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ABOUT THE JOURNAL

Journal of Functional Food and Nutraceutical (JFFN) is an official journal of **Perhimpunan Penggiat Pangan Fungsional dan Nutrasetikal Indonesia or Indonesian Society for Functional Food and Nutraceutical (P3FNI-ISFFN)** that has been established in collaboration with **Research Center for Food and Health Swiss German University (RC F&H SGU).** JFFN publishes review and research result on frontier research, development, and application in the scope of functional food and nutraceuticals**.** The journal is expected to bring together all stakeholders in relation to the food ingredients and nutraceuticals.

Scope of the journal Include:

- Interdisciplinary approach of food technology, food nutrition, and health
- Plant bioactive; dietary fiber, probiotics; functional lipids; bioactive peptides; vitamins, minerals and botanicals and other dietary supplements.
- Nutritional and technological aspects related to the development of functional foods and nutraceuticals.
- Food digestion, bioavailability, mechanism, efficacy, and safety of food ingredients and nutraceuticals.
- Food product development with health benefit
- Characterization of healthy foods and functional constituents
- Preparation of natural and synthetic ingredients for use in foods and supplement
- effects of processing (including packaging and storage) on functionality and improvement of product quality; verification, quality control and traceability of natural and synthetic functional food ingredients and nutraceuticals.
- The regulatory aspects of functional foods and related issues e.g. labelling, substantiation of health claims are also of interest together with those dealing with the value creation on the food chains based on the nutritional/healthy aspects.

JFFN publishes **2 times in a year,** August and February. JFFN adopting **Open Journal System** for fast manuscript management process. All authors are requested to register in advance and submit the manuscript online to support the fast managing and review process and to be able to track the real-time status of the manuscript.

All accepted manuscripts receive individual digital object identifier (DOI) and indexed by Google Scholar. The online PDF version of the journal is open access from **https://journal.sgu.ac.id/jffn**

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PREFACE

Welcome to the inaugural issue of *Journal of Functional Foods and Nutraceuticals (JFFN).* It is my great privilege and pleasure to present the inaugural volume of this new peer-reviewed journal, a joint publishing journal of Perhimpunan Penggiat Pangan FungsionaI dan Nutraceutical Indonesia (P3FNI) or Indonesian Society of Functional Foods and Nutraceuticals (ISFFN) and Research Center of Food and Health, Swiss German University (SGU). JFFN is a frontier publication devoted to strengthen the development of functional foods, from theoretical aspects to application-dependent studies and the validation of emerging technologies, which naturally complement each other, as well as any grass root issues for practitioners. JFFN aims to provide a highly readable and valuable contribution literature to emerging interest in functional foods science and technology in Indonesia Society. The journal is also dedicated to encourage early bird authors to experience publishing in an international journal by providing a friendly tutorial.

This first issue comprises five manuscripts, connected by a unifying theme: "Functional Food and Nutraceutical for Community Health". The presented articles can be categorized into the following groups:

- Basic research evaluating the functional activities
- Social studies on consumer trends on functional food

It is our hope that the articles of this first issue will become a valuable resource for the readers of JFFN, and will stimulate further research into the vibrant world of functional foods.

As the chairman of P3FNI, I would like to use this inauguration occasion to thank many people who supported the idea to create a new journal JFFN and provided the opportunity for the journal to be born, in particular Dr. Maria S. Gunawan-Putri. I also deeply appreciate the hearty support of SGU as we strive to make JFFN the most authoritative journal on the field of functional foods. Furthermore, as the editor in chief, I would like to extend my sincere thanks to all members of the editorial and the advisory boards, whose service, dedication, and commitment have made the creation of this journal possible. I would also like to acknowledge the highly appreciative effort to all of manuscript reviewers for providing valuable comments and suggestions to the authors. As we are working together, we aim to continue to strive for quality and excellence in published articles. It is without doubt that the success of our journal depends highly on the author contribution of articles. Through seamless collaboration with all of our authors, we aim to continue to strive for quality and excellence in publishing articles.

It is our hope that JFFN could deliver valuable and interesting information to the nationwide and worldwide community of food science and stimulate further exciting research in the diverse area of functional foods and nutraceuticals. I am certain that this first issue will be followed by many others, providing high quality reports on the most advanced developments in food science field. More information about JFFN guidelines for the preparation and submission of papers can be found at JFFN website: https://journal.sgu.ac.id/jffn/index.php/jffn/index .

Finally, as a newly established journal I do realize that there are still a lot of aspects that have to be improved. Therefore, we are sincerely waiting for your mutual suggestions and criticism.

July 2019,
C. Hanny Wijaya
Editor in Chief of Journal of Functional Food & Nutraceutical

Journal of Functional Food and Nutraceutical

USE OF BETA-CAROTENE PIGMENT TO IMPROVE FOOD PRODUCT CHEMICAL AND SENSORY QUALITIES: A REVIEW

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ABSTRACT

Beta-carotene is a pigment that occurs widely and abundantly in nature. Beta-carotene can be found in some fruits and vegetables. Commonly, beta-carotene consistently bonds to other carotenoid compounds. Beta-carotene is a powerful colorant with beneficial effects on human health due to its ability to radical scavenging. Beta-carotene is obtained through the process of extraction with suitable solvent. The extraction method influenced the profile and quality of beta-carotene. The sonochemical approach using ultrasound, microwaves, and ohmic heating is eco-friendly and helps decrease the amount of solvent used, reduce the extraction time, increase the yield of beta-carotene from the sources, and increase the efficiency of the food applied. It is easier for consumers to accept natural dyes with high stability and efficiency. The present review describes detailed information about the quality of beta-carotene extract, isolation methods, and factors that affect the efficiency of natural food colorants applied to food products, which is helpful for the further development of food product formulations.

Keywords: antioxidant activity; beta-carotene; colorant; extraction method; pigment; fruit and vegetables; sensory profile

ABSTRAK

Beta-karoten adalah pigmen yang terjadi secara luas dan melimpah di alam. Beta-karoten dapat ditemukan dalam buah dan sayuran. Umumnya, beta-karoten berikatan dengan senyawa karotenoid lainnya. Beta-karoten adalah pewarna yang kuat dengan efek menguntungkan bagi kesehatan manusia karena kemampuannya untuk menangkal radikal bebas. Beta-karoten di dapatkan melalui proses ekstraksi dengan pelarut yang tepat. Metode ekstraksi mempengaruhi profil dan kualitas betakaroten. Pendekatan sonokimia menggunakan ultrasound, gelombang mikro, dan pemanasan ohmik yang ramah lingkungan dapat membantu mengurangi jumlah pelarut yang digunakan, mengurangi waktu ekstraksi, meningkatkan hasil beta-karoten dari padatan, dan meningkatkan efisiensi dalam penggunaanya pada bahan makanan. Pewarna alami dengan stabilitas dan efisiensi tinggi yang tinggi merupakan hal yang konsumen butuhkan. Tinjauan ini menjelaskan informasi rinci tentang kualitas ekstrak beta-karoten, metode isolasi, dan faktor-faktor yang mempengaruhi efisiensi pewarna makanan alami yang diterapkan pada produk makanan, yang berguna untuk pengembangan formulasi produk makanan lebih lanjut.

Kata kunci: aktivitas antioksidan; beta-karoten; pewarna; metode ekstraksi; pigmen; buah dan sayur; profil sensori

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INTRODUCTION

Beta-carotene, a plant pigment, may reduce cancer risk and promote healthy eyes and bones (Liu & Tang, 2016). Over 700 naturally occurring carotenoids or beta-carotene isomers have been identified, and 80-90% of carotenoids are obtained through fruit and vegetable consumption. Human can absorb β-carotene, β-cryptoxanthin, α-carotene, lycopene, lutein, and zeaxanthin in their diet (Maiani et al., 2009). Since the identification of beta-carotene's potency and function as vitamin A, research has been conducted on ways to improve the yield and quality of beta-carotene extracted from plant sources using various techniques (Ghazi, 1999; Strazzullo et al., 2007).

Beta-carotene, a fat-soluble pigment, is found in fruits, vegetables, and microbes. It is soluble in nonpolar solvents such as petroleum ether, hexane, and tetrahydrofuran depending on its polarity (Rivera and Canela, 2012; Susan, 2014). According to Tiwari and Sarkar (2018), the concentration of beta-carotene in carrots extracted using organic solvents ranges from 371.90 mg/L to 631.90 mg/L. The solvent used significantly impacts the beta-carotene extraction process and may have negative health and environmental impacts for humans. Eco-friendly extraction methods, such as maceration, should be used to reduce the environmental damage caused by organic solvents (Rivera and Canela, 2012; Susan, 2014). However, conventional techniques, including maceration, often require a large amount of solvent (Tiwari and Sarkar, 2018). Alternative methods for isolating beta-carotene include ohmic heating extraction (Aamir and Jittanit, 2017), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (Chuyen et al., 2018; Kultys and Kurek, 2022).

Beta-carotene is commonly isolated from plant parts such as peel, seed, leaf, and flesh fruit, and it is also found in the metabolic pathway of algae and Escherichia coli (Kyriakopoulou et al., 2015; Yang and Guo, 2014). The main structure of beta-carotene isomers in food and human tissue is depicted in Figure 1. Algae are a preferred sustainable source of beta-carotene due to food safety and security. The extracted beta-carotene pigment should be added directly to food as a natural coloring agent. Beta-carotene is a pigment easily degraded by air and high temperatures, causing a color change from red to yellow (D'Evoli et al., 2013). The quality of beta-carotene can be assessed based on color, yield, and antioxidant activity using methods such as FRAP, ABTS, and liquid chromatography (Aamir and Jittanit, 2017; Chuyen et al., 2018; Mueller and Boehm, 2011).

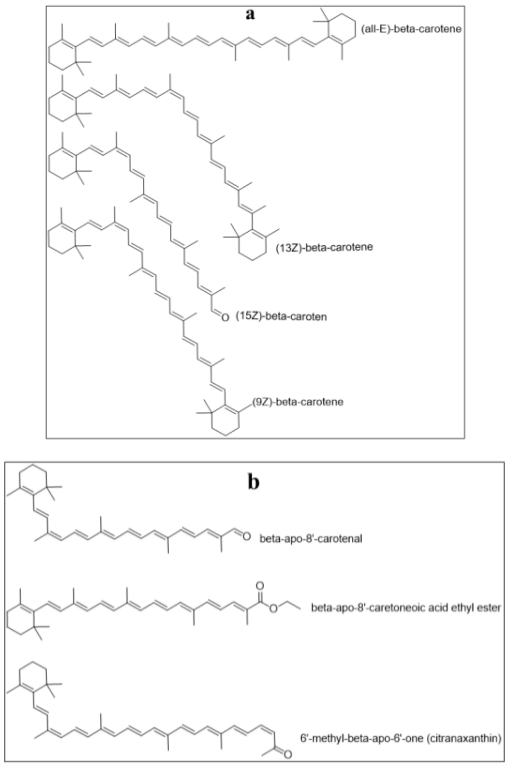

Figure 1. (a) Beta-Carotene Isomers in Food and Plant Tissue; and (b) Beta-Carotene Metabolite

The color of food products affects consumer perception and can impact their quality (Šeregelj et al., 2022) Using beta-carotene as a food colorant may have several benefits for a product. Its antioxidant activity can increase the functional aspects of the product, and the sensory and functional qualities of food products can be improved by using beta-carotene-derived natural colors to promote public health.

EXTRACTION OF BETA-CAROTENE

Extraction is the process of isolating a chemical or pigment from a plant using a solvent. The extraction of beta-carotene from plants can be

facilitated through the use of organic materials because extraction can be used to separate plants from pigments. Solvents with various solubilities have been used to enhance the yield of the extract (Dai and Row, 2019). Most carotenoids are insoluble in water and soluble in organic solvents such as acetone (Rivera and Canela, 2012), alcohol, tetrahydrofuran (D'Evoli et al., 2013; Tonucci et al., 1995), ethyl ether (Mumtaz et al., 2019), chloroform, and ethyl acetate (Ludwig et al., 2021). However, their solubility depends on the availability of specific functional groups. Beta-carotene and lycopene have similar solubility in hexane, but they can be separated using a combination of methanol and tetrahydrofuran (6:4 v/v) to increase the yield of beta-carotene from maize (Rivera and Canela, 2012).

In the past decade, various techniques have been developed for the isolation of beta-carotene. For example, a mixture of beta-carotene and oil from Gac aril fruit (*Momordica cochinchinensis*) can be obtained using a combination of water and hexane with a ratio of 1:6, with distilled water used as a conductive medium for heating. Ohmic heating is a technique that uses the principle of generating heat by applying electrical energy to materials with electrical resistance (measured in ohms) using a heat conductor. The electrical energy is converted into heat due to the material's electrical resistance, known as the Joule effect. (Aamir and Jittanit, 2017; Indiarto and Rezaharsamto, 2020). Ohmic heating can induce the rupture of the plant cell matrix, increasing the surface area between the powder and the solvent and allowing nonpolar components like beta-carotene to flow into the solvent. As a result, an ohmic heating method can be 245% more effective in extracting beta-carotene than a conventional extraction method (Aamir and Jittanit, 2017).

Conventional

The process of obtaining bioactive compounds from plant materials through the use of a solvent is referred to as solid-liquid extraction, though it is also known as leaching or lixiviation in chemical terminology (Sasidharan et al., 2018). Conventional extraction techniques can be divided into two categories: hot extraction by temperature, which involves the use of high temperatures (e.g., Soxhlet), and cold extraction, which does not use heat or high temperatures (e.g., maceration) (de Andrade Lima et al., 2019). Maceration, distillation, perforation, and reflux are conventional extraction methods that use a significant amount of solvent and have a prolonged duration, making them less efficient in the extraction process (Garcia-Vaquero et al., 2020; Khamitova et al., 2020; Uribe et al., 2015; Zhang et al., 2018), different methods will produce extracts with varying characteristics. Hot extraction (soxhlet, dist is faster but may result in lower antioxidant quality due to the sensitivity of the pigment to heat, while cold extraction takes longer but may produce extracts with higher antioxidant quality. Additionally, conventional extraction can be performed using a mechanical process, such as shaking and mixing at low temperatures (D'Evoli et al., 2013; Tonucci et al., 1995). One limitation of conventional techniques is the high solvent usage and the required prolonged extraction time. However, this method is simple for isolating beta carotene from solid matrices (Garcia-Vaquero et al., 2020). The quality of the beta-carotene yield extract may be influenced by various factors such as solid particle size, pressure, and co-solvent (Prado et al., 2013).

Modern method

Conventional extraction methods using solvents such as n-hexane, acetone, and ethyl alcohol for extracting beta-carotene from gac fruit flesh yield lower amounts of beta-carotene compared to microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) methods using ethyl acetate. Extraction using microwave-assisted (MAE) and ultrasound-assisted (UAE) methods yields higher amounts of beta-carotene compared to the maceration method, with values of 2623 ± 35 mg/L and 2677 ± 12 mg/L, respectively (Chuyen et al., 2017). Eco-friendly extraction methods yield nearly three times more beta-carotene than the maceration method.

The extraction of beta-carotene using microwave-assisted extraction (MAE) has several advantages including stable temperature conditions, pure yield extract, faster extraction compared to conventional

methods, and low energy requirements. (Chuyen et al., 2018; Elik et al., 2020; Kultys and Kurek, 2022). The use of ultrasonic waves, known as cavitation forces, in the UAE method results in increased diffusion of beta-carotene and high antioxidant activity. The cavitation effect causes bubbles to form on the solid surface of the material matrix in a liquid solvent medium, creating a microjet flow that increases the rate of mass transfer from inside the cell wall to the solvent. The UAE extraction process can be further accelerated by using suitable solvents. In comparison, the MAE technique involves the use of microwave energy and nonpolar solvents to extract beta-carotene, resulting in a purer yield extract and faster process with lower energy requirements (Abid et al., 2021; Chuyen et al., 2018; Jha et al., 2017; Lavilla and Bendicho, 2017; Li et al., 2013; Mumtaz et al., 2019). In addition, UAE extraction has a significant antioxidant activity due to various polyphenols extracted during the processing.

The yield of beta-carotene extract from vegetables and fruits is influenced by the number of components extracted during the extraction process. The suitability of the solvent with the ingredients being extracted plays a significant role in the success of the extraction process. Polar components dissolve in polar solvents and nonpolar components dissolve in nonpolar solvents. N-hexane, a nonpolar solvent, is effective for extracting nonpolar materials like beta-carotene (Arion et al., 2017; Güçlü-Üstündağ and Temelli, 2005) The solubility of beta-carotene in organic solvents, such as carbon disulfide, benzene, chloroform, acetone, ether, and petroleum ether, can be improved by the addition of acetone. The combination of polar acetone and nonpolar n-hexane is most effective for extracting beta-carotene. Some studies have shown that essential oils and certain combinations of semi-polar solvents can be used as alternatives to n-hexane to extract beta-carotene from cells surrounded by aqueous fluid (Grootaert et al., 2021; Ordóñez-Santos et al., 2015).

The concentration of beta-carotene in fruits and vegetables can vary due to factors such as cultivar, source, fruit clone, and extraction process. Dry extraction processes that use high heat and treatment can cause carotene degradation. These factors should be considered when using beta-carotene as a food additive (Lasunon et al., 2021). Carotene is highly vulnerable to isomerization and heat-induced oxidation by the conjugated double-bond system. The cis isomer of beta-carotene, which has provitamin A and lowers antioxidant activity, can be altered by high pressure, mechanical processes (such as mixing), the addition of essential oil, or replaced by more efficient methods. A summary of beta-carotene extraction methods for various sources can be seen in Table 1.

THE USE OF BETA-CAROTENE AS A FOOD DYE

The food industry often uses synthetic dyes to improve the appearance of products, as they are typically more cost-effective and efficient than natural colors. However, research suggests that excessive consumption of synthetic colors may negatively affect health, including allergies and abnormalities, as well as an increased risk of cancer. Natural colorants, such as beta-carotene, can be used to enhance the color of food without these potential negative consequences (Kobylewski and Jacobson, 2012).

Beta-carotene is a pigment colorant with the highest provitamin A activity. In addition to its role as a pigment, beta-carotene can be metabolized into the vitamin A by-products retinoic acid (RA) and retinal. RA is a regulator of various biological functions and is recognized by two classes of nuclear receptors: retinoic acid receptors (RARs) and retinoid X receptors (RXRs) (Grune et al., 2010; von Lintig et al., 2005). Since (9*Z*)-beta carotene is a precursor of (9*Z*)-RA, the ligand for RXRs, isomers (*all-E*)-beta carotene is a precursor for (*all-E*)-RA, it binds to RAR (von Lintig, 2020; von Lintig et al., 2005). Natural colorants also have biological features of antimicrobials. Applying natural colorants to food can have additional benefits for extending the product's shelf life (Assadpour et al., 2020; Cavalcanti et al., 2022). Beta carotene can be obtained naturally from raw materials like sweet potato, yam, rice, maize, pumpkin, and potatoes, or it can be added as a food

enhancer or emulsion product (de Andrade Lima et al., 2019; Jati et al., 2022; Rivera and Canela, 2012; Tang et al., 2012).

Table 1. Extraction of Beta-Carotene

Source	Part of plan	The Extraction method and solid ratio matrix by solvent	Condition extraction	Advantage	Total beta-carotene (mg/L)	References
Carrot	Whole	Maceration (1:5)	n-hexane at ambient temperature for 6 hours	Simplest without specialization apparatus	321.35	(Li et al., 2013)
Carrot	Whole	UAE (1:5)	Sunflower oil at 40°C temperature for 20 minutes	Rapidly processed and higher beta-carotene	334.75	(Li et al., 2013)
Carrots	Whole	Maceration	n-hexane and acetone (1:1) at ambient temperature	Simplest without specialization apparatus	33.7	(Rifqi et al., 2020)
Cherry tomatoes	Whole fruits	Maceration	Tetrahydrofuran, calcium carbonate, and dichloromethane	Simplest without specialization apparatus	10.0 ± 0.5	(D'Evoli et al., 2013)
Gac fruit	Aril pericarp	Maceration (1:7)	n-hexane at 50°C for 7 hour	Simplest without specialization apparatus	39.6	(Aamir and Jittanit, 2017)a
Gac fruit	Aril pericarp	Ohmic heating (1:7)	Water and n-hexane (1:6), 50 V at 50°C for 7 hours	Yield extract is 91% higher	582.2	(Aamir and Jittanit, 2017)
Gac fruit	Fresh peel	Maceration (1:10)	n-hexane:acetone:ethanol (2:1:1) at ambient temperature for 30 minutes	Simplest without specialization apparatus	907 ± 22	(H. V Chuyen et al., 2017)
Gac fruit	Dried peel	Maceration (1:80)	Ethyl acetate at 40.7°C for 150 minutes	Simplest without specialization apparatus		(H. V. Chuyen et al., 2017)
Gac fruit	Dried peel	MAE (1:80)	Ethyl acetate, 120 W at 60°C for 25 minutes	The temperature of extraction is relative stable, low energy needed, and rapidly processed by adding water as an ohmic heating medium	2623 ± 35	(Chuyen et al., 2018)
Gac fruit	Dried peel	UAE (1:80)	Ethyl acetate, 200 W at 60°C for 80 minutes	Great antioxidant activity, rapidly processed, higher yields, and without using an extra medium solvent or co-solvent	2677 ± 12	(Chuyen et al., 2018)
Orange fruit	Peel	Maceration (1:15)	Hexane and acetone (1:1) at ambient temperature for 15 minutes	Simplest without specialization apparatus	2700	(Ghazi, 1999)
Pandanus Conoideus Oil	Pericarp (pulp)	Maceration	Chloroform and methanol (2:1) at room temperature for 1 hour	Simplest without specialization apparatus	54.6	(Sarungallo et al., 2015)
Papaya	Flesh fruit	Maceration	Acetone: ethanol in petroleum ether (2:1:1) at ambient temperature	Simplest without specialization apparatus	42,2	(Mumtaz et al., 2019)
Peach fruit	Peel	UAE (1:2)	Sunflower oil, 80 W at 35°C for 30 minutes	The solvent was used as more eco-friendly with a yield similar to organic solvent and rapidly processed	1634.7	(Ordóñez-Santos et al., 2015)
Tomatoes	Whole fruit	Maceration	Tetrahydrofuran	Simplest without specialization apparatus	2.3 ± 0.4	(Tonucci et al., 1995)

Beta-carotene can be made more stable and soluble for use as a food colorant by evaluating it as an emulsion product. However, the use of a single emulsifier to create an oil-in-water emulsion of beta-carotene may not be suitable for industrial food production due to sensitivity to various factors

such as temperature, light, pH, heat, oxygen, and free radicals. Microencapsulation of beta-carotene can also be used to act as a fat replacer in cupcakes and enrich the product, potentially replacing up to 36% of the used fat. (Mueller and Boehm, 2011; Nayana et al., 2021). Table 2 summarizes the use of beta-carotene as a food dye.

Table 2. The use of Beta-Carotene as a Food Dye

Sample	Treatment	Parameter	Research result	Preferences
Flake product with the addition of Orange Sweet Potato and Red Rice	Ratio Orange Sweet Potato and Red Rice (100:0, 80:20, 60:40, 40:60, 20:80, and 0:100)	Total beta-carotene, antioxidant activity, color intensity, and sensory profile	Flake product with the addition of orange sweet potato and red rice (60: 40) has total beta-carotene of 25.77 mg/L; The antioxidant activity of 85% is in a strong category; The best color intensity is in flakes with a ratio of orange sweet potato and red rice (100: 0), as well as on the sensory profile of all flakes favored by consumers.	(Jati et al., 2022)
Ras Cheese with adding 99% beta-carotene of carrots pells	Ras cheese with 99% beta-carotene of carrots pells	Sensory evaluation of Ras cheese during 180 days of storage	Consumers enjoy the sensory characteristic of ras cheese, which has a yellowish hue and a smooth, slightly salty flavor after being held for 180 days.	(Al-Surmi et al., 2021)
Orange-fleshed sweet potato composite flour bread	Orange-fleshed sweet potatoes (56–70 grams in 100 grams)	antioxidant activity	Orange-fleshed sweet potato composite flour bread has antioxidant activity ranging from 12.31 mg/L to 40.36 mg/L.	(Oloniyo et al., 2021)
Incorporated beta-carotene in palm and flaxseed oil as an emulsion to develop cupcake	Replaced oil with microencapsulation powder oil for formulation cupcakes	Color texture profile and sensory properties	The texture, color, and sensory evaluation of cupcakes using microencapsulation oil are similar to butter as the fat.	(Nayana et al., 2021)
Functional mangiferin (mango peel extract) drink	The drink contains mangiferin extract	Antioxidant activity, beta-carotene content, physicochemical properties (pH, total soluble solid, total acidity) and sensory properties using the hedonic test (color, sweetness, sourness) during 2 months of storage	Mangiferin functional drink is more stable than control after 2 months of storage-based sensory evaluation and physicochemical properties	(Imran et al., 2016)

Nopal marmalades	Optimizing the base mix (raw material and water) and supplementary materials by: 87.40%, 79.40%, 66.30%, 66.00%	Antioxidant activity, beta-carotene, and sensory properties (color, odour, flavour, spreadability) at different temperatures	The formulation of 66.30% base mix, adding 33.0% lactitol, and aspartame 0.1%, has been more accepted by the customer than other formulations and is similar to standard marmalades	(Leopoldo et al., 2012)
Enrichment yoghurt with carrot juice and sugar	Yoghurt with added 10% and free sugar; concentration of carrot juice (0%, 15%, and 20%)	viscosity, syneresis, titratable acidity, pH, yeast and mould counts, color measurements, beta-carotene contents and sensory qualities during 21 days of storage time	The best treatment for all parameters is yoghurt with 10% sugar and 15% carrot juice; had beta-carotene content appropriate to 80 µg/100 g	(Cakmakci et al., 2014)
Beta-carotene fortified *Gari* based Orange-fleshed sweet potato (OFSP) root composite flour	Optimizing the substitution of OFSP composite flour and fermentation time	Swelling capacity, taste, texture, flavour, color, beta-carotene content	The optimation of OFSP composite adding is 10% with 43 hours of fermentation to provide *gari:* 16.72 µg/ml beta-carotene content, 85.16 brightness, 40.2 yellowness, 4.93 redness, 350% Swelling capacity, 6.75 scores for appearance, 7.0 for taste, 7.35 for texture, 7.0 for flavour, and 7.2 for overall acceptability	(Cakmakci et al., 2014)

Beta-carotene levels dropped in the flake product, including orange sweet potato and red rice. The heat degradation of beta-carotene may be the cause of this occurrence. Carotenoids are further recognized as being light- and heat-sensitive compounds. Red rice and orange sweet potatoes both contain different kinds of carotenoids. After boiling, there was an increase in carotenoids, which could be related to the thermal disruption of the protein-carotenoid system (Trono, 2019). They have sophisticated and matrix-based carotene release. The amount of orange sweet potato used in the flake formulation impacted the carotenoid level. The amount of carotenoid in the flakes increases with the percentage of orange sweet potato.

The fortifications of the food product by adding the sources of beta-carotene (fruit and vegetable) would change the chemical composition of polymer binding, i.e., protein, carbohydrate, and lipid to the hydrogen bound or matrices of the intramolecular network in a food product (Abano et al., 2020; Cakmakci et al., 2014). The inclusion of orange-fleshed sweet potato flour in Gari resulted in a low swelling capacity due to the weak protein and carbohydrate network's ability to absorb water. Beta-carotene, which is sensitive to heat and may change color as a result, significantly affects the intensity of the color. Observations show tbhat the hue of beta-carotene can range from yellow to red (Trancoso-Reyes et al., 2016). The Maillard process can alter the color of flakes, with more significant Maillard reaction products resulting in a darker color. Many panelists found the dark orange hue of beta-carotene-containing food to be unappealing, unappetizing, and less fresh in terms of sensory characteristics. Beta-carotene can improve the brightness and redness of a product, increasing color preference. Most panelists

preferred the reddish and brighter flakes. The white endosperm of the material is associated with a higher level of brightness

As shown in previous research, the breakdown of carotenoids during food processing is caused by heat treatment. This can also decrease the antioxidant activity of the carotenoids. The sensitivity of bioactive substances to heat can also impact their antioxidant activity, as there is a positive correlation between the decrease in bioactive substances and the decrease in antioxidant capability (Bagchi et al., 2021). According to a recent study, rising temperatures hasten the start of oxidation, impairing the effectiveness of antioxidants. Bioactive chemicals deteriorate, experience structural modifications, and ultimately become inert substances.

CONCLUSION

In summary, beta-carotene is a pigment found in plants with several health benefits, including reducing cancer risk and promoting healthy eyes and bones. It is commonly extracted from plant parts using organic solvents, but eco-friendly extraction methods, such as maceration, are also available. Alternative methods for extracting beta-carotene include ohmic heating, microwave-assisted, and ultrasound-assisted extraction. Beta-carotene can be used as a natural colorant in food products and has antioxidant activity. The solubility of beta-carotene depends on the solvent used and the presence of specific functional groups. Beta-carotene is easily degraded by air and high temperatures, and its quality can be evaluated based on color, yield, and antioxidant activity. The use of beta-carotene as a food colorant can improve the sensory and functional qualities of food products and promote public health.

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HYPOGLYCEMIC AND HYPOCHOLESTEROLEMIC EFFECTS OF LESSER YAM SYNBIOTIC YOGHURT (*DIOSCOREA ESCULENTA* L) ON METABOLIC SYNDROME WISTAR RATS

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ABSTRACT

This study aims to determine the effect of consumption of lesser yam synbiotic yoghurt on glucose levels and blood lipid profiles of metabolic syndrome rats. Synbiotic yoghurt has positive effect on health such as increasing body immunity, lowering blood glucose and cholesterol levels. In this study, lesser yam synbiotic yoghurt was made from cow's milk and lesser yam extract with (1:1) proportion, sugar, skim milk and starter of lactic acid bacteria and then followed by bioassay test using experimental rats. The results showed that lesser yam synbiotic yoghurt can reduce glucose levels and improve the blood lipid profile of rats. The best treatment is to give 4 ml of synbiotic yoghurt per day which can reduce glucose levels and total blood cholesterol of metabolic syndrome rats. It can be concluded that lesser yam synbiotic yoghurt which had high levels of dietary fiber and inulin, had hypoglycemic and hypocholesterolemic effect.

Keywords: hipoglycemic, hypocholesterolemic, lesser yam, synbiotic yoghurt

ABSTRAK

Penelitian ini bertujuan untuk mengetahui pengaruh konsumsi yoghurt sinbiotik gembili terhadap kadar glukosa dan profil lipid darah tikus sindrom metabolik. Yoghurt sinbiotik memiliki efek positif bagi kesehatan seperti meningkatkan kekebalan tubuh, menurunkan kadar glukosa darah dan kolesterol. Dalam penelitian ini, yoghurt sinbiotik gembili dibuat dari proporsi susu sapi: ekstrak gembili (1:1), gula, susu skim dan starter bakteri asam laktat kemudian dilanjutkan dengan uji bioassay menggunakan tikus percobaan. Hasil penelitian menunjukkan bahwa yoghurt sinbiotik gembili dapat menurunkan kadar glukosa dan memperbaiki profil lipid darah tikus. Perlakuan terbaik adalah pemberian yoghurt 4 ml per hari yang dapat menurunkan kadar glukosa dan kolesterol total darah tikus sindrom metabolik. Dapat disimpulkan bahwa yoghurt sinbiotik gembili yang kaya serat pangan dan inulin mempunyai efek hipoglikemik dan hipokolesterolemik karena tingginya kadar serat pangan dan yoghurt.

Kata kunci: hipoglikemik, hipokolesterolemia, gembili, yoghurt sinbiotik

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INTRODUCTION

Synbiotics are combination of probiotics and prebiotics. Both are interrelated where prebiotics selectively provide nutrition to probiotic bacteria, so that it will stimulate the growth of probiotic bacteria in the intestinal epithelium/mucosa (Hamed et al., 2012). Consuming probiotics, prebiotics and synbiotics affects the composition of the microflora, which is to restore the balance of microbes, so that this intake has the potential for human health (Morelli et al., 2003).

In the market recently, there are many synbiotic which contains prebiotics and probiotics. The advantage of the combination of prebiotics with probiotics is to increase the viability of the probiotics themselves because specific substrates are available for fermentation. In addition, consumers will get double benefits from both. The application of the synbiotic concept is often found in fermented beverage products. Fermented drinks often use Lactic Acid Bacteria (LAB) in the fermentation process. The fermentation process provides added value in terms of nutritional value and taste in the resulting product (Andriyani, 2005)

In this research, synbiotic yoghurt was made from lesser yam extract (*Dioscorea esculenta* L) and cow's milk. Lesser yam is minor tuber that has not been explored and utilized optimally because its presence is still rare compared to other types of tubers. Dioscorea tubers contain thick mucus consisting of glycoproteins and water-soluble polysaccharides. Glycoproteins and polysaccharides are bioactive ingredients that function as water-soluble dietary fiber and are hydrocolloid which are useful for lowering blood glucose levels and total cholesterol (LDL) levels (Trustinah et al., 2013). Lesser yam has the prospect to be developed as synbiotic yogurt because it contains a lot of inulin and dietary fiber or is often called prebiotic component (Surya, 2015). The results of the previous study showed that lesser yam synbiotic yoghurt, produced from proportion of milk: lesser yam extract (1:1), which had a total lactic acid bacteria of 7.23 logCFU/ml, dietary fiber (3.05%) and inulin content (1.2%) (Rosida et al. , 2021).

Synbiotic yoghurt has physiological effects that are beneficial for digestive health and can decrease blood cholesterol and LDL content (Surya, 2015) and blood triglyceride content (Febriansyah, 2015). Zana et al. (2017) stated that there is a decreasing in blood triglyceride, cholesterol total, LDL and an increasing in blood HDL on syndrome metabolic rats which are given synbiotic yoghurt after 2 weeks treatment. This study aims to determine the effect of the consumption of lesser yam synbiotic yoghurt on glucose levels and blood lipid profiles of metabolic syndrome rats

MATERIALS AND METHOD

The materials used in this study were lesser yam, cow's milk, sugar, skim milk obtained from Soponyono Market, Surabaya. Yoghurt starter (*Lactobacillus bulgaricus, Streptococcus thermophilus, and Bifidobacterium bifidum*) is obtained from the Biology Laboratory, Faculty of Science and Technology, Airlangga University, Surabaya and chemical reagents for analysis

Lesser Yam Yoghurt Production

Fresh cow's milk was pasteurized at 70°C for 15 minutes and added with 5% (w/v) skim milk and 8% (w/v) sucrose. The aim of skim milk and sugar addition is as food source for the bacteria starter. Cow's milk which is being heated and mixed with lesser yam tuber extract in ratio of 50:50 and then cooled. The mixture was inoculated with 5% (v/v) starter (*Lactobacillus bulgaricus, Streptococcus thermophilus, and Bifidobacterium bifidum*) and incubated at 37°C for 18 hours (Rosida et al., 2021).

Bioassay

As many as 30 white Wistar rats (aged 2 months, average weight 200 g) were acclimatized for 7 days, put in closed individual cages with the following cage conditions: light is not controlled, air ventilation in the cage is adequate, and cage temperature use room temperature. Experimental rats were injected with Streptozotocin-nicotinamide (intraperitoneally) at a dose of 50 mg/kg body weight to make the rats suffering diabetes. The experimental rats were also given high-cholesterol

diet (from cow's brain) for 7 days to make the rats become hypercholesterolemia. Then the rats were fasted for 12 hours and blood taken through the eyes (retroorbital plexus) to measure blood glucose and cholesterol levels and ensure that the rats were positive for diabetes and hypercholesterolemia (Metabolic Syndrome condition).

Then the rats were divided into 5 (five) feed groups, each of which consisted of 6 (six) rats, namely the control group and the treatment group which were given with lesser yam synbiotic yoghurt as much as 1, 2, 3, 4 , ml per day. All rats received standard diet and the manufacture referred to the AIN-93 standard diet formula (Reeves et al., 1993). The rat blood serum was analyzed for glucose levels, total cholesterol, HDL, LDL, and blood serum triglycerides on week 0, week 1, and week 2. This study used Nested Randomized Design. The data obtained were analyzed by analysis of variance and further test DMRT (Duncan Multiple Range Test) at the level of 5%.

RESULTS AND DISCUSSION

In this study, rats were conditioned to have metabolic syndrome. The condition of metabolic syndrome is characterized by the criteria of rats suffering hyperglycemia, hypertriglyceridemia and having low blood HDL. The results of the Bioassay test on the serum glucose levels of experimental rats can be seen in Table 1.

Blood glucose level

Table 1. Changes of Blood Glucose Level of Rats During 2 Week Treatment

Treatment	Blood glucose level (mg/dL)			Blood glucose level
	week 0	week 1	week 2	change (mg/dL)*
control(-)	268.2	269.4	270.4	-0.8
yoghurt 1 ml	271.3	171.3	130.5	51.9
yoghurt 2 ml	270.4	182.6	111.4	58.8
yoghurt 3 ml	270.5	141.3	102.4	62.2
yoghurt 4 ml	270.3	145.8	94.4	65.1

*deduction from week 2 to week 1

Based on Table 1, in week 0 shows that streptozotocin-nicotinamide injection is very effective in increasing the blood glucose levels of rats to the diabetic level (> 200 mg/dL). Kusumawati (2004) states that hyperglycemic rats are characterized by increased blood glucose levels that exceed normal (normal glucose levels <200 mg/dL) after 2 hours of eating.

The results showed the potential of lesser yam yoghurt in reducing serum glucose levels in hyperglycemic (diabetic) rats. Giving lesser yam yoghurt can reduce blood glucose level for 2 weeks as much as 51.9-65.1% in the treatment of giving 1-4 ml of lesser yam yoghurt/day. A sharp decrease in blood glucose levels in rats treated with lesser yam yoghurt was associated with relatively high levels of dietary fiber and inulin. According to Marsono (2000), dietary fiber affects the viscosity and absorption of blood glucose, thus affecting the potential for lowering blood glucose. Dietary fiber can reduce postprandial glucose levels because of its viscous nature and its ability to form a gel that can inhibit macronutrient absorption (Weickert and Pfeifer, 2008).

Furthermore, Weickert and Pfeiffer (2008) explained that it is not only the ability of polysaccharides to be fermented that plays a role in lowering blood glucose levels. Dietary fiber consumption contributes to a number of metabolic effects, including insulin sensitivity, modulation of hormone secretion in digestion and various metabolic processes associated with the metabolic syndrome.

Blood cholesterol total

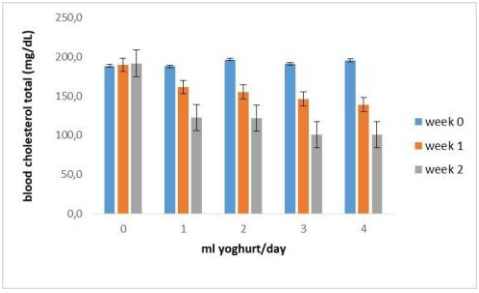

Figure 1. Changes of Serum Cholesterol Total of Rats Given Lesser Yam Yoghurt (1, 2, 3, 4 ml/day) for 2 Weeks

In Figure 1, at week 0, total blood cholesterol of all rats was relatively high (187.7-196.4 mg/dL) so that all rats had hypercholesterolemia. After the feeding intervention for 2 weeks, the serum total cholesterol level of the rats given the lesser yam yoghurt is decreased by 34.7-48.4%. However, the cholesterol level of the control group was constant (188.2 – 191.2 mg/dL).

The greatest decrease in total cholesterol levels occurred in the group of rats given 4 ml of yoghurt/day, which was 48.4%. It might be becaused lesser yam yoghurt is rich in fiber and inulin. The consumption of inulin can inhibit the absorption of cholesterol in the small intestine and ultimately lower cholesterol levels in human blood plasma (Anandharaj et al., 2014).

The hypocholesterolemic effect of probiotics is also associated with its ability to bind cholesterol in the small intestine. Sangeeta and Khaterpaul (2003), stated that probiotic bacteria found in fermented products can lower cholesterol in humans. The possible mechanism, according to Chiang et al. (2008), is the process of deconjugation of bile salts in the small intestine by probiotic bacteria. Cholesterol can also be converted in the intestine into coprostanol, which is directly excreted in the feces. This will lower the amount of cholesterol absorbed and lead to a decrease in physiological cholesterol concentrations. Cholesterol dehydrogenase/isomerase produced by bacteria such as: *sterolibacterium denitrificans* is responsible for catalyzing the transformation of cholesterol to cholest-4-en-3-one, an intermediate cofactor in the conversion of cholesterol to coprostanol. This serves as the basis for further research using probiotic bacterial strains.

Blood triglyceride level

In Figure 2, at week 0, the blood triglyceride levels of all rats had exceeded the normal value (>112 mg/dL) so that all rats had experienced hypertriglyceridemia (metabolic syndrome condition). The control group did not show a decrease in serum triglyceride levels (127.7-130.4 mg/dL), but all groups treated with yoghurt showed a significant decrease in serum triglyceride levels. The greatest decrease in triglyceride levels occurred in the group given 4 ml yoghurt/day, which was 35.8%. The decrease in blood triglyceride levels was in line with the decrease in blood cholesterol of experimental rats (Figure 2). It is in accordance with Kaur et al. research (2002) which showed that inulin could inhibit lipogenic enzyme which sinthesized fatty acid in the liver so it could reduce triglyceride content.

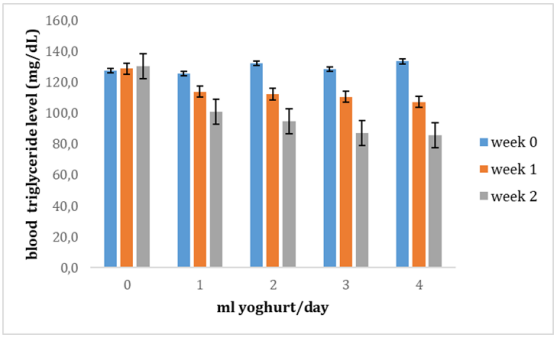

Figure 2. Changes of Serum Triglyceride Level of Rats Given Lesser Yam Yoghurt (1, 2, 3, 4 ml/day) for 2 Weeks

Blood LDL level

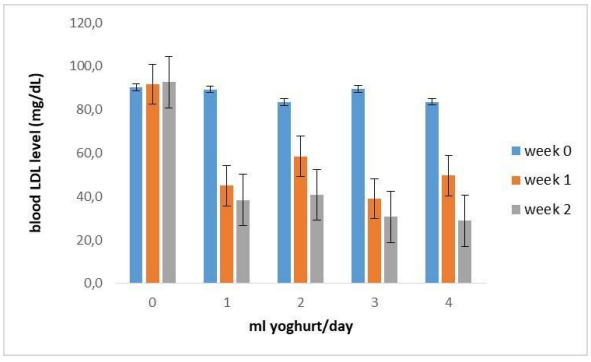

Figure 3. Changes of Serum LDL Level of Rats Given Lesser Yam Yoghurt (1, 2, 3, 4 ml/day) for 2 Weeks

After the hypercholesterolemic dietary intervention, all rats had high serum LDL (Figure 3). However, after 2-week intervention, the LDL levels of the rat group fed with the lesser yam yoghurt were lower than the control group. The greatest decrease in LDL cholesterol levels occurred in the group of rats fed with 4 ml yoghurt per day, which was 65.5%. This shows that lesser

yam yoghurt has the potential to lower blood LDL due to its fiber and inulin content.

According to Anderson et al (2009) high-fiber diet can increase the activity of LDL receptors in the liver. This activity is used to meet the availability of tissue cholesterol, so that more blood cholesterol is used, thereby lowering blood cholesterol levels (Anderson et al, 2009). Previous studies have shown that giving winged milk yoghurt to test animals can reduce the LDL lipoprotein profile of test animals. The mechanism of synbiotic yoghurt in reducing LDL content was that inulin was fermented by probiotic bacteria produced short chain fatty acid such as propionic acid. This acid reduced cholesterol synthesis in the liver by inhibiting the activity of HMGCoA reductase enzyme. The decrease in cholesterol production will hinder VLDL secretion and as a result it lowered LDL content (Akoma et al., 2000). Several studies have shown that synbiotics consisting of probiotics and prebiotics have an effect in improving lipid profiles (Ooi et al, 2010).

Blood HDL level

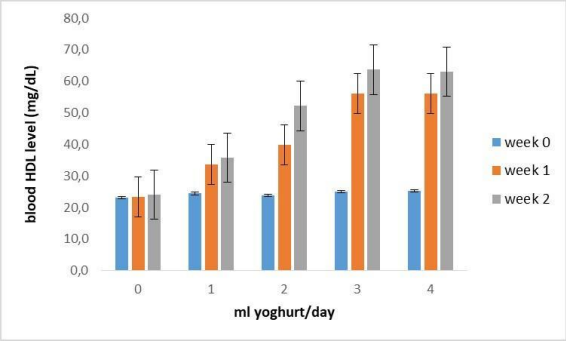

Figure 4. Changes of Serum HDL Level of Rats Given Lesser Yam Yoghurt (1, 2, 3, 4 ml/day) for 2 Weeks

Figure 4 shows that in the treatment group, there was a significant increase in HDL cholesterol levels ($p<0.05$) compared to the control group. The greatest increase in HDL cholesterol levels occurred in the group with 4 ml/day yoghurt treatment with an increase of 150.3%. The mechanism of this phenomena is Inulin in the lesser yam yoghurt could increase apolipoprotein A-1 as an enzyme cofactor and a ligand for interaction with lipoprotein receptor of HDL. So that in can increase HDL content (Mayes, 2003). According to Kai (2015) total cholesterol and triglyceride levels were reduced and HDL cholesterol levels increased in experimental rats consuming resistant starch and dietary fiber.

CONCLUSION

This research studied the effect of lesser yam synbiotic yoghurt intake on metabolic syndrome rats. The results of this study showed that after two weeks of giving lesser yam synbiotic yoghurt, metabolic syndrome rats had lower glucose, total cholesterol, triglycerides, and LDL levels, but higher HDL levels than the control group, especially those given 4 ml of lesser yam yoghurt per day. It can be concluded that lesser yam synbiotic yoghurt possibly has hypoglycemic and hypocholesterolemic effect due to its inulin and dietary fiber content.

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Journal of Functional Food and Nutraceutical

ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF HYDROLYSATE CHITOOLIGOSACHARIDES IN CRAB SHELL (*PORTUNUS PELAGICUS*) FROM DEGRADATION OF CHITOSANASE, A-AMYLASE, LIPASE AND CELLULASE ENZYMES

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ABSTRACT

The crab shell (*Portunus pelagicus*) has many benefits, one of the benefits is the used in the manufacture of chitooligosaccharides. The crab shell was chosen because it contains 20- 30% chitin inside. Chitin can be processed into chitosan and then hydrolyzed into chitooligosaccharides using enzymes. This study aims to determine the type of enzyme that is effective in the process of hydrolyzing chitooligosaccharides with various enzymes are the α-amylase, lipase, cellulase, chitosanase and a combination of enzymes are chitosanase α-amylase, chitosanase lipase, and cellulase chitosanase. The observation data were analyzed using ANOVA. The results showed that the best chitooligosaccharides obtained resulting from the hydrolysis of a combination of cellulase chitosanase enzymes which had the best characteristics, namely the highest degree of deacetylation of 94.95%, the lowest molecular weight of 3.54 KDa, the antioxidant activity of the DPPH method was 5.21 (µmol TE/g), the FRAP antioxidant method was 2.27 (µmol TE/g), the diameter of the inhibition area (DDH) was 7.30 mm in *Staphylococcus aureus* bacteria and 15.9 mm in *Salmonella typhosa* bacteria. From these results, it can be known that this chitooligosaccharide is more effective in inhibiting the growth. The Gram-positive bacteria was compared to gram-negative bacteria.

Keywords: chitooligosaccharide, antioxidant, antibacterial, enzyme combination

ABSTRAK

Cangkang rajungan (*Portunus pelagicus*) memiliki banyak manfaat salah satunya dalam pembuatan chitooligosakarida. Cangkang rajungan dipilih karena didalamnya mengandung sekitar 20-30% kitin. Kitin dapat diolah menjadi kitosan dan kemudian di hidrolisis menjadi chitooligosakarida. Penelitian ini bertujuan untuk mengetahui jenis enzim yang efektif dalam proses hidrolisis chitooligosakarida dengan berbagai jenis enzim yaitu enzim α-amylase, lipase, selulase, chitosanase dan kombinasi enzim diantaranya yaitu enzim chitosanase α-amylase, chitosanase lipase dan chitosanase selulase. Data hasil pengamatan dianalisis menggunakan *Analysis of Variance* (ANOVA). Hasil penelitian menunjukkan bahwa diperoleh chitooligosakarida terbaik yaitu chitooligosakarida hasil hidrolisis kombinasi enzim chitosanase selulase memiliki karakteristik yang paling baik yaitu derajat deasetilasi yang paling tinggi sebesar 94,95 %, berat molekul paling rendah 3,54 KDa, aktvitas antioksidan metode DPPH sebesar 5,21 (µmol TE/g), antioksidan metode FRAP sebesar 2,27 (µmol TE/g), nilai diameter daerah hambat (DDH) sebesar 7,30 mm pada bakteri *Staphylococcus aureus* dan 15,9 mm pada bakteri *Salmonella typhosa.* Dari hasil tersebut diketahui bahwa chitooligosakarida ini lebih efektif dalam menghambat pertumbuhan bakteri gram positif dibandingkan bakteri gram negatif.

Kata kunci: kitooligosakarida, antioksidan, antibakteri, kombinasi enzim

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INTRODUCTION

Indonesia is a maritime country that has 3.45 million km^2 ocean making the marine products very abundant, one of crustacean animal is the blue swimming crab (*Portunus pelagicus*). Blue swimming crabs have an important role in the Indonesian economy, especially in marine product export activities. In the January-February 2020 period, the export volume of Indonesian crab crabs reached 4,462 tons (BPS, 2020). However, currently, the crab is only used for meat, while the shells are simply thrown away. Even though the utilization of crab shell waste can provide added value because crab shells contain 30-40% protein, 30-50% minerals and 20-30% chitin (Amalia, 2018). One of the crab shell wastes can be made into Chitooligosaccharide (COS).

In the hydrolysis process of chitosan into chitooligosaccharides, there is a cleavage of the ß-(1,4) glycosidic chitosan bond chain, one of it using enzymatic techniques. Enzymatic hydrolysis is specific, controlled and environmentally friendly (Sarni et al, 2016). According to Abdel-Azi et al., (2014), the enzyme plays a role in specifically hydrolyze chitosan into chitooligosaccharides, the chitosanase enzyme. However, several enzymes are non-specific. Non-specific enzymes are relatively cheap and available for large-scale production, thereby reducing the cost of COS production. As for the non-specific nature of enzymatic cleavage, a series of COS products with various degrees of polymerization and high yields can be achieved by hydrolyzing chitosan through a combined enzyme system (Lin et al., 2009).

Therefore, for producing COS, this study uses treatments with different enzymes, including the enzymes chitosanase, lipase, cellulase, and α-amylase, where the combination of enzymes, namely chitosanase and lipase, chitosanase and cellulase as well as chitosanase and α-amylase to produce specific COS. This study aims to determine the type of enzyme that is effective in the process of hydrolyzing chitooligosaccharides with various enzymes and the effect of the type of enzyme and combination of enzymes (chitosanase, cellulase, lipase, and α-amylase) on the characteristics, antioxidant and antibacterial activity of chitooligosaccharide (COS) from crab shell chitosan.

MATERIALS AND METHOD

Materials

The material used in this manufacture of crab shells is crab shell obtained from PT. Kelola Mina Laut, Gresik. The enzymes are chitosanase used was imported by PT. Korean Genofocus of *Bacillus sp*. CAS no. 51570-20-8, Cellulase from novozymes viscozyme cassava CL, lipase from novozyme and α-amylase from sigma-aldrich. The chemicals used for analyzing are 2,2-diphenyl-1-picrylhydrazil (DPPH), methanol, distilled water, 96% ethanol solution, phosphate buffer, ascorbic acid, oxalic acid, TCA, K3Fe (CN)6, FeCl3, *Salmonella typhi*, *Staphylococcus aureus*, generic Amoxicillin (Kimia Farma), Nutrient Agar (Merck), Nutrient Broth (Merck), Blank paper disk (Oxoid), physiological NaCl, Aquades, Acetic Acid (Merck), pH Acetate Buffer 4.5 (Nitra Kimia).

Methods

Preparation of chitosan from crab shells (Butarbutar, 2018 Modified)

The process of isolating chitosan from crab shells was proceed chemically. First, the chitin isolation process is the demineralization process of the crab shells by adding 1.5L of 1.5M HCl into 100 g of shell powder and heating on the hotplate for 4 hours at 70^{o}C, and then the residue was filtered. Moreover, it was washed using distilled water until the pH was neutral. The residue obtained was baked in the oven for 3 hours at 70^{o}C. Furthermore, the deproteination process proceeded using 50g of the demineralized powder added with 0.5L of 3.5% NaOH, then heated for 4 hours at 70^{o}C, and then the residue was filtered and washed using distilled water until the pH was neutral. The residue obtained was baked in the oven for 3 hours at 70^{o}C to produce chitin. This process of deacetylation, namely 40 g of chitin plus 0.4L of 50% NaOH, was then heated for 4 hours. The temperature is maintained at 100^{o}C, then filtered, and the residue left behind is washed with distilled

water until the pH is neutral. The residue was baked in the oven for 3 hours at 70ºC, and dry chitosan was obtained.

Preparation of chitooligosaccharides from crab shells (Fawzya et al., 2009 Modified)

Preparation of 1% chitosan solution was carried out by dissolving chitosan into acetate buffer solution pH 4.5. Chitosan hydrolyzed with the various enzymes are chitosanase, cellulase, lipase, and α-amylase. The concentration each enzyme are α-amylase 1%, lipase 1%, cellulase 1%, chitosanase 1%, and hydrolyzed by combination enzymes are (1% chitosanase + 1% α-amylase), (1% chitosanase + 1% lipase) and (1% chitosanase + 1% cellulase) (w/w). Chitosan was incubated for 3 hours at 60ºC. The reaction was stopped by heating at 100ºC for 10 minutes. Then the sample was centrifuged at 9000 rpm to obtain a supernatant containing chitooligosaccharide. The reaction was stopped by heating at 100ºC for 10 minutes. Then the sample was centrifuged at 9000 rpm to obtain a supernatant containing chitooligosaccharide.

Physical analysis

The analysis used the Molecular Weight of the Mark-Khun Houwing method (Nasution, 2019) to determine the molecular weight of chitooligosaccharides, Degree of Deacetylation (Liu, et al., 2006) to determine the value of the number of the acetyl group or free amino groups after the deacetylation process. FTIR Functional Group (Muyonga, et al., 2004) to determine to identify functional groups in chitooligosaccharides, Antioxidant DPPH method (Munadiah, 2017) and antioxidant FRAP method (Munadiah, 2017) to determine the antioxidant activity of chitooligosaccharides, Antimicrobial disc diffusion method, Kirby-Bauer method (Nurhayati, 2020) to determine the antimicrobial activity through the zone of inhibition of bacteria.

Research design

The experimental design of crab shell chitooligosaccharide used a one-factor Completely Randomized Design (CRD), where using various enzymes with seven treatments such as 1% α-amylase, 1% lipase, 1% cellulase, 1% chitosanase, and also combination of enzymes such as (1% chitosanase + 1% α-amylase), (1% chitosanase + 1% lipase) and (1% chitosanase + 1% cellulase) (w/w) repeated three times, so there were 21 experimental units. The formulation in this design is determined based on preliminary research. The data obtained from the results of the analysis were processed using Analysis of Variance (ANOVA) and showed there were significant interactions and differences between each treatment. If there is a significant difference, a further test is carried out using the 5% DMRT (Duncan's Multiple Range Test) method. Data analysis used the help of the SPSS Statistics 17.0 for Windows program. From the data obtained, the best treatment was determined by looking at the results of each characteristic analysis performed.

RESULTS AND DISCUSSION

Molecular weight

Molecular weight is one of the essential parameters in chitooligosaccharides. The following is the table of the molecular weights of chitooligosaccharides hydrolyzed from various enzymes.

Table 1. The Molecular Weight of Chitooligosaccharides from Different Types of Enzymes

Sample	Molecular Weight (KDa)
COS α-amylase	$7.09^{a} \pm 0.09$
COS Lipase	$6.78^{b} \pm 0.06$
COS Selulase	$5.95^{c} \pm 0.11$
COS Chitosanase	$4.96^{d} \pm 0.04$
COS Chitosanase α-amylase	$4.73^{e} \pm 0.08$
COS Chitosanase lipase	$4.10^{f} \pm 0.06$
COS Chitosanase cellulase	$3.54^{g} \pm 0.07$

The data in **Table 1** shows that the chitooligosaccharides in this study have a molecular weight in the range of 7.09 KDa – 3.54 KDa. Chitooligosaccharides with the highest molecular weight were obtained from hydrolysis

with a combination of chitosanase and cellulase enzymes of 7.09 KDa. In comparison, the lowest molecular weight was obtained from hydrolysis with the α-amylase enzyme, which was 3.54 kDa. This is influenced by a specific enzyme that chitosanase. The chitosanase enzyme is only used for one reaction or the active part (the surface where the substrate is attached) is only attached to the surface of a particular substrate, which means that the specific enzyme. for chitosan hydrolysis is chitosanase. These specific enzymes can affect the hydrolysis process of breaking chitosan polymer bonds. In addition, the combination of enzymes makes the breaking of the glycosidic bond occur in two stages with a combination of specific and non-specific enzymes, it has an endo or Exo hydrolytic action so that the hydrolysis process can work optimally and affect the decrease in the resulting viscosity or molecular weight. According to the statement by Lodhi et al., (2014), chitosanase is one of the enzymes that hydrolyze chitosan to COS. According to Zhao (2019), this chitosanase has an end-type catalytic mode,endo-chitosanase often randomly breaks β-1,4-glycosidic bonds in the chitosan substrate to produce maximum chitooligosaccharides

This is supported by the statement of (Susilowati et al., 2015 in Nurhaeni et al., 2019) that the decrease in the molecular weight of chitosan after enzymatic hydrolysis occurs due to the breaking of bonds in the chitosan polymer chains to become shorter and the molecular weight of chitosan becomes lower. The combination of enzymes also influences the resulting molecular weight. According to Dong et al., (2015) that both chitosanase and cellulase enzymes are effective in hydrolyzing chitosan because their endo mode during the hydrolysis reaction process produces chitooligosaccharides. In addition, according to Je & Kim (2012), it happens because cellulase also has chitosanolitic activity in producing chitooligosaccharides. According to Poshina et al., (2020) that when compared to the amylase enzyme, the cellulase enzyme has the highest specific activity, which is shown by a rapid decrease in solution viscosity chitosan, which affects the resulting molecular weight due to the nature of this enzyme has endo and Exo capabilities that work synergistically.

Degree of deacetylation

The degree of deacetylation is also an essential parameter to determine the quality of chitooligosaccharides. According to Maidin (2017), deacetylation is the process of breaking the acetyl group from glucosamine. The degree of deacetylation indicates the number of acetyl groups that break off from the glucosamine group and the percentage number of amino groups in the polymer structure. The results of the degree of deacetylation in this study can be seen in the following table 2.

Table 2. Degree of Deacetylation of Chitooligosaccharides from Different Types of Enzymes

Sample	Degree of Deacetylation (%)
COS α-amylase	$81.78^{g} \pm 0.47$
COS Lipase	$85.01^{f} \pm 0.57$
COS Selulase	$87.90^{e} \pm 0.31$
COS Chitosanase	$90.54^{d} \pm 0.50$
COS Chitosanase α-amylase	$91.10^{cd} \pm 0.78$
COS Chitosanase lipase	$93.14^{b} \pm 0.23$
COS Chitosanase cellulase	$94.95^{a} \pm 0.77$

In **Table 2**, it can be seen that the degree of deacetylation of the chitooligosaccharides in this study ranged from 81.78% - 94.95%. Chitooligosaccharides with the highest deacetylation degree were obtained from hydrolysis with a combination of chitosanase and cellulase enzymes of 94.95%. In comparison, the lowest deacetylation degree obtained from hydrolysis with α- amylase enzyme is equal to 81.78%. The difference in the degree of deacetylation occurs due to the different types of enzymes because each enzyme has a special or specific ability to cut the existing acetyl groups. A study by Dong et al., (2015) stated that the enzymes chitosanase and cellulase were both effective in hydrolyzing chitosan because of their endo mode during the hydrolysis reaction process to produce chitooligosaccharides. The use of lipase enzymes is applied in the hydrolysis of chitooligosaccharides because the cutting mechanism can hydrolyze with endo-type and exo-

type. This is by the code (Lee, et al., 2008), namely lipase enzymes can hydrolyze chitosan into COS through exo- and endo-hydrolytic mechanisms.

Chitooligosaccharides that have a high degree of deacetylation is resulting from hydrolysis with a combination of chitosanase and cellulase enzymes. It is because the chitosanase enzyme can cut the acetyl group of glucosamine more effectively. After all, it cuts endo and Exo. The cellulase enzymes also have the characteristics of enzymes that can cut acetyl groups endo and Exo. So, they can optimally hydrolyze β-1.4 bonds and produce a large degree of deacetylation. According to Jiang et al., (2017), the degree of deacetylation (DD, %) is defined as the mole fraction of GlcN in the copolymer (chitosan), which is composed of GlcNAc and GlcN. It's also supported by Fouad's statement (2008) that the chitosanase enzyme is an enzyme that capable of endo-hydrolysis of β-1,4 bonds between GlcN (D-glucosamine) residues in the acetylation section of chitosan.

Chitooligosaccharides that have a high degree of deacetylation is resulting from hydrolysis with a combination of chitosanase and cellulase enzymes. It is because the chitosanase enzyme can cut the acetyl group of glucosamine more effectively. After all, it cuts endo and Exo. The cellulase enzymes also have the characteristics of enzymes that can cut acetyl groups endo and Exo so that they can optimally hydrolyze β-1.4 bonds and produce a large degree of deacetylation. According to Jiang et al., (2017), the degree of deacetylation (DD, %) is defined as the mole fraction of GlcN in the copolymer (chitosan), which is composed of GlcNAc and GlcN. This is also supported by Fouad's statement (2008) that the chitosanase enzyme is an enzyme capable of endo-hydrolysis of β-1,4 bonds between GlcN (D-glucosamine) residues in the acetylation section of chitosan.

According to Asha and Singh (2016), cellulase enzymes are enzymes glycoside hydrolase (GH) which utilize the hydrolysis acid-base catalytic mechanism. The first way, GHs with open active sites (grooves, gaps), which usually exhibit endo-cellulolysis (endo-cellulose), bind anywhere along the cellulose molecule and hydrolyze β-1,4 glycosidic bonds and other types exhibit exo-cellulolytic activity (cellobiohydrolases) which bind to the ends of the cellulose molecule and produce short chain oligosaccharides. Meanwhile, according to Liu & Xia (2006), cellulase has endo-type and exo-type activities, and the specificity of its cleavage includes GlcN-GlcNAc, GlcN-GlcN, and GlcNAc-GlcN bonds.

Antioxidant capacity DPPH method (2,2-diphenyl-1-picrylhydrazyl)

Testing the antioxidant capacity with the DPPH method is simple, fast, easy 2,2- diphenyl-1-pikrihydrazyl absorption method and uses a small amount of sample in a short time. The results of the antioxidant capacity of DPPH can be seen in the following table.

Table 3. The Antioxidant Capacity of Chitooligosaccharides from Different Types of Enzymes Using the DPPH Method.

Sample	Antioxidant Capacity (μmol TE/g)
COS α-amylase	$2.27^{g} \pm 0.05$
COS Lipase	$2.54^{f} \pm 0.06$
COS Selulase	$2.82^{e} \pm 0.04$
COS Chitosanase	$3.29^{d} \pm 0.08$
COS Chitosanase α-amylase	$3.34^{cd} \pm 0.07$
COS Chitosanase lipase	$3.72^{b} \pm 0.25$
COS Chitosanase cellulase	$5.21^{a} \pm 0.21$

Table 3 shows the average value of the antioxidant activity of DPPH resulting from the hydrolysis of chitooligosaccharides ranging from 2.27 to 5.21 μmol TE/g. In this study of antioxidant capacity using Trolox as standard, the unit of antioxidant capacity obtained was μmol TE/g. The lowest antioxidant capacity was obtained from the chitooligosaccharide treatment using the α-amylase enzyme, which was a 2.27 μmol TE/g sample. While, the highest antioxidant capacity was obtained from the chitooligosaccharide using a combination of chitosanase and cellulase enzymes, which was a 5.21 μmol TE/g sample. The greater the value of the antioxidant capacity of the sample indicates that more DPPH radicals are reduced. The

antioxidant capacity of the sample showed that the highest antioxidant capacity is chitooligosaccharide which used a combination of chitosanase and cellulase enzymes, which was a 5.21 µmol TE/g sample.

The difference in the antioxidant capacity produced is due to the different types of enzymes used. The highest antioxidant capacity of the chitooligosaccharides was produced by the combination of the enzymes chitosanase and cellulase. It's because the chitosanase enzyme can cut the acetyl group of glucosamine more effectively. After all, it cuts endo and Exo. The cellulase enzymes also have the characteristics of enzymes that can cut acetyl groups endo and Exo so that they can optimally hydrolyze β-1,4 bonds, catalyze glycosidic bonds in chitosan to produce glucosamine which has smaller monomers such as OH- (hydroxyl) groups) and amine groups (NH^+) become more numerous. The hydroxyl group and amine group play a role in the capture of free radicals. There are supported by Jung & Zhao (2012) that hydroxyl groups (OH-) and amino groups (NH^{2+}) contribute to the overall antioxidant capacity because they can react with unstable free radicals to form stable macromolecular radicals. According to the statement of Xie et al., (2001) that the mechanism from the binding of free radicals by chitosan. The OH^+ radical group from the lipid oxidation process can react with hydrogen ions from the ammonium ion group (NH^{3+}) in chitosan to produce a molecule that is more stable and produce antioxidant compounds.

Antioxidant capacity FRAP (Ferric Reducing Antioxidant Power) method

In this study, a synthetic antioxidant was used Trolox as a positive control. Trolox is similar to α-tocopherol and is used as a standard in the measurement of antioxidants. This test uses the FRAP (ferric reducing antioxidant power) method based on the ability of antioxidant compounds to reducing iron (III)-tripiridyl-triazine to iron (II)-tripiridyl triazine. This antioxidant test method used Fe $(TPTZ)_2^{3+}$ iron ligand complex 2,4,6-tripyridyl-triazine as a reagent. The blue Fe $(TPTZ)_2^{3+}$ complex will function as an oxidizing agent and will experience a reduction to yellow Fe $(TPTZ)_2^{2+}$. The results of the antioxidant capacity of FRAP are in the following table.

Table 4. The Antioxidant Capacity of Chitooligosaccharides from Different Types of Enzymes Using the FRAP Method.

Sample	Antioxidant Capacity (µmol TE/g)
COS α-amylase	$0.81^g \pm 0,03$
COS Lipase	$1.58^f \pm 0,05$
COS Selulase	$1.67^e \pm 0,01$
COS Chitosanase	$1.76^d \pm 0,03$
COS Chitosanase α-amylase	$1.89^c \pm 0,10$
COS Chitosanase lipase	$2.04^b \pm 0,03$
COS Chitosanase selulase	$2.27^a \pm 0,15$

Table 4 shows the average value of the antioxidant activity of FRAP resulting from the hydrolysis of chitooligosaccharides ranging from 0.81 to 2.27 µmol TE/g. The chitooligosaccharide treatment obtained the lowest yield using the α-amylase enzyme, which was 0.81 µmol TE/g sample. Moreover, the highest antioxidant capacity was obtained from the chitooligosaccharide treatment using a combination of chitosanase and cellulase enzymes, which is a 2.27 µmol TE/g sample. The results also show that different enzymes affect the antioxidant capacity produced. The combination of chitosanase and cellulase enzymes produced the highest antioxidant capacity of chitooligosaccharides. This is because the chitosanase enzyme itself can cut the acetyl group of glucosamine more effectively. After all, it cuts endo and Exo. Meanwhile, cellulase enzymes have the characteristics of enzymes that can cut acetyl groups endo and Exo so that they can optimally hydrolyze β-1,4 bonds, catalyze glycosidic bonds in chitosan to produce glucosamine which has smaller monomers such as OH^- (hydroxyl) groups. and amine groups (NH^+) become more numerous. The hydroxyl group and amine group play a role in capturing free radicals. This is supported by the statement of Jung & Zhao (2012) that hydroxyl groups (OH^-) and amino groups (NH^{2+}) contribute to the overall antioxidant capacity because they can

adsorb with unstable free radicals to form stable macromolecular radicals.

Antimicrobial activity with the inhibition zone diameter method

The inhibition zone test was processed using the disc-diffusion method. According to Hermawan et al., (2007), the disc-diffusion test or disc diffusion test was used to measure the diameter of the clear zone, which is an indication of a response to the inhibition of bacterial growth by an antibacterial compound. The gram-positive bacteria in this study used the *Staphylococcus aureus* bacteria, while the gram-negative bacteria used *Salmonella typhosa*. The following table presents the diameter of the inhibition zone of chitooligosaccharides hydrolyzed by various types of enzymes for two bacteria.

Table 5. The Diameter of the Chitooligosaccharide Inhibition Area of the Different Types of Enzymes for the Two Types of Bacteria

Sampel	Inhibition Zone Diameter (mm)	
	Staphylococcus aureus	***Salmonella typhosa***
COS α-amylase	$0.63^{g} \pm 0.15$	$1.03^{g} \pm 0.15$
COS Lipase	$1.17^{f} \pm 0.06$	$2.07^{f} \pm 0.15$
COS Selulase	$1.87^{e} \pm 0.06$	$3.13^{e} \pm 0.21$
COS Chitosanase	$2.47^{d} \pm 0.31$	$7.47^{d} \pm 0.51$
COS Chitosanase α-amylase	$3.00^{c} \pm 0.17$	$8.00^{cd} \pm 0.26$
COS Chitosanase lipase	$4.13^{b} \pm 0.68$	$11.47^{b} \pm 0.75$
COS Chitosanase selulase	$7.30^{a} \pm 0.79$	$15.90^{a} \pm 0.89$

In **Table 5,** it can be seen that the diameter of the inhibition zone of chitooligosaccharides against bacteria is indicated by the formation of an inhibition zone around the disc paper; for Staphylococcus aureus bacteria, the resulting inhibition zone ranges from 0.63 mm – 7.30 mm. Whereas in *Salmonella typhosa* bacteria, the range is from 1.03 to 15.90 mm. These results indicate that Chitooligosaccharide is effective in inhibiting the growth of gram-negative bacteria *Salmonella typhosa* compared to gram-positive bacteria *Staphylococcus aureus*. In this study, the effect of the type of enzyme on the hydrolysis of chitooligosaccharides on the bacterial inhibition zone was seen for two types of bacteria. Chitooligosaccharides derived from the hydrolysis of the cellulase chitosanase enzyme have the most significant inhibition zone effectiveness compared to other chitooligosaccharides. The different responses of the two groups of bacteria to chitooligosaccharides are caused by the different sensitivity of gram-negative and gram-positive bacteria. It is because gram-positive bacteria have a more straightforward cell wall structure than gram-negative bacteria, resulting in gram-positive bacterial cell walls being more easily damaged by antibacterial compounds from chitooligosaccharides than gram- negative bacteria. This follows the statement of Pilantanapak (2017), namely, chitooligosaccharides show better antibacterial activity against gram-positive bacterial species than against gram-negative bacteria. This is related to the chitooligosaccharide mechanism against gram-negative and positive bacteria, both of which are different. This is supported by Mei et al., (2015) statement that COS causes microbial cell death by changing the permeability of the cell membrane, which is a vital structure for protecting the release of cell constituents and controlling the entry of materials into cells from the environment. The positively charged COS can bind and absorb into the microbial cell wall through the negatively charged macromolecular components present in the microbial cell. ThiSampleke leads to their penetration into DNA and the blocking of RNA transcription.

Fourier-transform infrared spectroscopy (FTIR)

The change of chitosan into modified chitosan can be observed through the changes in its distinct functional groups. This FTIR test was carried out on samples of chitooligosaccharides derived from the results of hydrolysis using a combination of the enzyme chitosanase cellulase. This active group can be identified using Fourier-transform infrared spectroscopy.

Chitooligosaccharide has a distinctive FTIR spectral absorption band on the OH- and N-H

amine functional groups because the molecular formula of Chitooligosaccharide is (C6H8NO4)n. The FTIR test used the best treatment in this study, namely Chitooligosaccharide, which was the result of hydrolysis utilizing a combination of the cellulase chitosanase enzyme. The results of the OH- functional group from the FTIR absorption spectrum in this study showed a peak at a wavelength of 3417 cm^{-1}, which indicates that the wavelength of 3200-3600 cm^{-1} indicates the presence of the O-H functional group alcohol hydrogen bonding/phenol which usually appears at that wavelength. The wavelength of the O-H active group is not much different from Mourya et al., (2011), namely the O-H functional group of chitosan and its oligomers are located at a wavelength of 3450 cm^{-1}. Meanwhile, according to Singh et al., (2020), namely the FTIR chitooligosaccharide with a wavelength of 3434cm^{-1} shows the O-H functional group.

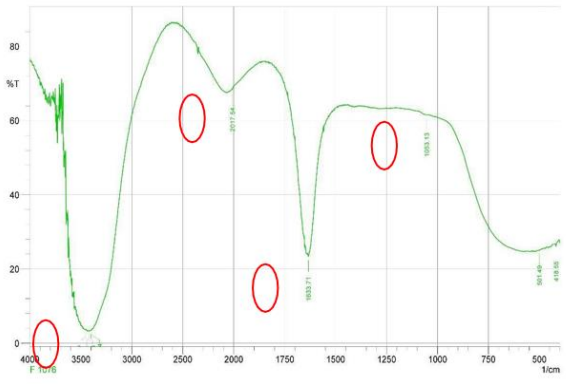

Figure 1. Chitooligosaccharide Functional Groups of Hydrolyzed Crab Shells Using a Combination of Chitosanase and Cellulase Enzymes

In this study, the peak at a wavelength of 3383 cm^{-1}, which is at a wavelength of 3300- 3500 cm-1, indicates the presence of the N-H amine/amide functional group, which usually appears at that wavelength. This amide group is also found in the statement of Mourya et al., (2011), that is, at a wavelength of 3120 cm^{-1}, it indicates the presence of a symmetrical N-H functional group. At 3320 cm^{-1}, there is an asymmetrical N-H functional group.

The peak wavelength of 1633 cm^{-1}, which is a wavelength of 1610-1680 cm^{-1}, indicates the presence of the C=O functional group, which usually appears at that wavelength. This is in accordance with the statement of Renuka et al., (2021) that Chitooligosaccharide has a C=O group at a wavelength of 1641 cm-1. In addition, there is also a functional group -C-O-C- at a wavelength of 1053.15 cm^{-1}, which indicates the presence of a 1,4-β- the glycosidic bond that has not been hydrolyzed. Thadatil (2014) explained that the enzymatic hydrolysis of chitosan into chitooligosaccharides could cut the (1,4)-β glycosidic bond from inside the chitosan structure and produce chitosan oligomers with a chain length of 2-10. This result is not much different from the results of Rokhati's research (2017), that the wavelength –C-O-C- is 1072.3 cm^{-1}.

CONCLUSION

Chitooligosaccharides from hydrolysis of the combination of cellulase and chitosanase enzymes had the best characteristics. It has the highest degree of deacetylation of 94.95% and the lowest molecular weight of 3.54 kDa. The most increased antioxidant activity of chitooligosaccharides using the DPPH method was in the chitooligosaccharides resulting from the hydrolysis of the cellulase chitosanase enzyme, which was 5.21 (µmol TE/g) and the antioxidant activity of chitooligosaccharides using the FRAP method was also found in chitooligosaccharides from the combination of the cellulase chitosanase enzyme, which was 2.27 (µmol TE/g). The best antimicrobial activity was found in chitooligosaccharides resulting from hydrolysis of the cellulase chitosanase enzyme, which had an inhibition area diameter of 7.30 mm in *Staphylococcus aureus* 15.9 mm in *Salmonella typhosa* bacteria. These results show that chitooligosaccharide is more effective in inhibiting the growth of gram-positive bacteria than gram-negative bacteria.

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CHOLESTEROL-LOWERING EFFECT OF SOY NUTS AND TEMPEH ON HYPERCHOLESTEROLEMIC SUBJECTS

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ABSTRACT

Exploration towards food with cholesterol-lowering property would be beneficial to reduce the incidents of cardiovascular caused by increasing number of people with hypercholesterolaemia. As much as 42 participants with total cholesterol (TC) levels ≥4.92 mmol/L were studied in a three-arms parallel intervention trial. As much as 72g soy nuts and 66g tempeh were consumed daily (25g soy protein/day) for six weeks, while control group was not given any sample. TC level and body composition were measured before and after the treatment for all groups. In soy nuts group, lower TC level was observed (-0.85±0.82 mmol/L, $p<0.05$) compared to the control group. Meanwhile in tempeh group, non-significant lower TC level was also observed (-0.40±1.19 mmol/L). Differences in body composition parameters were also measured and resulted in significant lower body weight, fat, and visceral fat in soy nuts group ($p<0.05$) while in tempeh group, only body weight and waist circumference were significantly decreased ($p<0.05$). This study suggested that daily consumption of soy nuts and tempeh containing 25g soy protein for six weeks showed a tendency to lower TC levels in hypercholesterolaemic-Indonesian participants. A more significant effect might be observed in subject with higher TC levels; and thus, further study is encouraged.

Keywords: body composition; hypercholesterolemia; soy nuts; tempeh; total cholesterol

ABSTRAK

Eksplorasi produk pangan dengan sifat penurun kolesterol sangat bermanfaat untuk menurunkan kejadian penyakit kardiovaskular pada mereka dengan hiperkolesterolemia. Studi intervensi ini melibatkan 42 peserta dengan kadar kolesterol total ≥4.92 mmol/L. Dua kelompok perlakuan diberikan 72gram kacang kedelai dan 66gram tempe per hari (25gram protein kedelai) selama enam minggu, sementara kelompok kontrol tidak. Kadar kolesterol total dan komposisi tubuh diukur sebelum dan setelah perlakuan pada semua kelompok. Penurunan kadar kolesterol total yang siginifikan (-0.85±0.82 mmol/L, $p<0.05$) teramati pada kelompok kacang kedelai dibandingkan dengan kontrol, sementara pada kelompok tempe, penurunan juga teramati meski tidak signifikan (-0.40±1.19 mmol/L). Perbaikan komposisi tubuh juga teramati secara signifikan pada parameter berat badan, lemak, dan angka lemak visceral pada kelompok kecang kedelai ($p<0.05$), sementara hanya parameter berat badan dan lingkar perut yang mengalami perbaikan signifikan pada kelompok tempe ($p<0.05$). Studi ini menyimpulkan bahwa konsumsi rutin kacang kedelai dan tempe sebesar 25gram protein kacang kedelai selama enam minggu cenderung dapat menurunkan kolesterol total pada orang Indonesia dengan kondisi hiperkolesterolemia. Bahkan penurunan kadar kolesterol total mungkin teramati lebih tinggi pada mereka yang memiliki kadar kolesterol total yang lebih tinggi sehingga diperlukan penelitian lebih lanjut.

Kata kunci: hiperkolesterolemia; kacang kedelai; kolesterol total; komposisi tubuh; tempe

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INTRODUCTION

Hypercholesterolaemia is a condition that is characterised by elevated total cholesterol levels and/or low-density lipoprotein cholesterol (LDL-C) concentration; which can be defined as total cholesterol level >5.17 mmol/L and/or LDL-C level is >3.37 mmol/L (Gardner et al., 2001; Gebreegziabiher et al., 2021; Hu et al., 2021; Yudin et al., 2022). In most cases, hypercholesterolaemia is caused by interaction of sedentary lifestyle and an increased intake of saturated and trans-fatty acids (Ibrahim and Jialal, 2019; Martinez-Hervas and Ascaso, 2019). Without proper treatment and effort to improve the cholesterol levels, hypercholesterolaemia could increase the risk of cardiovascular disease (CVD). Amongst other risk factors, CVD is caused by elevated LDL levels since LDL is very vulnerable to oxidation due to its interaction with free radicals. If LDL gets oxidised, it becomes ox-LDL (oxidised LDL) which is atherogenic and will lead to CVD (Gao and Liu, 2017; Jaishankar et al., 2021). Based on the data from Health Ministry of Indonesia, in 2016, 21.2% of Indonesian citizens were diagnosed with hypercholesterolaemia (mostly from range 19 to 64 years old). This situation led to the increase of CVD cases, where the number of coronary heart disease case is the highest. The prevalence of coronary heart disease is 1.5% with mortality rate of 7.4 million (Kemenkes RI, 2018).

Maintaining normal blood cholesterol concentrations is important to reduce future CVD risk. Having healthier lifestyle by increasing physical activity and incorporating healthy diets, such as decreasing saturated fat and increasing the amount of fibre, into daily life is beneficial for health in general and helps reducing an elevated cholesterol levels (Mann et al., 2014). Research on healthy nutrition that could lower serum total cholesterol levels and promote cardiovascular health in general is growing. One such ingredient is soybean, which is well-known for its health benefit to lower serum total cholesterol and LDL-C levels (Cho et al., 2007; Ramdath et al., 2017). The cholesterol-lowering properties of soybean is supported by its several interesting compounds such as soy protein, isoflavones, and phytosterols. Several studies have indicated that those compounds contribute to the cholesterol-lowering properties of soybeans (Ramdath et al., 2017).

A study by Gardner et al., (2001) showed a significant reduction in total cholesterol and LDL-C concentrations after supplementation of 42 g soy protein, containing 80 mg aglycone isoflavones, in hypercholesterolaemic postmenopausal women. Meanwhile, a study by Bakhit et al., (1994) showed reduction of total cholesterol in hypercholesterolaemic participants; but, not in normocholesterolemic participants; after consumption of 25 g/d of soy protein for four weeks. A meta-analysis conducted by Jenkins et al., (2019) concluded that a daily intake of 25 g/day soy protein is recommended to have CVD risk improvement. It was postulated that the components responsible to cholesterol-lowering property of soybeans is its soy isoflavones (Montgomery, 2013). However, most studies mentioned that the beneficial effects could not be attributed solely to isoflavones since the source of isoflavones used is usually isolated soy protein (ISP). Thus, the beneficial effects could not be concluded because of the isoflavones solely since both soy protein and isoflavones were presented (Demonty et al., 2003).

Since most studies evaluating the effects of soy protein intake on lipid profile were using ISP, the effects of commercially available soy foods have not been fully evaluated. A recent study by Ruscica et al., (2018) evaluated the effects of whole soy foods intake (in the form of soy nuggets, soy burgers, soy desserts, and soy drinks) with 30 g soy protein contained. The results showed that the soy foods diet significantly improved plasma lipid profiles by reducing TC by 0.50 mmol/L and LDL-C by 0.38 mmol/L after 12 weeks intervention. In Indonesia, soy is widely consumed in the form of tempeh, a fermented soybean cake. However, the effect of soy nuts and tempeh consumption is rarely explored in Indonesia. Thus, in the present study, the objective is to evaluate the cholesterol-lowering effect of soy nuts and tempeh consumption containing 25 g soy protein for six weeks on hypercholesterolaemic Indonesian participants.

MATERIALS AND METHOD

Participants

Participants were employees of Nutrifood Indonesia, office branches of Jakarta and Ciawi. Men and women aged ≥20 years with TC levels of ≥4.92 mmol/L and willing to consume either soy nuts or tempeh sticks for 6 weeks according to the guidelines were eligible to participate in this study. The exclusion criteria were pregnant or breastfeeding woman; presented with a current or previous medical history for example cancer and other chronic disease; taking medication to interfere lipid or cholesterol metabolism; suffering gastrointestinal disease for which a high-fibre supplement would be contraindicated; suffering from cardiovascular disorder and/or heart disease; suffering from diabetes and/or hypertension; allergic to soy; taking regular prescribed medicine such as acetylcysteine which possible side effect is lipoprotein-lowering. Participants who eligible to join the study were informed not to consume any supplementation (including certain food such as fish oil, green tea extract, oat etc) to lower cholesterol intentionally during the study period. Informed consent forms were signed by all participants prior the start of the study. The study protocol was approved by ethical committee for medical research with registration number 0534 /III/LPPM.PM.10.05/04/2020.

There were 54 participants involved in this study and they were divided into three groups (control, tempeh, and soy nuts groups) based on their preference towards the sample to increase participants' compliance. However, due to COVID-19 outbreak, 12 participants were excluded due to difficulty during data collection. At the end, there were only 42 participants which completed the data for the study: three in control group, 18 in tempeh group, and 21 in soy nuts group.

Study design and data collection

This study was a non-randomised controlled study with three arms. Recruited participants were grouped into three groups: control group, soy nuts group, and tempeh group. This study took place for six weeks. Participants in control group were not given any sample, whereas participants in intervention groups were given 60 g/d and 72 g/d of tempeh sticks and soy nuts, respectively. Tempeh sticks were made by cut the tempeh obtained from local seller in Jakarta into small thin size and fried using air-fryer (200ºC, 8 mins). Soy nuts were made by soaking the soy nuts in water for one night then baked in the oven until the nuts dried and crunchy. Control group was chosen from participants who did not willing to consume the soy nut and tempeh and offered to be in control group. The provided sample given each day contained 25 g of soy protein, based on calculation from proximate analysis (analysis was done by MBRIO Food Laboratory and PT. Saraswanti Indo Genetech) result in Table 1.

Table 1. Nutrition Contents of Soy Nuts and Tempeh Sticks

Nutrients (%)	Tempeh sticks (per 60 g)	Soy nuts (per 72 g)
Total calorie	270.11 Kcal	303.09 Kcal
Protein	25 g	25 g
Carbohydrate	16.34 g	26.34 g
Fat	11.64 g	10.86 g
Fibre	8.58 g	11.82 g

At the beginning and the end of study period, participants' total cholesterol levels and body compositions were measured. TC levels were measured by commercial clinical laboratory (Prodia) at the beginning of the study using fresh venous blood. Meanwhile, at the end of the study, TC levels were measured using finger prick test device (EasyTouch GCU®, Taiwan) due to the COVID-19 pandemic that caused health public facilities to be temporarily restricted. Even though the method was different, finger prick test is also accurate and has good clinical utility and the difference is not significant (Sblendorio et al., 2008; Sedgwick et al., 2011; Loch et al., 2017). Participants' body compositions were measured using Omron Karada Scan, Japan at the beginning and at the end of the study.

Statistical analysis

Statistical analysis was performed with SPSS statistics software version 23 by IBM®, United States. Non-parametric analysis was chosen since the number of participants were less than 30 in each group. Baseline characteristics were checked to see if all the baseline data is homogenous (no

significant difference between the three groups) with Mann Whitney U Test. To test whether there was any significant change from baseline to end-treatment, Wilcoxon test was used. Finally, to evaluate if there were any significant difference between control and treatment group, Mann Whitney U Test was used. Significance for all analyses was set at $p<0.05$.

RESULTS AND DISCUSSION

Forty-two adults with elevated cholesterol (Female: 47.6%; Age 35.36 ± 9.33 years) completed this study. To detect a 0.39 mmol/L difference in TC, with an 80% power and SD of 0.23 mmol/L based on similar previous study by Bakhit et al., (1994), minimal of 14 participants each group were required for the study, yet the amount of control group will be explained in discussion.

Anthropometric characteristics and total cholesterol levels of the participants from baseline to end treatment are summarised in Table 2 (control vs soy nuts group) and Table 3 (control vs tempeh group). Wilcoxon test was conducted to see if there is any significant change from week 1 to week 6 for every parameter within each group. Table 2 and 3 show that there were no significant changes in control group after six weeks period of study.

Variations in study designs, the form of soy products, and the length of intervention might contribute to the results inconsistency (Wong et al., 1998). A meta-analysis indicated that supplementation with soy protein on hypercholesterolaemia participants would be resulted in significant reduction of TC levels only if the participants initially had TC levels ≥6.22 mmol/L and would not show a significant effect if the participants had initial TC levels of <5.15 mmol/L (Anderson et al., 1995; Wong et al., 1998; Wofford et al., 2012). Cholesterol-lowering effect of soy protein also seems to be low or negligible in normocholesterolemic participants. Another study also showed a non-significant result from soy protein supplementation towards hypercholesterolaemic participants with moderately elevated LDL-C levels (3.37-4.89 mmol/L) (Gardner et al., 2001). Nevertheless, minimum TC levels of the subject of our study was set to 4.92 mmol/L, since TC levels between 4.66-6.22 mmol/L were considered as borderline high thus needs attention and effort to be lowered (Jeong et al., 2018). Also, it was more recommended for people in borderline high TC levels (4.66-6.22 mmol/L) to improve the condition by having a healthier dietary intake and lifestyle rather than taking medication to lower cholesterol such as statin (Loch et al., 2017).

Table 2. Anthropometric Characteristics of Subjects in Control vs Soy Nuts Groups (Data are Means ± Standard Deviations)

Parameters	Control	Soy nuts	P-value *(Mann Whitney U test)*
Weight (Kg)	*N=3*	*N=18*	
Week 1	58.17 ± 12.29	66.54 ± 12.41	0.947
Week 6	56.70 ± 12.66	65.04 ± 12.43	0.356
Δ	-1.47 ± 1.37	-1.49 ± 0.78	0.814
P value (Wilcoxon test)	0.180	0.00	
Fat (%)	*N=3*	*N=18*	
Week 1	27.80 ± 10.61	27.54 ± 7.90	0.316
Week 6	27.37 ± 14.21	27.07 ± 7.79	0.887
Δ	-0.43 ± 1.36	-0.47 ± 0.89	0.669
P value (Wilcoxon test)	1.000	0.029	
Visceral Fat (%)	*N=3*	*N=18*	
Week 1	8.67 ± 5.80	8.19 ± 5.32	0.286
Week 6	8.33 ± 5.69	7.67 ± 5.21	0.740
Δ	-0.33 ± 0.76	-0.53 ± 0.32	0.814
P value (Wilcoxon test)	0.414	0.000	
Waist Circumference (cm)	*N=3*	*N=18*	
Week 1	81.17 ± 12.79	84.25 ± 9.17	0.658
Week 6	83.17 ± 12.86	83.07 ± 9.24	0.740
Δ	2.00 ± 5.68	-1.40 ± 4.48	0.307
P value (Wilcoxon test)	1.000	0.266	
Total Cholesterol (mmol/L)	*N=3*	*N=18*	
Week 1*	5.89 ± 0.60	5.73 ± 0.55	0.814
Week 6**	5.95 ± 0.35	4.89 ± 0.61	0.017
Δ	0.06 ± 0.90	-0.84 ± 0.82	0.185
P value (Wilcoxon test)	1.000	0.003	

*venous sampling
** capillary sampling

Participants in soy nuts group had a significant difference ($p<0.05$) in body weight (-1.49 ± 0.78 kg), body fat (-0.47 ± 0.89 %), and visceral fat (-0.53 ± 0.32 %), and total cholesterol level (-0.85±0.82 mmol/L) after 6 weeks treatment

compared to baseline. Meanwhile, participants in tempeh group had a significant difference ($p<0.05$) in body weight (-0.61 ± 0.98 kg) and waist circumference (-2.07 ± 3.71 cm) only. To further analyse the change (Δ) for 6 weeks period between control and soy nuts group; and between control and tempeh group, Mann Whitney U test was used. No significant result was obtained.

Table 3. Anthropometric Characteristics of Subjects in Control vs Tempeh Groups (Data are Means ± Standard Deviations)

Parameters	Control	Tempeh	P-value *(Mann Whitney U test)*
Weight (Kg)	*N=3*	*N=21*	
Week 1	58.17 ± 12.29	67.28 ± 9.17	0.506
Week 6	56.70 ± 12.66	66.67 ± 9.51	0.172
Δ	-1.47 ± 1.37	-0.61 ± 0.98	0.202
P value (Wilcoxon test)	0.180	0.013	
Body Fat (%)	*N=3*	*N=21*	
Week 1	27.80 ± 10.61	29.37 ± 5.61	0.158
Week 6	27.37 ± 14.21	29.04 ± 5.53	0.742
Δ	-0.43 ± 1.36	-0.32 ± 1.29	0.680
P value (Wilcoxon test)	1.000	0.313	
Visceral Fat (%)	*N=3*	*N=21*	
Week 1	8.67 ± 5.80	8.67 ± 4.48	0.387
Week 6	8.33 ± 5.69	8.76 ± 4.37	0.935
Δ	-0.33 ± 0.76	0.10 ± 1.01	0.505
P value (Wilcoxon test)	0.414	0.979	
Waist Circumference (cm)	*N=3*	*N=21*	
Week 1	81.17 ± 12.79	88.23 ± 8.07	0.877
Week 6	83.17 ± 12.86	86.16 ± 6.94	0.783
Δ	2.00 ± 5.68	-2.07 ± 3.71	0.238
P value (Wilcoxon test)	1.000	0.019	
Total Cholesterol (mmol/L)	*N=3*	*N=21*	
Week 1*	5.89 ± 0.60	5.91 ± 0.72	0.310
Week 6**	5.95 ± 0.35	5.43 ± 1.12	0.202
Δ	0.06 ± 0.90	-0.4 ± 1.19	0.505
P value (Wilcoxon test)	1.000	0.092	

*venous sampling
** capillary sampling

After the intervention, it was shown that TC levels after six weeks period of soy nuts and tempeh consumption were lower compared to the baseline. TC levels were significantly lower by -0.85±0.82 mmol/L at the end of the study in soy nuts group ($p<0.05$). Meanwhile in tempeh group, routine consumption of tempeh could decrease the TC level. In this study, there was a slight decrease by -0.40±1.19 mmol/L although this is statistically not significant ($p=0.09$). This reduction of TC levels was beneficial to help improving participants' TC levels into desirable level. These results would also be helpful in general for people with moderately-hypercholesterolaemia that consumption of soy food products with at least 25 g soy protein per day will have favourable effect to their TC levels. However, it must be noted that although there was a trend of lower TC levels observed after six weeks treatment, these changes in TC levels were not significantly different compared to the control group.

Besides the TC levels, body parameters were also measured. In soy nuts group, participants' weight, body fat, and visceral fat were significantly lower after six weeks ($p<0.05$). Meanwhile in tempeh group, only the participants' weight and waist circumference were significantly lower ($p<0.05$) at the end of the study. Participants' protein intake was recorded using food dairy to make sure that any changes are due to the soy protein and not from other foods (Table 4). It was shown that there was no significant difference between groups and between baselines to end-treatment; and implying that the results were due to our treatments during study period. Even though it was not significant, the average of protein consumption was higher in tempeh and soy nuts group due to the soy protein than was added to their daily food consumption compared with control group.

Table 4. Food Diary for Protein Consumption (Data are Means ± Standard Deviations)

Parameters	Control	Tempeh	Soy Nuts	P-value *(Mann Whitney U test)*
Protein (g)	*N=3*	*N=21*	*N=18*	
Week 1	55.33 ± 26.96	72.13 ± 12.08	68.87 ± 16.01	0.436
Week 6	52.67 ± 6.08	68.79 ± 11.79	63.29 ± 15.56	0.056
Δ	-2.67 ± 23.25	-3.33 ± 17.21	-5.59 ± 17.07	0.829
P value (Wilcoxon test)	1.000	0.484	0.154	

One of limitations to our study was the difference of TC levels measurement methods between baseline and end treatment that could be subject to validity. TC levels measurement in this study was different between baseline and end-treatment due to the outbreak situation. This study used venous sampling in baseline and capillary sampling (prick test) in end-treatment. Although the blood-

sampling method was different between baseline and end-treatment (venous vs capillary blood), Sblendrio et al., (2008) and Sedgwick et al., (2011) concluded that capillary and venous blood lipid concentration were not significantly different. The other limitation was the amount of control group which only three participants had completed the study due to COVID-19 pandemic.

In addition, a comparative study between measurements of serum cholesterol by laboratory and by finger-prick test was conducted by Abdelmotaleb et al., (2017). The study concluded that EasyTouch® GCU is a promising device for quick and accurate total cholesterol measurement with there was no significant difference compared to laboratory test (0.01 mmol/L lower than laboratory test). Nonetheless, methods in research should be consistent and remain the same from the beginning until the end of the study. Another limitation of this clinical trial was the lack of measurement regarding soy phytosterol, isoflavones, and proteins in soy nuts and tempeh. The equol production in the gut, an active metabolite of soy isoflavone daidzein could bind estrogen receptors which associated with lipid-lowering efficacy (Simental-Mendia et al., 2018; Alshehri et al., 2021). Soy isoflavone also could serve as ligands for lipid-regulating proteins, such as liver X receptor, farnesoid X receptor and peroxisome proliferator activated receptors (PPARs), which would decrease hepatic lipid synthesis, bile acid synthesis, and cholesterol reabsorption (Ramdath et al., 2017). The other components are soy proteins such as 7S and 11S globulin. These proteins could regulate LDLR activity and inhibit hepatic ApoB synthesis (Mejia et al., 2019; Caponio et al., 2021). Measurement on phytosterol, isoflavones, and peptides contained in soy nuts and tempeh might explain the effect exhibited in the future studies.

CONCLUSION

This study showed that daily consumption of soy nuts and tempeh containing 25 g soy protein for six weeks had the tendency to lower TC levels in hypercholesterolaemic-Indonesian participants. Although the decrease in TC level after soy nuts and tempeh consumption in this study was not different significantly from control group, significant lower TC levels observed between the baseline to end-treatment should not be just ignored. Thus, daily soy nuts and tempeh consumption could be incorporated as part of healthy lifestyle recommendation for people with hypercholesterolaemia in attempt to reduce their TC levels.

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CONFLICT OF INTEREST

Authors 2-4 are the employees of PT Nutrifood Indonesia. Yet, the funder had no role in study design, data collection, analysis and interpretation.

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EFFECT OF ADDING LEMONGRASS STALKS ON CHARACTERISTICS OF HERBAL DRINK LEMONGRASS - PALM SUGAR AS A FUNCTIONAL FOOD

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ABSTRACT

Palm sugar is a product made from the sap of lontar tree flowers. Palmira Indonesia has started to develop palm sugar products in the form of powder and drinks. The addition of lemongrass stalks can be used as an alternative to improve product characteristics. This study aims to determine the effect of adding lemongrass stalks and to obtain the sensory characteristics of the lemongrass palm sugar herbal drink. This research was conducted using a completely randomized experimental design with the proportion of addition of lemongrass stalks (0%, 5%, 10%, 15%, 20%, and 25%). The data were analyzed by analysis of variance. If it gives a significant effect, then proceed with Duncan's Multiple Range Test. The results showed that the addition of lemongrass had a very significant effect on the scoring test of taste attributes, significantly on the hedonic test of the taste attributes and not significantly on the hedonic test of the color attribute, total acceptance, and the scoring test of the color attribute. The best treatment that can be used to make a lemongrass-sugar palm herbal drink is a 10% addition of lemongrass stalks. The panelist's acceptance of the treatment was liked for taste, color, and total acceptance attributes with the taste scoring criteria being balanced brown sugar and lemongrass and color scoring criteria being red. This treatment was also containing an antioxidant activity was 69.64% with an IC_{50} was 694.50 ppm, a total dissolved solid was 0.52 Brix, and a color characteristic of L* 26.9, a* 23.16, and b* 37.1 with red oxide criteria.

Keywords: adding lemon grass stalks; herbal drink; functional; palm sugar; phytochemical and sensory characteristic

ABSTRAK

Gula lontar merupakan produk gula yang terbuat dari nira yang didapat dari sadapan bunga pohon lontar yang saat ini di bali sudah mulai kembangkan produk gula lontar dalam bentuk powder dan minuman oleh Palmira Indonesia. Dalam pengembangan minuman tersebut, penambahan batang serai dapat digunakan sebagai salah satu alternatif untuk meningkatkan komponen bioaktif dan memperbaiki karakteristik produk, biasanya batang serai digunakan untuk bumbu masak ataupun minuman tradisional.Penelitian ini bertujuan untuk mengetahui pengaruh penambahan batang serai dan mengetahui karakteristik sensori dari minuman herbal serai-gula lontar. Penelitian ini dilakukan dengan menggunakan Rancangan Percobaan Acak Lengkap dengan persentase penambahan batang serai (0%, 5%, 10%, 15%, 20% dan 25%). Data yang diperoleh dianalisis dengan analisis varians. Jika memberikan pengaruh yang signifikan, maka dilanjutkan dengan Uji Jarak Berganda Duncan. Hasil penelitian menunjukkan bahwa penambahan serai berpengaruh sangat nyata terhadap uji skoring atribut rasa, berpengaruh nyata pada uji hedonik atribut rasa dan berpengaruh tidak nyata pada uji hedonik atribut warna, penerimaan total dan uji skoring warna. atribut minuman herbal serai-gula lontar. Perlakuan terbaik yang dapat digunakan untuk membuat minuman herbal sereh-gula lontar adalah penambahan batang serai 10%. Penerimaan panelis terhadap perlakuan adalah atribut rasa (suka) dengan kriteria penilaian rasa (gula lontar dan serai seimbang), atribut warna (suka) dengan kriteria penilaian warna (merah) dan penerimaan total (suka).Perlakuan ini juga mengandung aktivitas antioksidan 69,64% dengan IC_{50} 694,50 ppm, total padatan terlarut 0,52 ^{o}Brix dan karakteristik warna L* 26,9, a* 23,16 dan b* 37,1 dengan kriteria warna merah oksid

Kata Kunci: gula lontar; karakteristik fisikokimia dan sensori; minuman herbal; penambahan batang serai

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INTRODUCTION

Borassus flabellifer commonly known as Palmyra palm is easily found in Southeast Asia such as India and Indonesia. Palmyra palm known as *lontar* has many benefits and uses as a functional food. According to Gummadi et al., (2016) a product made from the palm plant was found to have anti-inflammatory, anti-rheumatic, antibacterial, analgesic, antipyretic, hypoglycemic, and antioxidant properties. One part of the *lontar* plant that can be used is palm sap. Saranya (2016) reported that palm sap is usually used as a sugar and has several benefits such as a diuretic agent, stimulant, laxative, anti-phlegmatic, tonic, and can function as an antidote and is good for liver health.

The main product of lontar is the sap which is obtained from flower leads and can be drunk directly or processed into sugar. The use of palm sap as sugar in Indonesia has been carried out. One of them was reported by Ledheng dan Naisumu (2020) to improve the quality of processing palm sap which is a product that is used as a regional superior resource in Maubeli Village, East Nusa Tenggara is excavated and developed into printed brown sugar.

In addition, in Bali, the manufacture of brown sugar powder from palm sap has begun in the Ban area, Karangasem. Utilization of palm sap into palm sugar has been carried out which is accommodated by Palmira Indonesia. This group is a social enterprise that produces high-quality organic palmyra sugar made from local natural resources, palmyra palm trees through empowering local communities including local farmers, women, and young people in remote villages of the Eastern part of Bali Island, Karangasem. The palm sugar production process by Palmira Indonesia has obtained a home industry food (*Pangan Industri Rumah Tangga* (P-IRT)) distribution permit and is distributed in retail. The high demand from the public regarding the development of palm sugar products requires Palmira Indonesia to develop products for functional food. Lemongrass is a special type of plant because it contains essential oils which are a supporting factor in the formation of their flavor of the lemongrass plant. Palmira Indonesia intends to develop palm sugar into functional food in the form of herbal drinks with the addition of lemongrass stalks.

The addition of lemongrass stalks in the process of making herbal drinks can increase the bioactive compounds that are good for health and provide unique organoleptic properties for palm sugar products. Evama et al. (2021) reported that the main active ingredients produced by the lemongrass plant are citronellal, geraniol, and citronellol essential oils. Zulfadhli et al., 2017 also reported that other bioactive compounds such as flavonoids, phenols, tannins, saponins, alkaloids, and steroids that support antioxidant activity are also contained in the lemongrass plant.

Based on this, functional food products were developed in the form of making herbal drinks with a combination of palm sugar and lemongrass stalks. The variables observed were the best product were analyzed for their physicochemical such as antioxidant activity, $IC_{50,}$ and the characteristics of the herbal drinks product included organoleptic, color using L*, a* and b*, also Total Dissolved Solids (^{O}brix).

MATERIALS AND METHODS

Material and equipment

The materials used in this study were palm sugar obtained from Palmira Indonesia, dried lemongrass stalks with size (2cm x 2cm) obtained at Badung Market, Aquades (Rofa, Indonesia), 2,2-diphenyl-1- pikrilhidrazil (DPPH) (Sigma Aldrich, USA) and Methanol Pro Analysis (Merck, Germany).

Equipment used in this study included a beaker glass (Pyrex, USA), cylinder (Pyrex, USA), oven (Cole Parmer, USA), analytical balance (Shimadzu, Japan), knife, blender (Phillips, Indonesia), Vortex (Barnsteadl Thermolyne Type 37600 Mixer, USA), UV-Vis spectrophotometer (Biochromsn 133467, UK), micropipette (Dragon Lab, Indonesia) and test tubes (Pyrex, USA).

Sample preparation

Sample preparation in this study was to ensure the availability of the materials used, especially palm

sugar obtained from Palmira Indonesia, and dried lemongrass stalks measuring 2cm x 2 cm. The process of making lemongrass- palm sugar herbal drink is done by adding lemongrass stalks that have been prepared according to the percentage (0%; 5%; 10%; 15%; 20%; 25%) in 200 grams of palm sugar. This study used a completely randomized design (CRD) with the percentage addition of lemongrass stalks. The making of herbal drinks is done by brewing the lemongrass-palm sugar herbal drink which has been formulated according to the treatment in boiling water for 5 minutes with a ratio of 1:15 (w/v). (Ardianta, et al., 2019) with modification.

Sensory properties

Research parameters are sensory properties (Soekarto, 1985) which include hedonic tests on the attributes of taste, color, and overall acceptance as well as scoring tests on taste and color attributes on 25 semi-trained panelists. For Hedonic test criteria: 1 = dislike very much, 2 = dislike, 3 = slightly dislike, 4 = neither like nor dislike, 5 = slightly like, 6 = like, 7 = very much like. For the Scoring test of taste criteria: 1 = Brown sugar, 2 = Brown sugar a little lemongrass, 3 = Brown sugar, and lemongrass are balanced, 4 = Brown sugar is very lemongrass and 5 = The taste of lemongrass is very strong and also for scoring test of color criteria: 1 = Brownish red, 2 = Red, 3 = Yellowish red, 4 = Reddish yellow and 5 = Yellow. Samples are presented in plastic cups in random order with specific sample codes, which is controlled to ensure that the panelists do not see all the samples. The sample presented is a sample of lemongrass-sugar palm herbal drink which has been brewed in boiling water for 5 minutes with a ratio of 1:15 (w/v).

Antioxidant activity

The best treatment according to the sensory properties test was continued by testing the Antioxidant Activity and IC_{50} (Shah and Modi, 2015). A total of 1 ml of 0.1 mM DPPH solution in methanol was dissolved with 2 ml of samples of the lemongrass-palm sugar herbal drink in a test tube. Samples were made by weighing 2 g of sample dissolved in 10 ml of methanol. The solution was vortexed and incubated for 30 minutes in the dark at room temperature. The absorbance was read at a wavelength of 517 nm using a spectrophotometer. The blanko used was methanol. The control was made according to the treatment given in the sample testing process but without adding a sample. The percentage of ability to ward off free radicals (antioxidant activity) is calculated by the formula:

$$\text{Antioxidant Activiy (\%)} = \frac{\text{Control Absorbance} - \text{Sampel Absorbance}}{\text{Control Absorbance}} \times 100\%$$

After testing the antioxidant activity, IC_{50} was tested. IC_{50} is the sample concentration required to inhibit 50% of DPPH free radicals. The sample used was a lemongrass-sugar palm drink with the best treatment. The sample concentration was varied from 0, 250, 500, 750, and 1000 mg/ml, then the antioxidant activity was measured. IC_{50} value can be obtained by linear regression equation (Pourmorad, et al., 2006)

RESULTS AND DISCUSSION

Taste attribute

The results showed that the percentage treatment of the lemongrass stalks addition had a very significant effect ($P<0.01$) on the taste scoring test of the lemongrass-palm sugar herbal drink and a significant effect ($P<0.05$) on the hedonic test of the taste of the palm-sugar lemongrass herbal drink. The average scoring and hedonic test scores for the lemongrass-sugar palm drink are shown in Table 1.

Table 1 shows the highest hedonic test value on the taste attributes of the lemongrass-sugar palm drink was obtained in addition to 10% lemongrass stalks and addition of 20% lemongrass stalks which is 6.05 (liked). The average value was not statistically different from the addition of 5% lemongrass stalks was 5.59 (liked), the addition of 15% lemongrass stalks was 6.00 (liked), and the addition of 25% lemongrass stalks was 5.59 (liked). The average value of the lowest hedonic test on the taste attributes of the lemongrass-palm sugar herbal drink was obtained in without the addition of

lemongrass stalks which was 5.00 (slightly liked). This can be interpreted that the panelists liking the taste of the sugar palm herbal drink with the addition of lemongrass stalks compared to those without the addition of lemongrass stalks. This is because lemongrass stalks have volatile oil compounds that can function as aroma compounds and affect the taste of the herbal drinks product. This is supported by the statement of Togatorop et al. (2015) who reported that the addition of lemongrass affects the hedonic value of the beverage's aroma because lemongrass has a strong aroma. This is supported by a statement from Evama et al., (2021) who reported that the main components contained in lemongrass stems were essential oils, such as citronellal, geraniol, and citronellol. The main component of lemongrass essential oil can affect the aroma of the product because it has a distinctive aroma (Satuhu and Yuliani, 2012). Besides the addition of lemongrass, several components contained in palm sugar also influence the taste of the resulting lemongrass-palm sugar herbal drink. Le et al., (2021) reported that a total of 38 volatile compounds were identified in palm sugar and the compound S-(R'R')-2,3-butanediol was thought to contribute to the unique taste of palm sugar.

Table 1. The Average Value of the Hedonic and Scoring Test of the Lemongrass-Palm Sugar Herbal Drink Taste Attributes

Treatments	Hedonic Taste	Taste Score
PO (Without Addition of Lemongrass Stalks (0%))	5,00±1,60b	1,00±0,00e
P1 (Addition of 5% Lemongrass Stalks)	5,59±1,26ab	2,23±0,69d
P2 (Addition of 10% Lemongrass Stalks)	6,05±0,90a	2,64±0,66cd
P3 (Addition of 15% Lemongrass Stalks)	6,00±1,20a	2,82±0,91c
P4 (Addition of 20% Lemongrass Stalks)	6,05±0,95a	3,41±0,91b
P5 (Addition of 25% Lemongrass Stalks)	5,59±1,50ab	4,00±1,02a

Note: Different letters behind the mean value indicate a very significant difference (P<0.01). Hedonic test criteria: 1 = dislike very much, 2 = dislike, 3 = slightly dislike, 4 = neither like nor dislike, 5 = slightly like, 6 = like, 7 = very much like; Scoring test criteria: 1 = Brown sugar, 2 = Brown sugar a little lemongrass, 3 = Brown sugar and lemongrass are balanced, 4 = Brown sugar is very lemongrass and 5 = The taste of lemongrass is very strong.

The results of the scoring test on the taste attributes of the lemongrass-sugar palm herbal drink shown in Table 1 indicate that the panelists can distinguish the taste of herbal drink products that are added with lemongrass stalks. The taste was more bitter when more lemongrass was added. The highest average value was obtained with the addition of 25% lemongrass stalks which was 4.00 (very lemongrass brown sugar) and the lowest average value was obtained in without the addition of lemongrass stalks which was 1.00 (brown sugar). Taste is the process of selecting food products from the cooperation of the five kinds of human senses which include taste, smell, touch, sight, and hearing which can be distinguished from taste or several attributes of food products which include appearance, aroma, taste, texture, and temperature. The higher lemongrass stalks are added, the lemongrass flavor will be stronger in the herbal drink product. This is because lemongrass stalks contain volatile oil compounds. (Evama, et al., 2021) reported that citronellal, geraniol, and citronellol essential oils are the main components of lemongrass. In addition, Satuhu and Yuliani (2012) also reported that the main components of lemongrass essential oil such as citronellal, geraniol, and citronellol act as aroma compounds so that lemongrass has a distinctive aroma.

Color attribute

The results showed that the percentage treatment of the lemongrass stalks addition had no significant effect (P>0.05) on the scoring test and the hedonic test for the color of the lemongrass palm sugar herbal drink. The average value of the scoring and hedonic color tests for the lemongrass-sugar palm herbal drink is shown in Table 2.

Table 2 showed that the panelist's assessment of the hedonic test of the lemongrass sugar palm drink color attribute ranged from 5.27-5.68 with the criteria of slightly liked to like. The average value of the hedonic test on the color attribute of the lemongrass-palm sugar herbal drink was highest in the addition of 20% lemon grass stalks which was 5.68 (liked) while the lowest average was found in the addition of 5% lemongrass stalks which was 5.27 (slightly liked). The addition of lemongrass stalks did not affect the hedonic color test of the

resulting palm sugar lemongrass herbal drink and that indicates the panelist can accept the color of the product.

Table 2. The Average Value of the Hedonic and Scoring Test of the Lemongrass-Palm Sugar Herbal Drink Color Attributes

Treatments	Hedonic Color	Color Score
PO (Without Addition of Lemongrass Stalks (0%)	5,41±1,10	1,55±0,91
P1 (Addition of 5% Lemongrass Stalks)	5,27±1,12	1,55±0,67
P2 (Addition of 10% Lemongrass Stalks)	5,59±0,91	1,77±0,87
P3 (Addition of 15% Lemongrass Stalks)	5,41±1,18	1,95±0,90
P4 (Addition of 20% Lemongrass Stalks)	5,68±0,99	2,14±1,21
P5 (Addition of 25% Lemongrass Stalks)	5,36±1,14	2,27±1,16

Note: Hedonic test criteria: 1 = dislike very much, 2 = dislike, 3 = slightly dislike, 4 = neither like nor dislike, 5 = slightly like, 6 = like, 7 = very much like; Scoring test criteria: 1 = Brownish red, 2 = Red, 3 = Yellowish red, 4 = Reddish yellow and 5 = Yellow.

The results of the color attributes scoring test of the lemongrass-palm sugar herbal drink as presented in Table 2 show that the panelist's assessments ranged from 1.55-2.27 with the Red criteria. The highest average value in the assessment of the color attribute scoring test was obtained in addition of 25% lemongrass stalks which was 2.27 (red) and the lowest average value was obtained in the sample without the addition of lemongrass stalks and also the addition of 5% lemongrass stalks which is 1.55 (red). The addition of lemongrass stalks at a certain percentage causes an increase in the average value of the color attribute scoring test of the resulting lemongrass-sugar palm drink that became darker. The color produced by the lemongrass herbal drink in each treatment is included in the red color criteria. This is because the color of palm sugar dominates more than the color given by lemongrass. Maillard reaction is one of the factors that affected the formation of color in sugar. This is because glucose and fructose with amino groups play an important role in the formation of color in sugar. In addition, the addition of lemongrass stalks has not been able to affect the color. This could be because the percentage of lemongrass added was not optimal to change the color of the herbal drink produced. According to (Omarta et al., 2020) lemongrass in the form of an extract has a characteristic pale yellow color.

Overall acceptances

The results showed that the percentage treatment of the lemongrass stalks addition had no significant effect (P>0.05) on the hedonic test of the lemongrass-palm sugar herbal drink overall acceptance attribute. The average value of the hedonic test on the overall acceptance attribute of the lemongrass-sugar palm drink is shown in Table 3.

Table 3. The Average Aalue of the Hedonic Test of the Lemongrass-Palm Sugar Herbal Drink's Overall Acceptance

Treatments	Overall Acceptance
PO (Without Addition of Lemongrass Stalks (0%))	5,14±1,67
P1 (Addition of 5% Lemongrass Stalks)	5,55±1,26
P2 (Addition of 10% Lemongrass Stalks)	6,05±1,00
P3 (Addition of 15% Lemongrass Stalks)	5,91±1,11
P4 (Addition of 20% Lemongrass Stalks)	5,95±0,95
P5 (Addition of 25% Lemongrass Stalks)	5,68±1,36

Note: Hedonic test criteria: 1 = dislike very much, 2 = dislike, 3 = slightly dislike, 4 = neither like nor dislike, 5 = slightly like, 6 = like, 7 = very much like

Table 3 showed that the panelist's assessment of the hedonic test of the lemongrass-sugar palm drink's overall acceptance attribute ranged from 5.14 to 6.05 with the criteria of slightly liked to like. The average value of the hedonic test on the overall acceptance attribute of the lemongrass-sugar palm drink was highest in addition to 10% lemon grass stalks which was 6.05 (liked). The lowest value on the overall acceptance attribute of lemongrass herbal drink - palm sugar was found in the sample without the addition of lemongrass stalks which was 5.14 (slightly liked). The assessment of the overall acceptance attribute of the panelists was influenced by several factors such as the color and taste of the lemongrass-palm sugar herbal drink.

Physicochemical characteristics of herbal drinks with the best treatment

The physicochemical characteristics of the palm sugar herbal drink with the best treatment were tested on color criteria with L*, a*, and b* using a colorimeter application, total dissolved solids (^{o}Brix) using a refractometer, antioxidant activity, and IC_{50}. The addition of 10% lemongrass stalks was selected as the best treatment based on the best sensory characteristics that had been tested on semi-trained panelists. The results of the physicochemical characteristics test which include antioxidant activity, total dissolved solids (^{o}Brix), IC_{50}, and color criteria which include L*, a*, and b* lemongrass- palm sugar herbal drink shown in Table 4.

Table 4. The Average Value of the Lemongrass-Palm Sugar Herbal Drink Physicochemical Test

Treatment	Antioxidant Activity (%)	IC_{50} (ppm)	Total Dissolved Solids (^{O}brix)	L*	a*	b*
P2 (Addition of 10% Lemongrass Stalks)	69,64±2,81	694,50±48,36	0,52±0,08	26,9±7,03	23,16±2,96	37,1±8,04

Table 4 shows the average value of the physicochemical test of the lemongrass palm sugar herbal drink in the best treatment was a 10% addition of lemongrass stalks. Sample testing with the best treatment was carried out with 5 replications. The physicochemical characteristics obtained antioxidant activity of 69.64% with an IC_{50} value of 694.50 ppm (very weak), a total dissolved solids (TSS) value of 0.52 ^{o}Brix with color criteria L* 26.9, a* 23.16 and b* 37.1 which has the color criteria of red oxide.

The average value of lemongrass- palm sugar herbal drink antioxidant activity was influenced by the bioactive compounds that function as antioxidants contained in each ingredient. This is supported by the statement of Silou et al., (2017) who reported that lemongrass stalks contain essential oils such as citronellal (40-48%), geraniol (10-22%), citronellol (10-12%), limonene (2-3%), geranyl acetate (1-2%), linalool (1%) which can function as antioxidants. In addition, (Najmah et al., 2021) also reported that the n-hexane fraction of lemongrass contains ar-turmerone as an antioxidant, as well as beesioside N and notohamosin A that can function as antidiabetic. In addition, the palm sugar used also contributes several compounds as antioxidants. This is supported by the statement from Le et al., (2021) who reported that palm sugar contains total phenolics ranging from 1.78-5.15 mg GAE/g and contains several vitamins such as vitamins B1, B2, B3, B5, B6, vitamin C, vitamin D, folic acid and vitamin E which can function as antioxidants.

The IC_{50} value obtained from the lemongrass-palm sugar herbal drink with the best treatment was 694.50 ppm. The criteria for the IC_{50} value are very weak. This is because, in the production process of lemongrass-palm sugar herbal drinks, the ingredients go through several processes such as heat treatment. The presence of bioactive compounds that functions as an antioxidant which is indicated by the identification of the percentage of antioxidant activity and the IC_{50} of the product indicates that the herbal drink produced from the combination of lemongrass stalks and palm sugar can be categorized to be an herbal drink.

CONCLUSION

The conclusion of this study is the additional percentage of lemon grass stalks has a very significant effect on the scoring test of the taste attribute, significantly affects the hedonic test of the taste attribute, and has no significant effect on the hedonic test of the color attribute, overall acceptance and the color attribute scoring test of the lemongrass-sugar herbal drink. The best treatment that can be used to make a lemongrass-sugar palm herbal drink is a 10% addition of

lemongrass stalks. The panelist's acceptance of the treatment was hedonic taste attribute (liked) with taste scoring criteria (balanced brown sugar and lemongrass), hedonic color attribute (liked) with color scoring criteria (red), and overall acceptance (liked). This treatment was also able to produce an antioxidant activity value of 69.64% with an IC_{50} value of 694.50 ppm, a total dissolved solid of 0.52 ^{o}Brix, and a color characteristic of L* 26.9, a* 23.16 and b* 37.1 with red oxide criteria.

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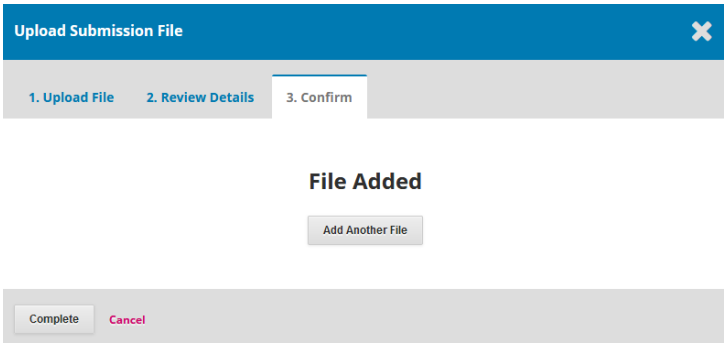

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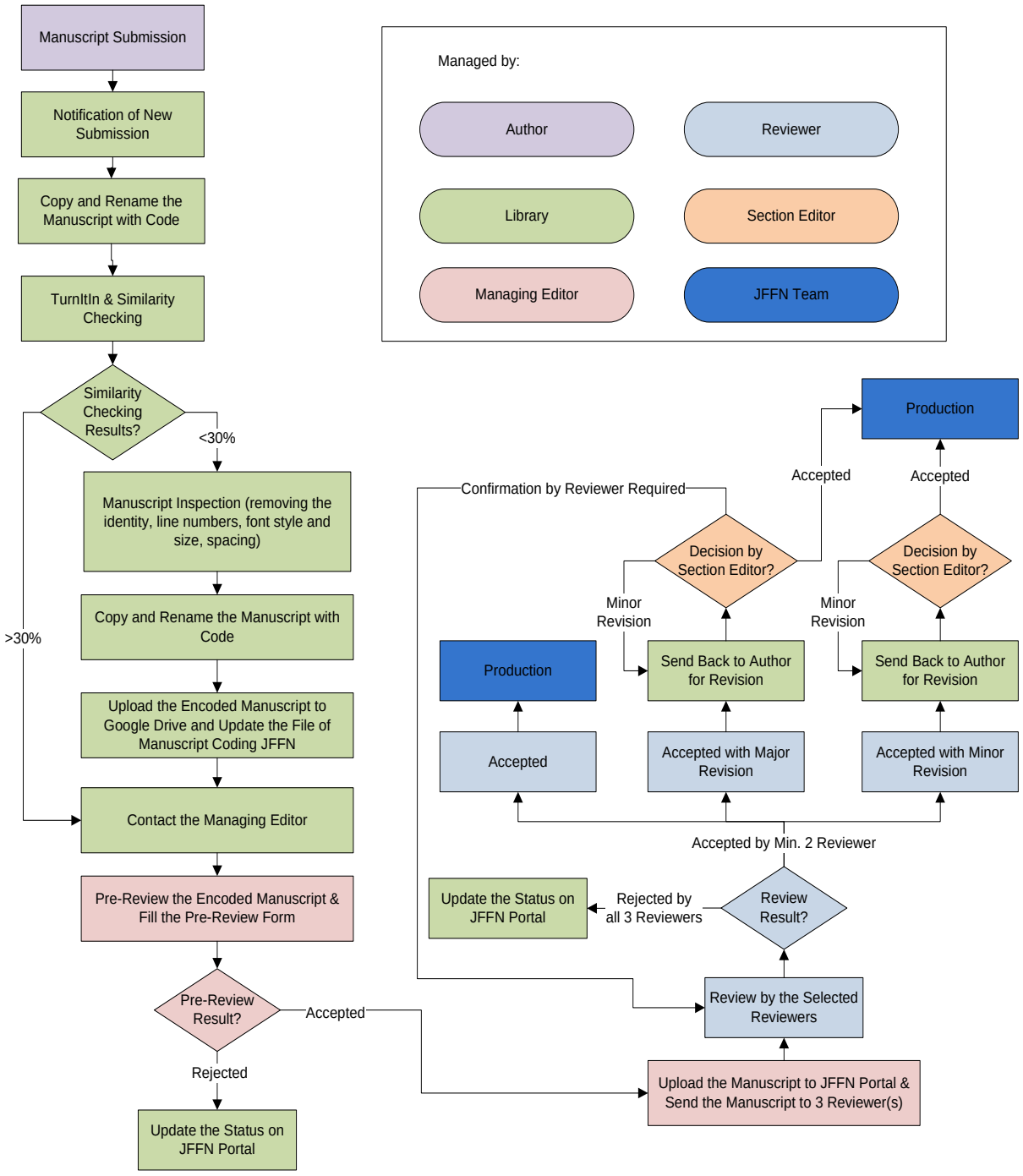

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Peer-review is an important step to maintain the high quality of a journal. Reviewers provide scientific critiques based on their expertise that assist editors to make acceptance decision professionally. Therefore, the Editors would like to acknowledge our reviewers listed below who have contributed their valuable time for maintaining the quality of Journal of Functional Food and Nutraceutical.

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