidates. *Lactobacillus plantarum* Dad 13 probiotic which was isolated from *dadih*, a traditional fermented buffalo milk has been studied extensively. It can be used as a single starter culture or mixed cultures to produce probiotic fermented milk. Consumption of this probiotic fermented milk increased the amount of lactic acid bacteria and *L. plantarum*, and decrease *Enterobacteriaceae* and *E. coli* in the faecal of healthy Indonesian volunteers. Method for production of the probiotic powder has been developed, and its application in various food as probiotic carrier has been studied. The effect of consumption of either probiotic powder or probiotic powder supplemented in some food to the gut microbiota and intestinal health of various selected volunteers show promising results.

Keywords: Lactic acid bacteria, Health benefit, Probiotics

ABSTRACTS OF ORAL PRESENTERS

The Presence of Lactic Acid Bacteria (LAB) on 'Inasua' Originated from Teon, Nila and Serua, Central Maluku District

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ABSTRACT

'Inasua' is a traditional fermented fish produced by communities in Teon, Nila, and Serua (TNS) Villages at Central Maluku District. This fermented fish has been determined as one of the intangible cultural heritages (ICH) of Maluku by the local government since 2015. 'Inasua' is defined as fermented fish where processed by using salt solution, and it is often called wet salt fish. The raw fish used were a kind of fishes that live around coral reefs, such as 'satan' or 'pig' fish (*Ruvettus pretiosus* Cocco). One of the microbial groups that play a role in fish fermentation is Lactic Acid Bacterias (LAB) that categorized as the Generally Recognized as Safe (GRAS). The aim of this study was to determine the total colonies of lactic acid bacteria (LAB) on 'inasua'. The method used is a descriptive method by identifying the total LAB colonies from the 3 villages named Teon (T), Nila (N) and Serua (S) where each village was represented by 2 samples. The research showed that total colonies of LAB in sample N1, N2 ; T1, T2 and S1, S2, were 1.3 x 10^4 (CFU/g), 1.6 x10^6 (CFU/g); 1.4 x 10^6 (CFU/g), 3.0x10^5 (CFU/g) and 1.7 x 10^6 (CFU/g), 1.6 x 10^4 (CFU/g) respectively.

Keywords: Inasua, Fermented, Lactic acid bacteria, Teon, Nila, Serua

Isolation and Characterization of Lactic Acid Bacteria in Fermentation of Brown Rice as An Antibacterial

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ABSTRACT

Brown rice has a high carbohydrate content. Brown rice has the potential as a source of lactic acid bacteria. This study aims to determine the lactic acid bacterial isolates obtained, characterization and antibacterial activity test from the fermentation of red rice washing water for 3 days in a sterile glass bottle and isolation of the fermentation results using MRS media to obtain 2 lactic acid bacteria isolates. The characterization of lactic acid bacterial isolates showed negative results on the Catalase, Motility, Indol, VP, Citrate and positive tests on the TSIA Test. Antibacterial activity test on the growth of *Staphylococcus aureus* and *Escherichia coli* using disc diffusion method gives an average inhibition zone value of 15.77 mm, 16.95 mm in *Staphylococcus aureus* and 12.07 mm, 12.12 mm in *Escherichia coli*. This shows that lactic acid bacterial isolates included in the strong category of the test bacteria *Staphylococcus aureus* and *Escherichia coli*.

Keywords: Antibacterial activity, Lactic acid bacteria isolation, Red rice fermentation

Antimicrobial Activity of Bacteriocin from *Pediococcus pentosaceus* Strain 2397 and Its Application as A Natural Preservative in Fish Meatballs

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ABSTRACT

Bacteriocin, a peptide produced by lactic acid bacteria (LAB), is widely used as a natural and safe preservative in food products. The objectives of this study were to determine antimicrobial activity bacteriocin produced by *Pediococcus pentosaceus* strain 2397 at various sources of carbon and nitrogen against *E. coli* and *S. aureus,* and to evaluate the effect of bacteriocin as a natural preservative on the microbial counts of fish meatballs during frozen storage. The antimicrobial activity of bacteriocin from strain 2397 against pathogenic microbes was carried out by the well diffusion method. Bacteriocins at various concentrations were applied to fish meatballs as a natural preservative, and observed the microbial counts during frozen storage. The results showed that the addition of various nitrogen and carbon sources affected the growth and antimicrobial activity bacteriocin from *Pediococcus pentosaceus* strain 2397. Optimal conditions for growth and increased antimicrobial activity of strain 2397 against *E. coli* and *S. aureus* were obtained at the addition of N sources such as peptone, beef extract, yeast extract, ammonium sulfate, and C source as lactose. The increase in the concentration of bacteriocin used in the manufacture of fish balls showed a decrease in Total Plate Count and *E. coli* at each frozen storage time. *S. aureus* was not found in fish meatballs with or without the use of bacteriocin during frozen storage. The use of 0.6% bacteriocin as a natural preservative could maintain the microbiological quality during frozen storage as per Indonesian National Standard of fish meatballs.

Keywords: Bacteriocin, Antimicrobial activity, *Pediococcus pentosaceus,* Natural preservative, Fish meatballs

Effect of Soybean Germination on The Growth of *Lactobacillus kefiri* YK4 and *Lactobacillus rhamnosus* BD2

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ABSTRACT

Lactic acid bacteria (LAB) have involved in production of many fermented foods around the world. The presence of LAB provide advantages in improving food safety, quality, and health benefits. Several LAB has been recognized as probiotic with significant health benefits. Yoghurt is the most popular lactic fermented foods to deliver probiotic bacteria. Soybean has been used as alternative to milk. Beside rich in protein, soybean also contain isoflavone. Germination can improve bioavailability of nutrient and bioactive compound of soybean. During germination, complex carbohydrate is converted into simple sugar that more available to the growth of LAB. The aim of this research is to study the effect of germination on the growth of LAB. Two LAB isolates namely *Lactobacillus kefiri* YK4 and *Lactobacillus rhamnosus* BD2 were used to ferment soybean milk. The LAB are isolated from kefir grains and have been evaluated for their potential as probiotic. The soybeans were germinated prior to processing into soymilk. The highest total LAB count amounted to $9.20 \pm 0.6 \times 10^8$ and $7.68 \pm 0.6 \times 10^9$ CFU/ml respectively for *Lactobacillus kefiri* YK4 and *Lactobacillus rhamnosus* BD2 was reached in soymilk obtained from 72 hours germinated-soybean. Those amounts were higher than non-germinated one. The pH value and total soluble solid are not affected by germination time, but they are decreasing after lactic fermentation, both using *L. kefiri* YK4 and *L. rhamnosus* BD2 starter.

Keywords: Lactic acid bacteria, Germinated soybean, *Lactobacillus*

Effect of Fermentation Using *Lactococcus lactis, Lactobacillus bulgaricus, and Lactobacillus paracasei* on Edamame Protein

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ABSTRACT

Attempt to increase the bioactivity properties of functional foods are increasingly becoming a necessity, including protein foods such as edamame (*Glycine max (L.*) Merrill) which contains high protein but its functional properties are still relatively low compared to other proteins. The functional properties of the protein can be improved through the derivation of the primary protein into shorter peptides.In this study, protein derivation was carried out by hydrolysis of edamame protein isolate using proteolytic bacteria, lactic acid bacteria (LAB) *L. bulgaricus, L. paracasei* and *L. lactis* for 24 hours. The proteolytic activity of these bacteria was qualitatively proven by the presence of a clear zone around the bacterial colonies on skim milk agar (SMA) media. Sodium odecyl sulphate poly-acrylamide gel electrophoresis (SDS-PAGE) analysis of fermented protein profiles showed the presence of proteins with lower molecular weights than unfermented proteins, indicating hydrolysis of proteins by proteases produced during fermentation by LAB. Free radical scavenging were conducted to determine the antioxidant activity (ABTS•+, OH•). The IC50 value of $ABTS^{\bullet+}$ of fermented edamame using *L.bulgaricus*, *L. paracasei* and *L. lactis* were 10.63 g/mL; 9.21 g/mL and 8.56 g/mL lower than that of control (before fermentation, 15,43 g/ml). These results indicate that the fermentation of edamame protein isolate using *L.bulgaricus*, *L. paracasei* and *L. Lactis* were able to generate a shorter peptide derivatives which were more bioactive rather than its native protein.

Keywords: Protein, Edamame, Antioxidant

Various Volumes of Palm Sugar in Making Yogurt Toward Characteristics of Yogurt (Pre-organoleptics Test)

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ABSTRACT

The purpose of this research is to determine: characteristics of yogurt (pre- organoleptic test) in making yogurt using various volumes of palm sugar. There are 5 treatments and replications are using palm sugar 1 ml (t 1), 1.5 ml (t 2), 2 ml (t 3), 2.5 ml (t 4) and 3 ml (t 5). The results of the pre-organoleptic test were analyzed descriptively. The results of the pre-organoleptic test showed that in treatment 1, the viscosity was 100% thick, 100% less red in color, 100% less sour with the aroma of tuac, 100% sour taste with slight of tuac. Pre-organoleptic of treatment 2 showed that 20% slightly thick, 20% less viscous and 60% viscous. The color is 100% slightly red. The aroma of yogurt is 20% very sour with the aroma of tuac, 40% sour with the aroma of tuac and 40% slightly sour with the aroma of tuac. The taste of yogurt is 20% very sour with tuac taste, 40% sour with tuac taste and 40% slightly sour with tuac taste. Treatment 3 are: viscosity 60% very thick, and 40% thick, color 100% slightly red, 100% less sour with the aroma and taste of tuac. The results of the 4 treatments were 100% viscous, 100% red color, 100% less acidic with the aroma and taste of tuac. Treatment 5 obtained 100% very viscous and very red color, 100% slightly sour with the aroma of tuac. Yogurt treatment was compare with control yogurt using 1 ml white sugar.

Keywords: Various volumes of palm sugar, Yogurt, Pre-organoleptic test

Types of Acidifier Impact Microbiome, Nutrient Composition, Antioxidant and Anti-Diabetic Activity of Soy Tempeh

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ABSTRACT

Tempeh is traditional fermented soy food from Indonesia that has anti-diabetic activity. Usually, tempeh was made by reducing the pH of soy to prevent the growth of unwanted bacteria as well to promote the growth of mold prior to addition of *Rhizopus* mold. Several tempeh producer used different types of organic acid, water or lactic acid bacteria that may affect nutrient, microbiome, antioxidant and anti-diabetic activity of soy tempeh. Five types of tempeh (TA, TG, TP, TL, and TLp) were subjected to proximate, dietary fiber and DPPH radical scavenging activity. Total DNA were isolated from all tempeh and V3-V4 16S rDNA sequencing were performed to identify microbiome composition of tempeh. Diabetes mellitus was induced by injection of streptozotocin (65 mg/kg bw) and nicotinamide (230 mg/kg bw). Diabetic rats were supplemented with each types of tempeh, replacing 15% of casein in the diet. Blood was collected before and after 4 weeks of treatment for blood glucose and lipid profile. There is significant different ($p<0.05$) on moisture, protein, fat, dietary fiber and DPPH-reducing activity between tempeh. Tempeh TLp has the highest bacteria diversity with Proteobacteria was the major composed phyla in tempeh. There is significant different ($p<0.001$) on glucose, total cholesterol, triglyceride, HDL- and LDL-cholesterol among group. Tempe TG has the highest metabolic profile improvement compared with other tempeh.

Keywords: Acidifier, Lactic acid bacteria, Tempeh, Nutrient, Antioxidant, Diabetes mellitus

The Possible Role of Probiotics in Alleviating Inflammation during The COVID-19 Infection

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ABSTRACT

COVID-19 pandemic has attacked the whole world for more than one year. All efforts are implemented to treat the infection. However, little is known about the possible role of probiotics in alleviating inflammation during the COVID-19 infection. This paper is a literature review. Literature was identified from the archives from PubMed, Elsevier, and Science Direct databases. All the literature was published in 2020. The inclusion criteria were the literature should be peer-reviewed and related to the topic of the paper. Results revealed that probiotics regulate immune cells in the intestinal tract. Probiotics maintain the equilibrium of the immune response. The role in enhancing immune functions is the possible role of probiotics in alleviating inflammation during the COVID-19 infection. However, the potential roles of probiotics against the COVID-19 infection need further investigations. Conclusion: the possible role of probiotics in alleviating inflammation during the COVID-19 infection is by regulating the immune cell in an equilibrium state, especially in the human intestinal tract. However, it needs further investigation and clinical research to establish the roles.

Keywords: COVID-19, Inflammation, Probiotic

"Can Probiotics Be A Promising Therapeutic Agent On Bone Health?"
A Gut-bone Axis Perspective Between Calcium Bioavailability And Microbiota Modulation

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ABSTRACT

Dietary supplementation as a therapeutic agent for preventing bone disease is preferred treatment by clinicians' and patients', and it is becoming increasingly popular. Beneficial microbes, such as probiotic, have been used in food for centuries due to their ability to administer a health benefit on the host with adequate amounts including potential clinical effects in treating osteoporosis. Hence, the objective of this review was to explain the dietary probiotic affected on bone health and mineral uptake by considering the correlation between calcium and gut microbiota modulation. Web of Science, Scopus, and PubMed by employing keywords for examining the calcium bioavailability and microbiota across the research spectrum. The literature studies revealed that *Lactobacillus* and *Bifidobacterium* could regulate the transcellular pathway in the calcium uptake, improve the imbalance of microbiota proportion in the gastrointestinal tract, and develop the immune system on bone health. Nevertheless, further experiments of probiotics on bone health are still needed to confirm the association between calcium bioavailability and gut microbiota balance.

Keywords: Probiotic, Bone health, Calcium Bioavailability, Microbiota

Nanoencapsulation of Probiotics: An Updated Review

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ABSTRACT

Nanoencapsulation is a promising technology in the development of functional food as it potentially enhances the bioactivity of functional material loaded in the capsule. Following the success story of probiotics microencapsulation, some recent studies attempted to develop nano-probiotics. Encapsulating probiotics in nano-scale material is a technological challenge as many factors must be considered. This study reviewed recent updates on the nanoencapsulation of active ingredients as well as the factors affecting the quality of the nano-capsule. Previous researches regarding the nanoencapsulation of probiotics are also discussed. Challenges in the nanoencapsulation of probiotics are also presented to give an alternative direction in the development of nano-probiotics.

Keywords: Nanotechnology, Encapsulation, Probiotics

The Effect of Probiotics Number in Fermented Bitter Melon (*Momordica charantia*) Juice in Decreasing Blood Sugar and Lipid Profiles

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ABSTRACT

Momordica charantia (MC) has anti-diabetic and anti-oxidative properties. The pathogenesis and progression of diabetes are induced by oxidative stress and followed by elevated triglyceride and cholesterol level. This study was aimed to evaluate blood glucose level and lipid profile before and after the treatment. A total of 24 male Sprague-Dawley rats were used in the experiments. Diabetes type 2 was induced by a single dose (60 mg/kg) of streptozotocin (STZ) and 120 mg/kg of nicotinamide, intraperitoneally. Three days after induction, the animals were randomly divided into four groups. Group 1, 2, 3, and 4 were given MC 10 ml/kg bw, fermented MC (FMC1) 10 ml/kg bw contained 3-7x10^{7} CFU/ml probiotics, fermented MC (FMC2) 10 ml/kg bw contained 3-7x10^{14} CFU/ml probiotics, and distilled water, respectively, for 28 days. The difference between pretest and posttest data were analyzed using pair t-test. Data were analyzed using ANOVA and Tukey HSD for post hoc analysis. The level of significance was set at 0.05. Groups treated with FMC2 significantly decreased both glucose and lipid profiles compared to MC and FMC1 ($p < 0.001$). The results showed that the probiotics number in MC was able to decrease both blood sugar and lipid profiles.

Keywords: Fermented bitter melon, Diabetes mellitus type 2, Probiotics, Hypolipidemic

Isolation and Characterization of Yeast from Fermented Sumbawa Mare's Milk with Probiotic Properties

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ABSTRACT

This study was aimed to determine the potency of yeast isolated from fermented Sumbawa mare's milk as a probiotic candidate and to identify the most potential yeast species as probiotic based on the ITS rDNA sequence. Mare's milk sample from each regency (Bima, Dompu, Sumbawa) were isolated using serial dilution method and inoculated in YEPD agar medium, then the yeast density was determined using Total Plate Count method and purified. The probiotic properties of the isolates were evaluated namely tolerance to low pH (pH 2 and 3) and bile salts (0,5% and 2%), antimicrobial activity, sensitivity to antibiotics, and hemolysis test. The experiments were carried out using a completed randomized design in triplicates. The data were analyzed using One-Way ANOVA ($p<0,05$) and isolate with the best probiotic potential was molecularly identified based on the ITS rDNA sequence. A total of 19 yeast isolates (5 isolates from Bima, 11 isolates from Dompu, 3 isolates from Sumbawa) were obtained, six of which (D1, D2, D6, B1, B4, B5) exhibited high tolerance to acidic conditions (pH 2) and bile salt (2%) with the survival rate ≥ 100% and ≥ 96%. The six isolates were resistant to tested antibiotics and no hemolytic property was demonstrated. Moreover, two isolates (D2 and D6) were able to inhibit the four tested pathogenic microbes, with the highest inhibitory activity was demonstrated by isolate D2 against E. coli ATCC 25922 by 496 AU / mL. Isolate D2 was the best probiotic yeast isolate that identified as Kluyveromyces marxianus.

Keywords: Fermented Sumbawa mare's milk, ITS rDNA, Kluyveromyces marxianus, Probiotics, Yeast.

Functional Effects of Probiotic Candidates, Isolated from Local Sources (Indonesia), on Human Health

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ABSTRACT

Isolation and identification of potential Probiotics used to improve human health has significantly increased worldwide in the last two decades. In Udayana University, Bali-Indonesia, particularly at the Integrated Laboratory for Biosciences and Biotechnology, intensive research on the isolation, characterization, and identification of potential probiotic candidates with a view to develop Bali local probiotics have been conducted since 2005. Until recently, hundreds of lactic acid bacteria have been successfully isolated from many sources, including from milk of various animals as well as human, fermented foods, feces of healthy infants, and Bali local fruits. Among those, only limited numbers of potential isolates have been identified molecularly and deeply or comprehensively studied. In this talk, their potential to improve blood lipid profile, to reduce blood cholesterol level, to alter microbial composition in human intestinal tract, as well as to suppress non beneficial bacteria (those potential as pathogens) in the intestine of animal model are discussed.

Keywords: Probiotics, Bali, Fermented foods, Human health

Antimicrobial Activity and Colour Properties of Annatto Extract Using *Bacillus subtillis* as A Pathogen

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ABSTRACT

Bixa orrellana L. known as annatto is a plant that rich in carotenoid pigment namely bixin and norbixin. Three main commercial processes used to extract the pigment from annatto seeds were direct extraction into oil or aqueous alkali, and indirect extraction using organic solvents. Annatto extraction using aquadest is profitable since aquadest is an edible solvent, easy to obtain, and cheap. This study aims to evaluation antimicrobials activity and natural colorants of annatto extracts. Aquadest was used as solvent and *B. subtillis* as the pathogenic bacteria. The extraction was carried out by maceration for 10 minutes, at various extraction temperatures (70, 80, and 90^{o}C). The antibacterial activity was tested by measure the inhibition zone and MIC (minimum inhibitory concentration). Munsel Colour Chard was used to determining the level of hue (colour), value (brightness), and chroma (intensity). The results showed that annatto extracts potential to inhibit *B. subtillis*. Increasing the extraction temperature causes an increase of inhibition while decreased of hue value, and chroma. The diameter of the clear zone was 4.33-5.17 mm (weak to moderate inhibitory activity category) and MIC resulted in 20% of extract concentration. The range hue, value, and chroma of extracts were 2.5-5 YR (yellow-red); 4.0-4.3, and 8-10 respectively.

Keywords: Annatto, Aquadest, Antimicrobial activity, B. *subtilis*, Colour, Extraction,

Effect Of Synbiotics Powder (*L. Plantarum* Dad-13 and Frukto-Oligosaccharide) on Fefecation Pattern and Stool Acidity in Stunting Children in Yogyakarta

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ABSTRACT

Stunting is caused by insufficient food intake and infectious diseases. Diarrhea is an infectious disease caused by pathogenic bacteria that can cause inflammation and damage to the intestines. The sample consisted of 39 stunted toddlers who were divided into 19 subjects as a synbiotics group given synbiotic (*L. plantarum* Dad-13 1x 1010 cfu and Fructo-oligosaccharide 700 mg) powder and 20 subjects as a placebo group given skim milk. The intervention was carried out for 90 days. Parents were asked to fill out a structured questionnaire. The pattern of defecation includes the frequency of defecation, stool consistency (based on the Bristol Stool Scale), and stool color. Stool acidity was measured with a pH meter. The results showed statistically there was a significant difference in the frequency of defecation and stool consistency between the synbiotics and placebo groups ($p<0.05$), but not in stool color ($p>0.05$). Acidity values in the synbiotics group during the 90-day intervention reduced stool acidity from 6.76±0.26 to 6.31±0.16, on the other hand, stool acidity in the placebo group increased from 6.69±0.26 to 6.74± 0.41. Increased stool acidity can cause inflammation. There was a significant difference in stool acidity between the synbiotics and placebo groups ($p<0.05$). Synbiotics can improve the quality of stool frequency and consistency and decrease stool acidity so as to reduce inflammation on stunting children.

Keywords: Defecation pattern, Stool acidity, Stunting children

Critical Control Point Analysis of Rendang Cooking in Padang Restaurants for Food Safety and Maintenance of Consumer's Gut Microbiota Balance

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ABSTRACT

This study aims to observe the application of Critical Control Point (CCP) analysis in the production process of Padang Restaurant rendang on *E.coli* contamination, moisture content, a_W value, and organoleptic properties test to improve food safety and prevent disturbances in the balance of the consumer's digestive microbiota. The parameters observed were the comparison of the application of CCP analysis and without CCP analysis on *E.coli* contamination, water content, a_W value, and organoleptic properties test of rendang dishes at Padang Restaurant. The research data were analyzed using *the Independent Sample T Test*. Data analysis on the organoleptic test used the *Kruskal Wallis non-parametric test* and continued with *the Mann Whitney test*. The results showed that rendang produced with CCP analysis was better than rendang without CCP analysis. The application of CCP analysis had a significant effect ($P<0.05$) on *E.coli* contamination, moisture content, a_W value, and organoleptic properties test of rendang dishes. This shows that the application of CCP analysis is needed to improve food safety and prevent disturbances in the balance of the consumer's digestive microbiota.

Keywords: CCP analysis, *E.Coli,* Moisture content, $a_{W,}$, Organoleptic properties test

ABSTRACTS OF POSTER PRESENTERS

The Influence of Different Temperature of Extraction Annatto Seeds (*Bixa orellana L*) as Natural Colourant and Antibacterial *Staphylococcus aureus*

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ABSTRACT

Annatto seed (*Bixa orellana L*) or Kesumba keling is commonly used as a colorant for textile and cosmetics. The pigment from Annatto is obtained from various carotenoid pigments that can be a coloring agent in food. In addition, Annatto seeds also have antibacterial activity because it contains saponins, tannins, steroids, and glycosides. Extraction of Kesumba seeds using water solvent (aquadest) pH 7 with different extraction temperatures are produced high color intensity. Annatto extraction using aquadest edible and profitable. The objectives of this study were to evaluate the influence of different temperatures of annatto seed extraction in preventing antibacterial activity and color intensity. The Extraction was carried out by maceration for 10 minutes, mixing speed of 2000 rpm and various temperatures includes 70, 80, 90 ℃. The color potential of the extract was measured using a color reader to determine the level of brightness (L), redness (a), and yellowness (b). The potential of the extract as antibacterial was tested by looking at Its ability to inhibit pathogenic bacteria using measure the inhibition zone. The pathogenic bacteria used were *Staphylococcus aureus* (Gram-positive bacteria). The results of this study include a brightness was 27.07-43.80; redness was 9.37-12.93; yellowness was 32.70- 42.50, and antibacterial activity with an inhibition zone was 4.00- 6.10 (weak to moderate inhibitory activity category).

Keywords: Annatto, Antibacterial, *S. aureus,* Brightness, Redness, Yellowness

Effect of Annatto Seed Extraction Temperature (*Bixa orellana L*) as Antimicrobial *Escherichia coli*

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ABSTRACT

Kesumba keling (*Bixa Orellana L*) commonly called annatto is a plant that has long been known and used for medicine and health. Annatto is rich in carotenoid pigments, namely bixin (nonpolar) and norbixin (polar). In addition, annatto also has antibacterial activity because it contains saponins, phenols, and tannins. The three main commercial processes commonly used to extract the active compound from dried annatto seeds are direct extraction into oil, direct extraction into aqueous alkali, or indirect extraction with organic solvents. Annatto extraction using aquadest is one of the profitable alternatives because aquadest is an edible, easy-to-obtain, and inexpensive solvent. This study aimed to obtain the antimicrobial activity of annatto seed extract. Extraction was carried out by maceration for 10 minutes, with a stirring speed of 2000 rpm at various extraction temperatures (70, 80 and 90˚C). Aquadest as solvent and *E.coli* as pathogenic bacteria (test bacteria). The potential of annato seed extract as an antibacterial was tested by measuring the clear zone and minimum inhibitory concentration (MIC). The results showed that annatto seed extract had the potential to inhibit E.coli. Increasing the extraction temperature causes an increase of inhibition. The diameter of the clear zone was 4.33-6.10 mm (weak to moderate inhibitory activity category) and MIC resulted in an extract concentration of 10%.

Keywords: Antimicrobial, Seed annatto, Temperature

Angiotensin I Converting Enyzme Inhibitory Activity of Jambal Roti with *Lactobacillus plantarum* 307 Application

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ABSTRACT

Jambal Roti is a traditional fermented fish product which famous in Java. The raw material used is catfish (*Arius thalassinus*). The manufacturing process includes weeding, salting for 3 days followed by drying with sun drying for 3 days. During the manufacturing process, there is an overhaul of proteins, fats and so on. Protein degraded will produce bioactive compounds that have the potential as Angiotensin I Converting Enzyme inhibitors. The aimed of this study was to examine the role of *Lactobacillus plantarum* 307 during the process of making jambal roti with 25% salt content. The results showed that the ACE inhibitory activity increased compared to the fresh product from 53% to 78.30%. The number of lactic acid bacteria decreased in number at the end of the study. The levels of peptide, soluble protein and total protein increased, indicating that fish protein was degraded into simpler components.

Keywords: Jambal roti, *Arius* thalassinus, Angiotensin converting enzyme inhibitor, *Lactobacillus plantarum 307*

Growth Pattern of *Bacillus* sp. (NrLtF5) Isolated from The Gastrointestinal Tract of The Nypa Worm (*Namalycatis rhodochorde*) at Different pH and Salinity Values

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ABSTRACT

Bacterial isolates of the genus *Bacillus* (code NrLtF5) have been isolated from the gastrointestinal tract of nypa worms (*Namalycastis rhodochorde*). This isolate is known to have probiotic potential and is cellulolytic, so it can be developed in nypa worm feed formulas. This study aims to determine the optimal salinity and pH for the growth of *Bacillus* sp. (NrLtF5). Salinity and pH are environmental factors that affect microbial growth, so optimization is necessary to obtain optimum values for cell production. The research was conducted using a microplate shaker culture method on Glucose Yeast Peptone (GYP) medium. The treatment of pH values was adjusted by the addition of 5M NaOH and 5M HCl to obtain pH values of 4, 5, 6, and 7, while the salinity value was adjusted by adding NaCl to media with salinity values of 5%, 10%, and 15%. Measurement of the growth pattern of Bacillus on salinity and pH values used the response surface methodology (RSM) with the central composite design (CCD) method at a wavelength of 585 nm. The results showed that the optimum growth value of *Bacillus* sp. (NrLtF5) was at a salinity of 15% with a pH value of 6. This is closely related to the growth character of nypa worm and has the potential to be developed as a probiotic for nypa worm culture.

Keywords: Bacillus, Nypa worm, Probiotic, Growth.

Influence Fermentation of Chocolate Tempeh on Antioxidant Activity and Blood Sugar Reduction In Vivo

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ABSTRACT

Chocolate on the market is generally at risk of diabetes because of the high content of glucose and fat. This is what urges the need to engineer a new chocolate processed product that is safe for consumption and plays a role in the therapy of diabetics through fermentation into tempeh. The purpose of this study was to determine the best fermentation time in increasing the bioactive compound content of cocoa beans which have the potential anti-diabetic agents as an innovative solution for reducing glucose for people with diabetes mellitus in vivo. This study used a randomized block design (RAK) with 1 factorial, the incubation time of cocoa bean tempeh fermentation, and 3 levels fermentation, control, 24 hours, 48 hours. The variables analyzed were proximate (moisture content, ash content, protein content), phytochemicals (phenols, flavonoids, tannins), antioxidants and in vivo tests on wistar. The results of proximate levels showed that the fermentation time had no significant effect on the water, ash and protein content, while the phytochemical and antioxidant tests showed the best fermentation time at 24 hours with a phenol content of 237.41 mg GAE/g , tannin 27,100 mg TAE/g, antioxidant 72.14% and for flavonoid levels the best results were shown at 48 hours fermentation 18,87 mg QE/g. In vivo testing on Wistar by measuring blood sugar using an glucometer on days 0, 7, and 14. It is known that the administration of 24-hour cocoa bean tempeh fermented extract can significantly reduce the blood glucose levels of Wistar by 25.80 mg/dl.

Keywords: Antidiabetic, Indiction, Tempeh, Cacao seed

In Silico Analysis of Antibiotic Resistance Genes in *Lactobacillus plantarum* T3

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ABSTRACT

Lactobacillus are widely used as probiotics in yogurt, cheese, wine. They are generally recognized as safe (GRAS). Probiotics used in food products must be considered for food safety, one of which is resistant to antibiotics because probiotics must not carry antibiotic resistance genes. The strains used in this study were *Lactobacillus plantarum* T3 from *gatot* (fermented dried cassava). We analyzed their levels of antibiotic resistance using a minimum inhibitory concentration (MIC), their antibiotic resistance gene profiles, and the transferability of some of the detected resistance markers using Rapid Annotation using Subsystem Technology (RAST) and Comprehensive Antibiotic Resistance Database (CARD). Phenotypically *L. plantarum* T3 is resistant to chloramphenicol (64 µg/mL), kanamycin (256 µg/mL), streptomycin (1 µg/mL), erythromycin (8 µg/mL), clindamycin (8 µg/mL) ciprofloxacin (1 µg/mL) and sensitive to tetracycline (32 µg/mL), ampicillin (1 µg/mL). The result from our WGS analysis revealed 3 tetracycline, 2 fluoroquinolones, and 4 β-lactamase resistant bacteria that carried the tet(W), otr(A), fus(A), gyr(A), gyr(B), ampC1, ampH, blaF, and EXO-1 genes, respectively. There was no transfer of antibiotic resistance genes in the probiotics used which were analyzed using RAST because they did not have transposable elements and plasmids. Overall, this study produced antibiotic resistance profiles of *L. plantarum* T3 to assessed their risk of transferring an antibiotic gene to other bacteria. This study can provide important data on the safe use of probiotics.

Keywords: *Lactobacillus plantarum*, Antibiotic resistance, Gene transfer, Minimum inhibitory concentration, Whole-genome sequencing

Physicochemical Properties and Volatile Compound of Yoghurt Powder Encapsulated by Spray Drying Method

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ABSTRACT

Yoghurt contains many volatile compounds. However, in the drying process into yoghurt powder using spray dying, high temperatures can damage the volatile components of yoghurt. Encapsulation technique is believed to be a solution to protect yoghurt powder from losing flavor during the drying process, but its ability depends on the type of encapsulation used. This study investigated the volatile components of yoghurt powder that were encapsulated using 2 types of encapsulation. **Y**oghurt powder was prepared by adding skim and maltodextrin encapsulants with concentrations of 5%, 10% and 15% using spray drying at an inlet temperature of 110˚C and outlet temperature of 68-80˚C. Cell viability, pH, total titrated acid, moisture content, and volatile compounds were investigated. The result showed that the variation of encapsulant and concentration affect the volatile compound and physicochemical of yoghurt powder. Yoghurt powder with the addition of 15% maltodextrin encapsulant is able to retain a number of the main volatile compounds of yoghurt (aldehyde, acetoin, acetic acid, and some aromatic compounds), has a water content according to SNI (3.85%), able to maintain the production of yoghurt powder (pH 3.92 and total acid titrated 0.87) after undergoing a drying process using spray drying, and has a solubility percentage of 89.10% after the spray drying process.

Keywords: Yoghurt powder, Encapsulation by spray drying, Volatile compound

Cold Stress Response Gene of *Lactobacillus plantarum* Mut-3 and *Lactobacillus plantarum* Mut-7 Supports Their Ability to Survive in Freezing Condition

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ABSTRACT

Probiotics are currently widely consumed in a mixture of several food matrices and provide health benefits to the host. The viability of probiotic cells is influenced by several factors, including exposure to high temperatures during the production process and low temperatures during storage. In this study, we report on the stress response of *Lactobacillus plantarum* Mut-3 and Mut-7 after 24 hours of storage at 4 ℃ and -20 ℃. The cold stress response of cold shock protein genes was evaluated through whole genome sequencing. Results revealed that the viability of *L. plantarum* Mut-3 and *L. plantarum* Mut-7 had no significant differences between control (11,99 log CFU/ml and 11,76 log CFU/ml, respectively) and at 4℃ (11.68 log CFU/ml and 11.39 log CFU/ml, respectively) also at -20 ℃ (11.30 log CFU/ml and 11.11 log CFU/ml, respectively). Meanwhile, through whole genome analysis there is one gene *csp*A/*csp*L which have the possibility of actively playing a role in maintaining cell resistance at low temperatures. This gene in *L. plantarum* Mut-3 and *L. plantarum* Mut-7 has 84,85% similarity with *Listeria monocytogenes* EGD-e. Then, based on multiple alignment on amino acid sequence of *csp*A/*csp*L both strain have the same conserved motif compared with *Listeria monocytogenes* EGD-e.

Keywords: Cold shock, Stress response, *Lactobacillus plantarum* Mut-3, *Lactobacillus plantarum* Mut-7, *csp*A, *csp*L

Viability and Volatile Compound of *Lactobacillus plantarum* Dad-13 and *Lactobacillus plantarum* Kita-3 as Starter Cultures in Probiotic Cream Cheese

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ABSTRACT

Lactobacillus plantarum Dad-13 and *Lactobacillus plantarum* Kita-3 are Indonesian indigenous probiotic obtained from the isolation of Indonesian fermented foods. In this study, probiotic *L plantarum* Dad-13 and *L plantarum* Kita-3 were used as starter cultures in the production of cream cheese and compared with starter commercial of cream cheese. Their viability was evaluated over 35 days of storage at 4 ^{0}C. The results showed that the viability of lactic acid bacteria in cream cheese with probiotic starter *L. plantarum* Dad-13 and *L. plantarum* Kita-3 were higher than the commercial starter and the amount of viable cells were maintained at 10^8 CFU/g during storage at 4 ^{0}C for 35 days. A total of 36 volatile compound were identified by SPME/GC-MS and 9 fatty acid, 9 ketone, 5 alcohols, 6 ester and 7 lactones were the 5 major contributors to the characteristic flavours of the cheeses. Additionally, high levels of acetic acid (vinegar), hexanoic acid (pungent/sour), butanoic acid, octanoic acid, and decanoic acid (rancid, fatty) were also identified in the samples. The use of probiotics as starter cultures increased the diacetyl compounds (buttery flavour) and produced more ester and lactone compounds than commercial starters.

Keywords: Cream cheese, Probiotic, *Lactobacillus plantarum* Dad-13, *Lactobacillus plantarum* Kita-3, Viability, Volatile compound.

Effect of Purple Sweet Potato (*Ipomoea batatas* L) Paste Concentration on Lactic Acid Bacteria Growth in Soygurt

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ABSTRACT

Soygurt is a type of probiotic beverage made from soy milk fermented by lactic acid bacteria (LAB). Purple sweet potato contains various bioactive compounds which potent to improve the functionalities especially health benefits of soygurt. Purple sweet potato is also a source of carbohydrates which can contribute to the lactic acid bacteria growth. The purpose of this research was to investigate the effect of purple sweet potato paste concentration on lactic acid bacteria growth in soygurt. This experiment used a starter contained *Streptococcus thermophilus*, *Lactobacillus bulgaricus,* and *Lactobacillus acidophilus*. The purple sweet potato paste was added at six concentration levels i.e. 0%, 3%, 6%, 9%, 12%, and 15%. The total plate count of LAB cells of soygurt is 7,9900-9,7459 log cfu/g and the pH value is between 4,735-4,298. Statistical analysis with Anova at α of 5% showed that the concentration of purple sweet potato paste affects significantly the total plate count LAB and pH of soygurt. The higher concentration of purple sweet potato paste, the total plate count of LAB increase, and the pH decrease. Various compounds in purple sweet potato such as carbohydrates (starch, sugars i.e. sucrose, maltose, and glucose), protein, minerals, and vitamins might be contributing to the LAB growth. In conclusion, the purple sweet potato has a positive impact on LAB growth.

Keywords: Soygurt, Purple sweet potato, Lactic acid bacteria

Weissella confusa F213 Modulated Tumor Necrosis Factor-α in DSS-induced Colitis Rats

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ABSTRACT

Inflammatory bowel disease (IBD) is an inflammatory disorder of the digestive tract. Increased intestinal level of the proinflammatory cytokine TNF-α is associated with disease severity. IBD occurs as results of many factors, including dysbiosis. It is well-established that gut microbiota plays role in mucosal immunity, then modulation of gut microbiota may help in alleviating IBD symptoms. Probiotics could modulate gut microbiota. *Weissella confusa* F213 (WCF213) has been found to successfully enhance mucosa integrity *in vitro*. The role of this local probiotic in immunomodulation on intestinal mucosa has not been investigated yet. Therefore, the aim of this study was to evaluate the effect of WCF213 on TNF-α level in colon mucosa of DSS-induced colitis rats. Sixteen male Wistar rats (± 100 g) were divided into 4 groups; control, WCF213 (1 x 10^8 CFU/BW) for 14 d, 2.5% Dextran Sulphate Sodium (DSS) for 7 d and WCF213 (1 x 10^8 CFU/BW) for 14 day, and 2.5% DSS for 7 d. After sacrificing the rats, the colon was collected, and mucosal TNF-α level was measured using ELISA. It was found that TNF-α level of DSS-WCF213-treated group was significantly lower than that of DSS-treated group (p=0.011). In IBD, there is an elevation of TNF-α tissue level in the mucosa and lamina propria of colon. Therefore, this preliminary finding indicated that *W. confusa* F213 may play a role in IBD management. Further investigation on other inflammatory markers and mucosal integrity are needed to study the role of this probiotic in IBD.

Keywords: *Weissella confusa*, Inflammatory Bowel Disease, Colitis, Dextran Sulphate Sodium, TNF-α

Comparison of Food Habbits and Gut Microbiota in Type 2 Diabetes and Healthy Women in Yogyakarta Indonesia

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ABSTRACT

Association of food intake with intestinal microbiota, short chain fatty acid and metabolic markers in Indonesian women with type 2 diabetes (T2D) has been investigated. The case control study that used subjects of healthy women and women with T2D. The intake were taken with a semi-quantitative food frequency questionnaire (SQ-FFQ) and physical activity data with IPAQ for a month. Then after 1 month, anthropometric data and metabolic markers were taken. Feces were taken for analysis of pH, gut microbiota and SCFA. HbA1c, fasting blood glucose (FBG) and age were different in healthy and T2D women. Macronutrient intake (calories, carbohydrate, protein, fat) and fiber were not different but T2D women had more water intake significantly than healthy women. T2D had more sugar intake but less meat & poultry product intake and fish & seafood product intake than healthy women. Total short chain fatty acid (SCFA), acetic acid, propionic acid, butyric acid, valeric acid and gut microbiota (*L. plantarum, Bifidobacterium and Prevotella*) were not different significantly in healthy and T2D women. There was correlation between FBG with age, HbA1c, waist hip ratio (WHR) and protein intake. HbA1c correlated with age, waist circumference, WHR. Stool pH correlated with waist circumference, WHR, acetic acid, propionic acid, total SCFA and *Prevotella*. Acetic, propionic and butyric acid correlated with *Prevotella* and just propionic acid correlated with carbohydrate intake. Microbiota markers of T2D is *Bacteroidetes* and Microbiota markers of healthy is *Ruminococcaceae*.

Keywords: Gut microbiota, SCFA, T2D women

Effectiveness of Jelly Candy Formulation Nipah Leaves (*Nypa fruticans*) Fermented by *Lactobacillus plantarum* To Lower Blood Triglycerides Levels In Vivo

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ABSTRACT

Obesity is a condition of excess body fat due to increased activity of the pancreatic lipase enzyme. Cases of obesity in the world, including in Indonesia, continue to increase every year, especially during the pandemic, patients are vulnerable to being infected with COVID-19. Treatment of obesity such as consuming synthetic fat diet drugs (sibutramine and orlistat) in the long term has dangerous side effects. Nipah (Nypa fruticans) plants, especially their fermented leaves, contain high levels of bioactive compounds (saponins, flavonoids and tannins). The purpose of this study was to determine the best dose of fermented nipah leaf powder in a jelly candy formulation that can effectively reduce blood triglyceride levels and remain delicious for consumption. This study used a Completely Randomized Design with 4 jelly candy formulations, namely fermented nipah leaf powder, sorbitol and carrageenan, including F1 (1% : 75% : 8%), F2 (8% : 75% : 8%), F3 (16% : 75% : 8%) and F4 (0% : 75% : 8%). The results of this study showed that the fermented nipah leaf of *Lactobacillus plantarum* had a phenol value of 32.94 mg/g; flavonoids 1974.84 mg GAE/g; tannins 44.66 mg/g; toxicity with an LC50 value of 130.81 mg/mL, and an antioxidant 34.76%. Jelly candy formulation with the addition of fermented nipah leaf powder as much as 16% (F3) was able to reduce body weight of obese rats by 1.91 g from 29.03 g to 27.12 g, and triglyceride levels by 39.8 mg/dL from 240 mg/dL to 200.2 mg/dL.

Keywords: Obesity, Nipah palm (*Nypa fruticans*), Fermentation, Jelly candy, Lower blood triglycerides

Gut Microbiota Profile Between Normal and Moderate Malnutrition Children in Yogyakarta, Indonesia

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ABSTRACT

Malnutrition has been associated with the gut microbiota composition and the gastrointestinal environment. This study aimed to evaluate whether there is a difference in the gut microbiota profile between the normal and undernutrition (considered moderate malnutrition) children. Ten days' observations were done between normal (n:13) and undernutrition (n:15) children. The subject's diet was recorded using a food record. Analysis of the gut microbiota was performed using 16S rRNA gene sequencing targeting the V3-V4 variables region, while the specific bacterium's number (*L. plantarum*, *Bifidobacterium* and *Enterobacteriaceae*) was analyzed using qPCR. The result shows that the undernutrition group's energy intake was lower than in the normal group. Although there was no difference in diversity index and overall gut composition, overexpression of the genera *Methanobrevibacter*, *Anaerococcus*, *Eubacterium*, and *Succinivibrio* was observed in the undernutrition group. Meanwhile, in the normal group, *Ruminococcus* and *Fusobacterium* were found. In both groups, there was also the dominant of *Prevotella* enterotype. *Bifidobacterium* was significantly higher in the normal group, and there was no difference in the number of *L. plantarum* and Enterobacteriaceae in both groups.

Keywords: Gut microbiota, Dysbiosis, Moderate malnutrition

Indigenous Lactic Acid Bacteria from Halloumi Cheese as A Probiotics Candidate of Indonesian Origin

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ABSTRACT

This study aimed to isolate lactic acid bacteria (LAB) from Halloumi cheese produced by Mazaarat Artisan Cheese Indonesia, to evaluate their potential as a probiotics, and to identify them based on molecular characteristics. LAB were isolated from fresh milk, curd, and Halloumi cheese and identified their morphology and physiology. Screening of LAB isolates for probiotics candidates was mainly based on the resistance of simulated gastric juice, bile salts, antibacterial activities against pathogens, and the adhesion properties by hydrophobicity test. The selected LAB strains as probiotics candidates were then identified molecularly using 16S rRNA. From this study, 13 LAB isolates were obtained. Among 13 isolates, K3 isolate was the most potent as a probiotics candidate. This isolate had high tolerance for simulated gastric juice and bile salts, good ability to protect the gut against pathogenic bacteria since it has antibacterial activities and had ability to adhere to human epithelial cells. Based on molecular identification, this probiotics candidate was identified as *Lactobacillus plantarum*.

Keywords: Halloumi cheese, Lactic acid bacteria*, Lactobacillus plantarum*, Probiotics

Effect of Sucrose and Skim Milk Addition on The Acid Production during Black Soybean Milk fermentation by *L. plantarum* Dad-13

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ABSTRACT

The objectives of this research were to study the pattern of acid production and pH decrease during the fermentation of black soybean milk with various concentration of sucrose and skim milk by lactic acid bacteria. The black soybean milk with 0%, 2%, 4%, 6%, and 8% (w/v) addition of sucrose and skim milk were inoculated with 1% (v/v) *Lactobacillus plantarum* Dad-13. The titratable acidity and pH were examined in every 3 hours of fermentation at 37°C. In original black soybean milk, acid and pH change occurred during the 12 hours of fermentation and likely to remain same afterwards. In variation of sucrose and skim milk concentration, acid and pH change occurred during the 24 hours of fermentation. In the end of fermentation of black soybean milk with sucrose addition, acid levels were in the range of 1.13–1.63% and pH value were in the range of 3.07–4.24. In the end of fermentation of black soybean milk with skim milk addition, acid levels were in the range of 1.07–1.86% and pH value were in the range of 4.08–4.68. Based on the research results, the time required for the fermentation of black soybean milk using *L. plantarum* Dad-13 was for 9–12 hours.

Keywords: Black soybean milk fermentation, *Lactobacillus plantarum* Dad-13, Acid production

Screening and Effect of MSG on The Increase of Aminobutyric Acid (GABA) *Lactobacillus plantarum* Fermented Food Isolates

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ABSTRACT

γ-aminobutyric acid (GABA) is a non-protein amino acid, with various effects of functional health, produced from decarboxylation of glutamate catalyzed by glutamate decarboxylase (GAD). This enzyme is produced by various microbes like lactic acid bacteria (LAB) as starters to increase the concentration of GABA in fermented foods. Screening was conducted on 34 strains of LABs (30 *Lactobacillus rhamnosus* and 4 *Lactobacillus plantarum*) previously isolated from fermented foods. These LAB strains were grown on MRS broth and GABA production was detected using TLC methodology. Strains produced positive GABA spots were confirmed from GAD existence. The results of this study showed that 21 out of 34 strains tested (17 *L. rhamnosus* and 4 strain *L. plantarum)* demonstrated their potential as GABA producers. *L. plantarum* U201, *L. plantarum* PH715, *L. plantarum* PH719 and *L. plantarum* PH18bb, produced GABA with concentrations of 14,43±2,54 µg/ml, 31,30±4,11 µg/ml, 28,57±4,14 µg/ml, dan 25,70±1,03 µg/ml on MRS, respectively. In MRS medium supplemented with 2% MSG, GABA production of *L. plantarum* U201, *L. plantarum* PH715, *L. plantarum* pH719 and *Lactobacillus plantarum* PH18bb were 177,57±3,25, 279,80±5,75, 139,46±2,56 and 233,40±2,68 following incubation for 48 hours at 37^{0}C. Production of GABA which was different in each strain was influenced by the activity of fermentation of microorganisms, the addition of MSG as a precursor of GABA and its growth medium. It can be concluded from this research that PH715 was the best GABA producing strain and had potential to be used as a starter in food fermentations, such as “rebung” fermentation.

Keywords: GABA, *L. plantarum*, MSG

Inhibitor Alpha Glucosidasion Activity of Fermented Milk from *Lactobacillus plantarum* Dad-13 in Various Fermentation Time

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ABSTRACT

The high number of people with diabetes in the world especially in Indonesia, lead to study of functional food with alpha glucosidase inhibitor activity (AGI) capabilities. The proteolytic activity of lactic acid bacteria (LAB) during fermentation is considered capable of producing peptides with AGI capabilities. Fermentation time is one of the factors that can affect the results of fermentation. In this study, AGI was studied in skimmed milk fermentation using Lactobacillus plantarum DAD13 with fermentation times of 0, 6, 12, 18 and 24 hours. Apart from AGI, the number of bacteria, pH, total peptides and degree of hydrolysis were also studied. Protein pattern showed that the majority of molecular weight of fermented milk was between 10-34 kDa, which was suitable for AGI. The results showed that the alpha glucosidase inhibitor activity of fermented milk for 18 hours was higher among others (0,36%), although the total peptide and the degree of hydrolysis were lower than 24 hours fermented milk.

Keywords: Lactic acid bacteria, Fermented milk, Alpha glucosidase inhibitor

Viability, Acid, and Bile Tolerance of Spray-dried Synbiotic Microcapsules using Various Encapsulating Materials from Iles-iles (*Amorphophallus oncophyllus)* and a Protective Agent

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ABSTRACT

This study aims to determine the influence of the encapsulating material of Iles-iles fractions and protective agents on the viability of spray-dried synbiotic microcapsules in acid and bile salts. Despite, to obtain spray-dried synbiotic microcapsules with the highest number of *Lactobacillus casei* cells and have high viability to acid and bile salts. The randomized complete block design was used as an experimental design with two factors. The first factor is the encapsulating materials consist of three levels, namely A1 (iles-iles glucomannan), A2 (iles-iles starch), and A3 (iles-iles flour). The second factor is the type of protective agents consists of three levels, namely B1 (skim milk), B2 (maltodextrin), and B3 (Arabic gum). The results showed that the encapsulating material affected the decrease in *L. casei* during storage and a decrease in viability of acid and bile salts by 2%. This type of protective material does not affect the decrease in *L. casei* during storage and a decrease in viability to acid and bile salts of 2%. The iles-iles glucomannan and arabic gum produce synbiotic microcapsules with the highest viability of L. *casei* and tolerance in acid and bile salts.

Keywords: Viability of synbiotic microcapsules, Iles-iles, Protective agents, Acid, Bile salts

Mother's Knowledge of Probiotic's Benefit for Their Children in Java Indonesia

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ABSTRACT

Mothers play a vital role in choosing their child's diets. Therefore, a mother's knowledge about the benefits of probiotics for children will affect the consumption of probiotics for their children. Meanwhile, the potential benefits and ways of using probiotics in children have been widely studied globally. This study will explore mother's basic knowledge of the benefits of probiotics for their children. Data was gathered using a questionnaire that was participated in by 100 mothers who have children under 17 years old and live in Java, Indonesia. This study showed majority (90%) respondents had already known the term of probiotics, (83%) understand that probiotics contain live bacteria. However, only 14% of respondents believe that not all LAB (Lactic Acid Bacteria) are probiotic; the rest assume that all LAB are probiotic. On the knowledge of the use of probiotics in children, respondents only believe in the benefits of probiotics to cure diarrhoea (64%) and help increase stool frequency (84%), besides that respondents do not have enough information to determine the benefits of probiotics for children and choose doubtful options (53-63%) about their benefits. Therefore, mothers' knowledge about the benefits of probiotics is limited to the popular ones, while mothers still doubt the other benefits for children.

Keywords: Probiotics, Mother's knowledge, Probiotic's benefit, Children

Isolation and Identification of Lactic Acid Bacteria from Halloumi Cheese Produced by SME Mazaraat Artisan Cheese Yogyakarta

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ABSTRACT

This study was intended to isolate the Lactic Acid Bacteria (LAB) present in Halloumi Cheese made from local fresh milk. Isolation is carried out on fresh milk, curd before milk boiling process, and Halloumi Cheese. The isolates obtained were identified by their morphology, physiology, and molecular. Morphological identification is carried out by microscope observation to determine the shape and arrangement of cells. Physiological identification is determined by the nature of the Gram; catalase; the ability to grow at certain temperature conditions (10 ^{0}C, 37 ^{0}C, and 45 ^{0}C), pH (4.4 and 9.6), and salt concentration levels (6.5% and 18%); and the type of fermentation. Molecular identification is carried out by PCR amplification method of 16s rRNA sequence. The isolation results in 13 isolates that were molecularly identified as *Lactobacillus plantarum* and *Enterococcus faecium*. The most dominant phenotype of *Lactobacillus plantarum* is rod-shaped, non-chain; Gram-positive; non-catalase; heterofermentative which is characterized by the formation of CO_2 in the fermentation of sugar; able to grow at 10 ^{0}C, 37 ^{0}C or 45 ^{0}C; able to grow at salt concentration of 6.5%; and able to grow at pH 4.4. The phenotype of *Enterococcus faecium* is round-shaped with a duplo or tetrad arrangement; Gram-positive, non-catalase; homofermentative which is shown from the absence of CO_2 in sugar fermentation; able to grow at 10 ^{0}C, 37 ^{0}C or 45 ^{0}C; able to grow at salt concentration of 6.5%; and able to grow at pH 4.4 and pH 9.6.

Keywords: Halloumi cheese, Fermentation, Lactic acid bacteria, Curd, Milk

Consumption of Probiotics and Gut Health of Students of Faculty of Agricultural Technology Universitas Gadjah Mada During Pandemic Covid-19

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ABSTRACT

Students are one of the aspects needed in the realization of Health Promoting University launched by Gadjah Mada University. However, with a dense activity makes many students who do not pay attention to a healthy lifestyle. Whereas at the moment there is also a Covid 19 pandemic that requires a healthy lifestyle of students. A healthy environment is a state that includes physical, mental and social health is not only a state free from disease. To provide information on the gut health of student can be conducted survey of fecal quality and probiotic consumption. The survey was conducted for 10 days by 60 respondents by filling out a questionnaire on the google form. The data obtained is then processed with Microsoft Excel, and SPSS. From the survey obtained probiotic consumption 70% of students never/rarely consume probiotics. In the frequency of defecation, 90% of students had a normal frequency, 93% of students had a normal stool consistency and 95% of students had a normal stool color. This shows no relationship between probiotic consumption and defecation frequency according to spearman's statistical analysis of 0.05 significance.

Keywords: Probiotics, Students, Gut health

Effect of *Spirulina platensis* Addition on Physicochemical and Microbiological Properties of Fermented Milk by Indonesian Probiotics Bacteria

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ABSTRACT

Spirulina platensis contained valuable compounds such as essential amino acids, minerals, vitamins, and other bioactive compounds. This work aimed to study the effect of *S. platensis* powder addition on physical, chemical, and cell growth during milk fermentation by *Lactobacillus plantarum* Dad 13 and *Streptococcus thermophilus* Dad 11. Milk added with various *S. platensis* powder concentrations was pasteurized and inoculated with cultures *L. plantarum* Dad 13 and *S. thermophilus* Dad 11, and incubated at 37 °C for 24 hours. The cell's growth, acid production, and antioxidant activity were analyzed after incubation. The sensory characteristics were described by the authors. The viscosity was monitored during milk fermentation with the addition of selected *S. platensis* concentration. The results showed the *S. platensis* addition into fermented milk by combination of two cultures increased cell growth along with the increase of *S. platensis*, from 8.63 log CFU/mL (without *S. platensis*) to 8.79-9.16 log CFU/mL (0.15-0.6% *S. platensis*). An increase of cell growth also occurred in *L. plantarum* Dad 13, which is a probiotic, from 8.21 log CFU/mL (without *S. platensis*) to 8.38-8.88 log CFU/mL (0.15-0.6% *S. platensis*). The increase of *S. platensis* concentration also increased the acid production from 0.85% to 1.09-1.33% and antioxidant activity from 33.60% to 35.01-42.63%. Moreover, the selected concentration of *S. platensis* increased viscosity during the fermentation process, from 0.35-2.66 Pa.s (without S. platensis) to 0.42-4.05 Pa.s (0.3% *S. platensis*). *S. platensis* has been successfully added to milk fermentation by combination of *L. plantarum* Dad 13 and *S. thermophilus* Dad 11.

Keywords: Antioxidant activity, Fermented milk, Probiotic, *Spirulina platensis*

Adhesion Properties of *Lactobacillus plantarum* Dad-13 and *Lactobacillus Plantarum* Mut-7 on Rat Intestine

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ABSTRACT

Adhesion capacity has been considered as one of the selection criteria for probiotic strains. The aim of the study was to determine the adhesion properties of two candidate probiotics, *Lactobacillus plantarum* Dad-13 and *Lactobacillus plantarum* Mut-7. The evaluation included hydrophobicity of the cell surface, autoaggregation, and adhesion of *L. plantarum* Dad-13 and *L. plantarum* Mut-7 to the intestinal mucosa accompanied by genomic analysis. All strains tested showed high surface hydrophobicity and medium autoaggregation ability. Exposure of both isolates to the surface of the rat intestine increased the total number of lactic acid bacteria, indicated the attachment of lactic acid bacteria on the surface of the rat intestine and reduced the indigenous *E. coli*. Both strains have genes related to adhesion properties that could play an important role in increasing the adherence of probiotics to the intestinal mucosa. Based on this findings, *L. plantarum* Dad-13 and *L. plantarum* Mut-7 have the ability to attach to the intestinal mucosa in the rat intestine model system.

Keywords: Probiotic, *Lactobacillus plantarum*, Hydrophobicity, Autoaggregation, Adhesion ability

Inclusion of Lebui Nut (*Cajanus sp*) Extract to Increase the Functional Value of Goat Milk Yogurt

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ABSTRACT

Lebui nut (*Cajanus* sp) has been known to have antioxidant activity. The results of Devi *et al*., (2016) showed that the total content of phenolic compounds, the total content of flavonoid compounds and the IC_{50} value of lebui nuts on antioxidant activity against DPPH free radicals were 57.20 (mg GAE/g extract); 49.43 (mgQE/g extract); and 0.84 (mg/ml extract). The purpose of this study was to determine the effect of the addition of lebui nut extract on the viability of lactic acid bacteria and the antioxidant activity of yogurt produced. The results indicated that the phenolic compounds contained in yogurt added with lebui nut extract did not contain components that had specific bactericidal activity on the culture starter. It was seen that on the first day of refrigerator storage (4^{o}C), the number of lactic acid bacteria remained at 10^8 log CFU/g and only showed a decrease in the number of lactic acid bacteria to 10^7 log CFU/g until the end of the storage process in the refrigerator (4^{o}C). Antioxidant activity of yogurt with the addition of lebui nut extract occurred at the 7th day of storage in the refrigerator. It was assumed that on the 7th day, lactic acid bacteria were able to metabolize phenolic compounds through hydrolysis of phenolic glycosides to aglycone so as to increase antioxidant activity until the 7th day of storage in the refrigerator.

Keywords: Yogurt, Goat milk, Lebui nut (*Cajanus* sp.), Lactic acid bacteria, Antioxidant activities

Weissella confusa F213 is A Promising Probiotic Strain

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ABSTRACT

This study was aimed to find out the effect of the administration of a promising probiotic strain, *Weissella confusa* F213 (WCF213), on gut microbiota (GM). The study design was pre- and post-analysis, which has been approved by the Ethics Committee of the Faculty of Medicine, Udayana University. Faecal samples were collected from 9 healthy subjects, which were collected before and after 28 days of probiotics consumption and after 14 days washed-out period. Profile of gut microbiota was analysed using Next-Generation DNA sequencing (NGS). Furthermore, defecation frequency, stool form and colour, and side effects of probiotics consumption were tightly monitored. The results showed that the administration of WCF213 increased the diversities of GM among the study group (beta diversity) and in individual subjects (alfa diversity). Administration of WCF213 increased Firmicutes and lowered the proportion of Proteobacteria and Actinobacteria. *Enterobacteriaceae, Bifidobacteriaceae*, and *Corriobacteriaceae* were decreased that coincided with an increase of *Lactobacillaceae, Leuconostoccaeae,* and *Bacillaceae* due to WCF213 administration. *Verrucomicrobia, Saccharibacteria*, and *Bacteriodetes* were also observed that gradually increasing during WCF213 administration. There were no inconvenience and any adverse effects during the probiotic's consumption, but conversely gave a positive effect on defecation frequency and stool form. In conclusion, this study demonstrates that the administration of WCF213 did not result in a dramatic change in the composition of gut microbiota and elicited a good effect on defecation. Further studies are required to explore more evidence on the safety issues and functionality of WCF213 as a promising probiotic for humans.

Keywords: Probiotic, Gut microbiota, *Weissella confusa* F213, Next Generation Sequencing

Antiadherence And Antimicrobial Activity Crude Extracts Bioactive Peptides Traditional Dayak Fermented Food Pekasam Black Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Pekasam black tilapia is a functional food from fish fermented spontaneously with other ingredients such as the addition of salt and rice. Pekasam is a typical food Dayak tribe of West Borneo, and is known as a diarrhea-reducing food. This is due to the presence of potential bioactive compounds, bioactive peptides produced by lactic acid bacterial community during fermentation. Bioactive peptides are small proteins that are inactive when bound to parent protein complex. The bioactive component of this peptide is thought to act as an antidiarrheal through its antiadherence activity which prevents the attachment of ETEC to the intestinal wall. This study aims to determine the bioactive potential of pekasam black tilapia as antiadherence to ETEC and its chemical characteristics. Extraction bioactive peptides was carried out through fractionation and purification of ammonium sulfate using ion exchange dialysis membrane, which will measure protein content, as well as antimicrobial and anti-adhesive activity using *Saccharomyces cerevisiae* model cells. Results showed that the pH value of tilapia was 5,95. This is in accordance the taste which's relatively sour and sweet. The highest bioactive peptide protein content was found in the 70% fraction of bioactive peptides pekasam black tilapia of 90%. Bioactive peptide pekasam black tilapia has best inhibition against the pathogenic bacteria *Bacillus subtilis* 80% with an average inhibitory diameter of 2,78 mm, besides that also affects attachment of ETEC bacteria to *Saccharomyces cerevisiae* model cells antiadherence percentage of 98% against ETEC bacteria.

Keywords: Bioactive peptide, Antiadherence, Antimicrobial

Catfish Waste (Pangasius sp.) Fermentation by Probiotics for Essential Amino Acid and Fatty Acid Production

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ABSTRACT

Catfish waste (Pangasius sp.) has a high nutritional content of 22.96% carbohydrates, 35.81% protein, and 12.47 % fat, so it is very potential as raw material for the production of amino acids and essential fatty acids through fermentation. In the study of catfish waste fermentation using L. plantarum, L. curvatus, and B. subtilis, the aim was to analyze L. plantarum, L. curvatus, and B. subtilis to produce amino acids essential fatty acids through solid-state fermentation (SSF). Experimental research using Completely Randomized Design (RAK), consisting of stages of probiotic validation, fermentation, and measurement of amino acid and fatty acid levels at optimal fermentation conditions. The results showed that there was an increase in amino acid levels in the fermentation of catfish waste for 84 hours by L. curvatus by valine 2.43%; L-leucine 4.96%; L-phenylalanine 3.60%; L-arginine 5.89%; L-threonine 3.95%; and L-histidine 2.43% with an increase in the number of essential amino acids by 27.68%. The highest increase in L-lysine levels in fermented fish waste by B. subtilis was 3.73%. The most significant increase in fatty acid levels in the fermentation of catfish waste by L. plantarum ATCC 8014 was 0.2% oleic acid, omega-6 15.03%, and omega-3 30.88%. Fermentation of catfish waste by L. curvatus increased omega-3 by 5.94%. Fermentation of catfish waste by B. subtilis increased omega-6 4.22% and omega-3 7.98%, but the L curvatus, L. plantarum, B. subtilis consortium did not improve the fatty acid content.

Keywords: Catfish waste, Probiotics, Solid state fermentation, Amino acids, Fatty acids

The Existence of Bacteriophage in Lactic Acid Fermentation: How Far They Can Fail The Fermentation?

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ABSTRACT

Lactic acid bacteria (LAB) is a group of microaerophilic bacteria that can metabolize carbohydrates with fermentation to form lactic acid as its end product. Lactic acid bacteria are generally recognized as safe (GRAS) and have been used for centuries in food processing (either plant-based or animal-based). Lactic acid fermentation can be bothered by the existence of bacteriophage in its medium. The effects are a failure in forming flavor, taste, and texture, delaying lactic acid production, and leading to fermentation failure. This paper is discussed how bacteriophage infects LAB and disrupt their cell and how to minimize the amount of bacteriophage in the fermentation medium. Hopefully, this paper can help to reduce lactic acid fermentation failure, especially that caused by bacteriophage.

Keywords: LAB, Bacteriophage, Fermentation, Failure

Exploration of Lactic Acid Bacteria from Chao Products

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ABSTRACT

Chao, a traditional fermented fish from South Sulawesi. The purpose of this study was to explore the proteolytic Lactic Acid Bacteria (LAB) from the *chao* traditional fermentation of tembang fish. This study consisted of two stages, namely isolation and identification proteolytic LAB from *chao* traditional fermentation and apply it as a starter in the *chao* fermentation. LAB isolation was done using the scratch method. Proteolytic LAB selection was carried out using the skim agar medium. Identification of proteolytic LAB was carried out with API 50 CHL kit and analysis of 16S rRNA gene sequences. The application of proteolytic LAB was carried out in 3 stages of fermentation, namely salt fermentation, LAB fermentation, and *chao* fermentation. The results showed that 15 proteolytic LABs were obtained from *chao* traditional fermentation. Two proteolytic LABs identified as *Lactobacillus plantarum* Ags 1-3 and *Pediococcus acidilactici* Ags 7-3. The results of the application of proteolytic LAB showed that in salt fermentation, there was no growth of microorganisms, no changes in lactic acid and pH, but there is an increase in dissolved proteins and activity of ACE inhibitors. In the LAB fermentation, there is an increase in total bacteria, LAB, yeast, dissolved proteins and the activity of ACE inhibitors, but the mold not grow, not change in the value of pH and lactic acid. In the *chao* fermentation, there is an increase in the number of bacteria, LAB, yeast, dissolved proteins, activity of ACE inhibitors, and lactic acid. The fungi and pH value were decreased.

Keywords: *Chao*, Fermented, Fish

The Fortification of Purple Sweet Potato (Ipomea batatas Blackie) on The Quality of Soyghurt

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ABSTRACT

Yogurt is a drink made from fermented milk with lactic acid bacteria, tastes slightly sour. In its processing, it can be fortified with purple sweet potato. This study aims to determine the effect of purple sweet potato fortification on the soyghurt quality. This study was arranged using a completely randomized design with fortification treatment of (0, 15, 30, 45, and 60)% in the processing of soyghurt. The results were analyzed with 5% ANOVA and further test with 5% HSD. The results showed that fortification of purple sweet potato had a significant effect on chemical properties (protein content, lactic acid content) and organoleptic properties (color, texture, and taste scores) but had no significant effect on the flavor and antioxidant activity. The best treatment was obtained at 45% fortification treatment with protein content (3.16%), lactic acid content (2.46%), and antioxidant activity (82.68%). The flavor and taste are a bit like, the color is light purple color, and the texture is a slightly thick.

Keywords: Soyghurt, Purple sweet potato, Fortification

Current Taxonomic Name of Indigenous Probiotic Strains

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ABSTRACT

Probiotics defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2002). Speciation of bacteria strain identity must be established using the most current valid methodology. Strain identity was important to link a strain to a specific health effect as well as to enable accurate surveillance and epidemiological studies. It was recommended that a combination of phenotypic and genetic tests must be used. Besides, nomenclature of the bacteria must conform to the current, scientifically recognized names. Several lactic acid bacteria which isolated from traditional fermented foods and phenotypically identified as *Lactobacillus plantarum-pentosus* (2003) were screened their potential benefits as probiotic candidates. In the same year, probiotic candidate was also screened from fecal material of healthy infant baby, and phenotypically identified as *Lactobacillus acidophilus* (2003). Five of these probiotics' candidates were then genotypically identified based on sequence gene of 16s rRNA and *RecA* gene profile for *Lactobacillus plantarum* group, as well as *pepR* gene profile for *Lactobacillus casei* group. Based on these four strains of genotypic method, i.e., Mut-7, Mut-13, T-3, Dad-13 were identified as *Lactobacillus plantarum,* while SNP-2 was identified as *Lactobacillus paracasei* (2015). All these strains were deposited at Food and Nutrition Culture Collection (FNCC) Universitas Gadjah Mada. According current taxonomy revision of lactic acid bacteria (Zheng *et al*., 2020), four probiotics candidate strains, i.e., Mut-7, Mut-13, T-3, Dad-13 belong to *Lactiplantibacillus plantarum* subsp. *plantarum,* while SNP-2, belongs to *Lacticaseibacillus paracasei* subsp. *paracasei.*

Keywords: Probiotic, Lactobacillus, Nomenclature

Study of Characteristic and Shelf-life of Cream Cheese using *Lactobacillus plantarum* Dad-13 as Starter

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ABSTRACT

Increase cheese consumption in Indonesia not yet supported by availability of raw materials, especially culture starter. *Lactobacillus plantarum* Dad-13 isolated from Dadih have potency as a starter culture for making probiotic cheese. In this research, probiotic cream cheese was analyzed chemical and sensory characteristic compared cream cheese using commercial starter Flora Danica. Microbial characteristics and viability of *Lactobacillus plantarum* Dad-13 in it were also analyzed. In addition, the shelf life was determined under the typical refrigerator/cold storage conditions (± 2.5°C). Result shows ($T<0.05$) yield, moisture on fat-free basis, total solids, fat content of cream cheese respectively with commercial starter 43.50%, 90.42±0.60%, 51.99±0.32%, 90.20±2.85%, and with *Lactobacillus plantarum* Dad-13 38.15%, 84.59±1.17%, 55.73±0.61%, 85.53±1.63%. Result of sensory analysis show cream cheese using commercial starter and *Lactobacillus plantarum* Dad-13 have little white color ($T<0.05$), acid flavor ($T<0.05$), little soft ($T>0.05$), and little easy to spread ($T>0.05$), and hedonic analysis in overall not significantly different. The amount of viable *Lactobacillus plantarum* Dad-13 were able to be maintained at the functional level of 10^7-10^8 CFU/gr after keeping for 6 weeks at 2.5°C. The storaging treatment had a significant effect on changes in the viability of *Lactobacillus plantarum* Dad-13, but the significant effect was only seen at one week of storage or less. The cream cheese samples contained coliform that exeeded the acceptable safe limit (≤10^5 CFU/gr) on the first day of storage, although decreased to reach the acceptable safe limit during further storage time. The shelf life is determined to be 74 days.

Keywords: Cream cheese, *Lactobacillus plantarum* Dad-13, Probiotic

CURRICULUM VITAE

Prof. Fransisco B. Elegado

- Research Professor & Program Leader of the National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños (UPLB).
- Researches on the functionalities of Philippine fermented foods, utilization of lactic acid bacteria and superior yeast for the production of improved starter cultures/ functional food products, process development in ethanol fermentation.

Prof. Dr. Hooi-Ling Foo

- Professor at Universiti Putra Malaysia (UPM).
- Research interests: purification and characterisation of enzymes; application of lactic acid bacteria and bioprocess development.
- Executive Board member of Asian Federation Societies of Lactic Acid Bacteria since 2009. She also has a long contribution record for Malaysian Society for Microbiology (MSM).

Prof. Jiro Nakayama

- Professor at Department of Bioscience and Biotechnology Faculty of Agriculture, Kyushu University, Japan.
- The Japan Bioscience, Biotechnology, and Agrochemistry Society Awards for the encouragement of young scientists. “Bio-organic chemical and molecular biological studies on pheromone signaling in Enterococcus faecalis”.
- Research topics: Human GI-tract microbiota (join Asian Microbiome Project)

Dr. rer. nat. Agus Wijaya

- Doctor in Food Microbiology and he is currently the University Lecturer at Agricultural Product Technology Study Program, Universitas Sriwijaya, Palembang, Indonesia
- He published 15 articles in scientific journals in the last 5 years
- Research interests : Fermentation food, lactic acid bacteria, probiotics, bakery products, sanitation, functional food, and other food matter

Prof. Dr. Ir. Tyas Utami

- Secretary of Center for Food and Nutrition Studies (2019-2020).
- Head of The Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada.
- Professor in Food Microbiology, Faculty of Agricultural Technology, Universitas Gadjah Mada.
- She published 35 articles in scientific journals and proceedings in the las 5 years.
- Research interest: Food fermentation, lactic acid bacteria, probiotic, media formulation (halal media) for lactic acid bacteria; production and application of probiotic in food, food safety.

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ABSTRACT ACCEPTANCE LETTER

This is the confirmation that the abstract entitled:

"Antimicrobial Activity and Colour Properties of Annatto Extract Using *Bacillus subtillis* as A Pathogent"

Authors: Isti Handayani*, Pepita Haryanti*, Susanto Budi Sulistyo**

Has been accepted as oral presentation in The 6th International Conference of Lactic Acid Bacteria and Gut Microbiota

We look forward to your presentation at 6th IC-ISLAB-GM

Warm regards,
Aprilia Nur Khasanah
Organizing Committee

ANTIMICROBIAL ACTIVITY AND COLOUR PROPERTIES OF ANNATTO EXTRACT USING *Bacillus subtillis* AS A PATHOGENT

Isti Handayani, Pepita Haryanti, Susanto Budi Sulistyo

The 6th International Conference of Lactic Acid Bacteria and Gut Microbiota
13 August 2021

Introduction

Annatto (*Bixa Orellana L*)

Phytochemical:
- Flavonoid
- Saponin
- Tanin
- Alcaloid

Carotenoid
- Bixin (non polar)
- Norbixin (polar)

- edible solvent,
- easy to obtain
- cheap

Aquadest

Maceration

Commercial process extraction:
- direct extraction into oil
- indirect extraction using organic solvents

Annatto extract

Colour
- Hue
- Value
- Chrome

Antimicrobia

B. subtillis

Factors: temperature, solvent pH, extraction times

Ekst

Objectives of this research:

To evaluate antimicrobia activity and natural colorants of annatto extracts using aquades as solvent extraction at various temperatures

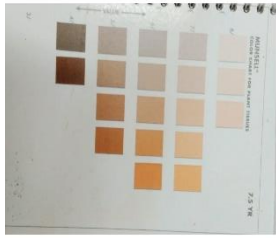
Methode

Materials:

- Annatto seeds
- Aquades
- *B. subtilis* FNCC 0060
- Nutrient broth/ agar
- NaCl

Equipments:

- Magnetic Stirrer
- Spektofotometer
- Petridish
- Thermometer
- Munsel colour chart
- Glass apparatus



Factor :

- **Extraction temperature (70, 80 ,90 ° C)**

Variables observation:

1. **Colour of extract (Munsel Colour Chart)**
   a. Hue (colour)
   b. Value (brighness)
   c. Chrome (intencity)
2. **Antimicrobia activity**
   a. Clear Zone (agar well diffusion)
   b. MIC

Data analysis:

The data were statistically analyzed using **ANOVA**, if the results of the analysis showed a significant effect, then continued with the **Duncan Multiple Range Test** at the 5% level.

Research Flow Chart

Annatto seeds (25 g)

Aquades (90 ml)

Maceration extraction (T: 70;80; and 90°C, t: 10 minutes;)

Filtering

Annatto extract

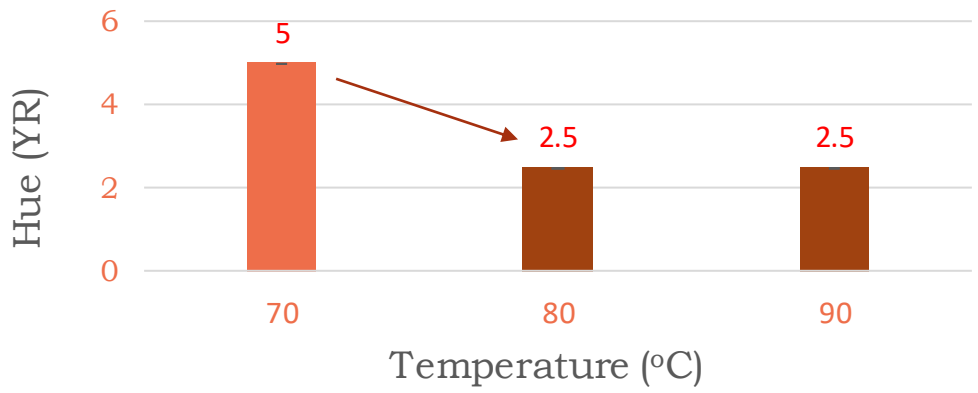
Measurement:

- Colour (Munsel colour chart) :
  - Hue
  - Value
  - Chroma
- Antimicrobia activity:
  - Clear Zone
  - MIC

Result and Discussion

1 **Colour of extract (Munsel Colour Chart)**

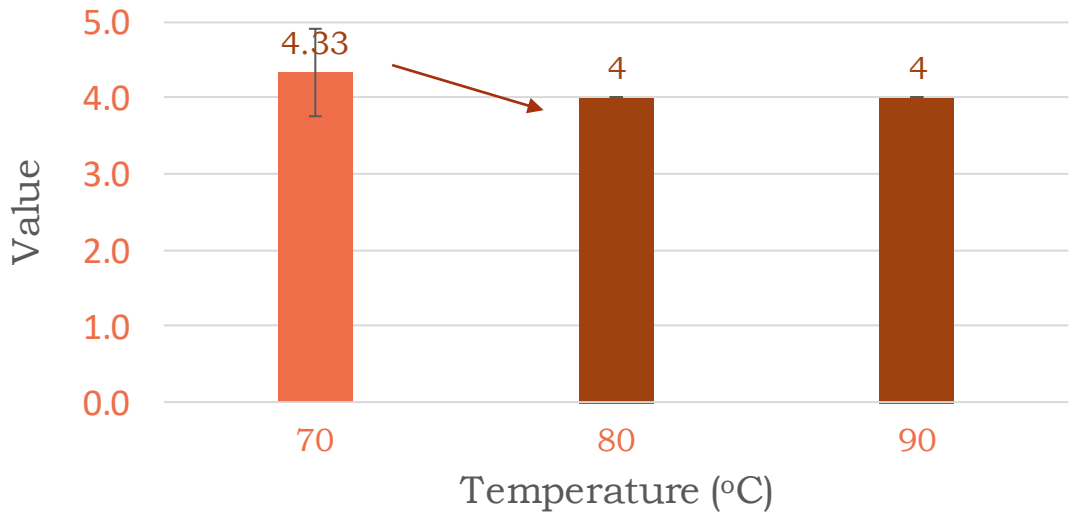
1. a. Hue



- Hue YR (yellowish red /orange)
- The color component of the extract in aquadest was norbixin in the form of cis norbixin or trans-norbixin (Satyanarayana *et al.* (2003).
- It is suspected high temperature can change cis norbixin to trans-norbixin

1 Colour of extract (Munsel Colour Chart)

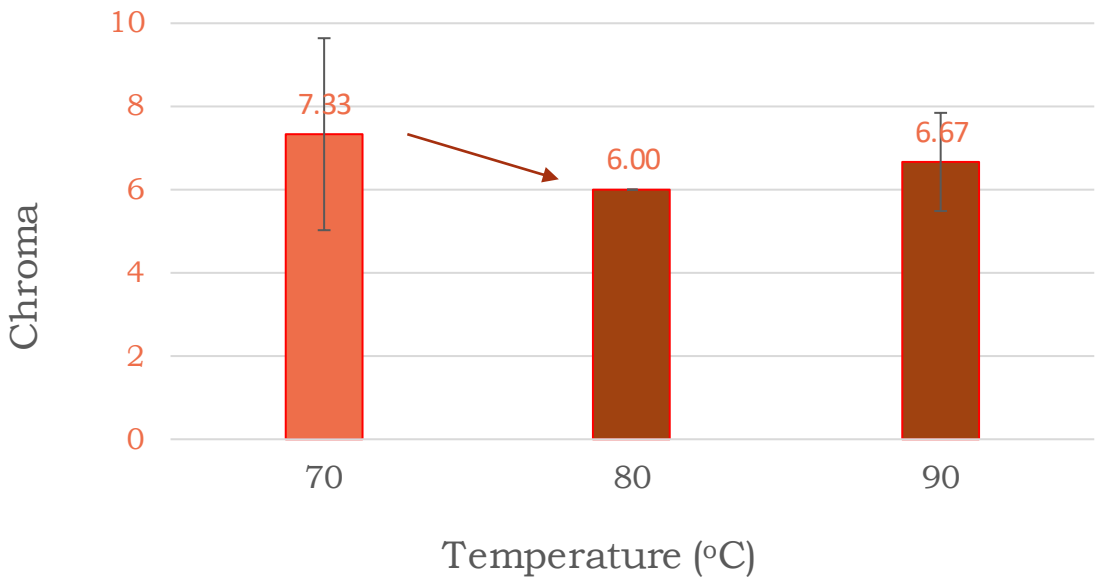
1. b. Value



- Increasingly of temperature couse decreasing of value
- The higher of temperature, more dark the colour
- The lower temperature, more light the color
- It is suspected that cis norbixin has a higher ability to reflect light

1 Colour of extract (Munsel Colour Chart)

1. c. Chrome

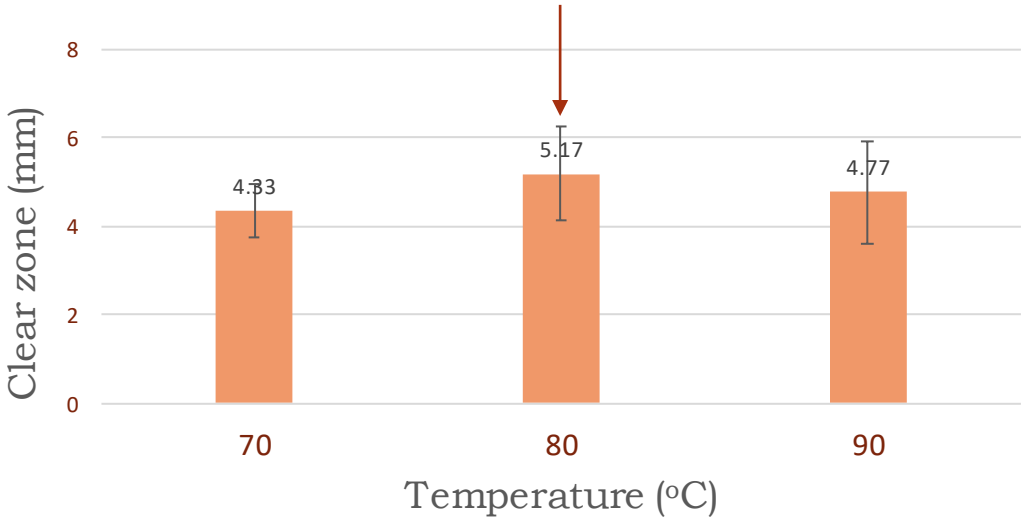


- The higher of crome, the stronger of color
- Low temperature resulted the stronger of yellowish–red of the extract

2

Antimicrobia Activity

2. a. Clear zone



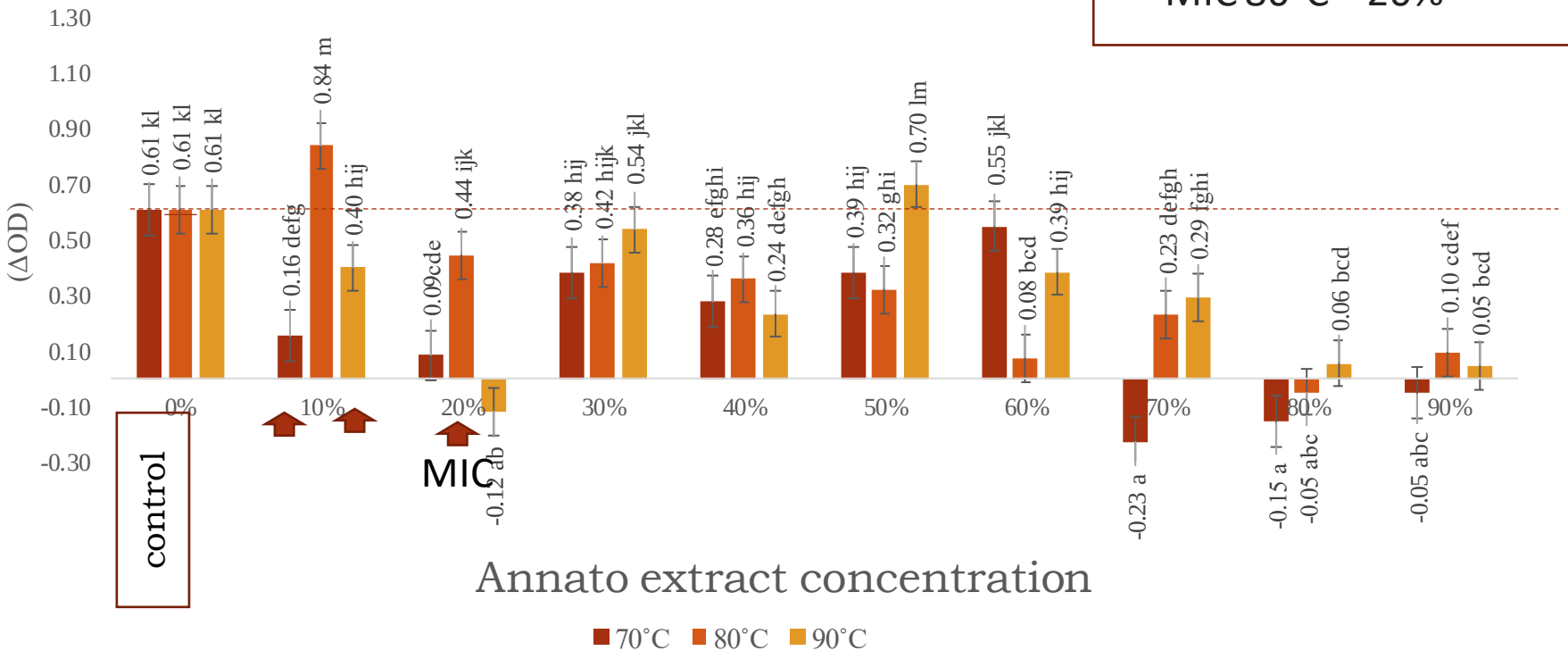
- The 80°C is the optimum extraction temperature
- An increase in temperature can cause the degradation of phytochemical components that act as antibacterials
- The diameter of the clear zone was 4.33-5.17 mm (weak to moderate inhibitory activity category)

2

Antimicrobia Activity

2. b. Minimum Inhibitory Concentration

- The lower the value of Δ OD, the higher the activity of aktibacteria
- MIC 70 & 90 °C = 10%
- MIC 80°C = 20%



Conclusion

- Increasing the extraction temperature from 70°C to 80°C decreases the hue, value and chroma

- Annato extraction at 80°C produced the highest inhibition zone but lower MIC values were produced at 70 and 90°C extraction temperatures.

Thank You

Indonesian Society of Lactic Acid Bacteria and Gut Microbiota

Center for Food and Nutrition Studies Universitas Gadjah Mada

Department of Food and Agricultural Product Technology Universitas Gadjah Mada

Center of Excellence in Science and Technology

Center of Excellence for Probiotics

CERTIFICATE OF APPRECIATION

46/UN.1/PSPG/PROBIOTIK/VIII/2021

PRESENTED TO

Isti Handayani

Universitas Jenderal Soedirman Purwokerto

for Participation as Oral Presenter in

The 6th International Conference of Indonesian Society For Lactic Acid Bacteria and Gut Microbiota

Given on the 13th of August, 2021

Prof. Dr. Ir. Endang S. Rahayu, MS

Head of Center For Food and Nutrition Studies
Universitas Gadjah Mada

LIST OF ORAL PRESENTER

15.30-15.45	OR12	Yoga Dwi Jatmiko	Isolation and Characterization of Yeast from Fermented Sumbawa Mare's Milk with Probiotic Properties
	Oral Session 3 **Moderator : I Nengah Sujaya, PhD**		
16.00-16.15	OR13	Yan Ramona	Functional effects of probiotic candidates, isolated from local sources (Indonesia), on human health
16.15-16.30	OR14	Isti Handayani	Antimicrobial Activity and Colour Properties of Annatto Extract Using *Bacillus subtilis* as A Pathogen
16.30-16.45	OR15	Delima Citra Dewi Gunawan	Effect Of Synbiotics Powder (*L. Plantarum Dad*-13) and Frukto-Oligosaccharide on Defecation Pattern and Stool Acidity in Stunting Children in Yogyakarta
16.45-17.00	OR16	Yoyok Budi Pramono	Critical Control Point Analysis of Rendang Cooking in Padang Restaurants for Food Safety and Maintenance of Consumer's Gut Microbiota Balance