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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

A REVIEW ON THE HEALTH BENEFITS OF KALAKAI *(STENOCHLAENA PALUSTRIS)*

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ABSTRACT

Kalakai (*Stenochlaena palustris*) is a common plant in Kalimantan and Sumatra which has been consumed locally as a vegetable. Information about the benefits of kalakai as a functional food has spread widely in the community. However, scientific information and research investigating the potency of kalakai are scarce, and the scientific literature is dominated by local Indonesian-language journals. This review aims to explore and compile the potential health benefits of kalakai based on its nutritional and bioactive content. Kalakai is reported to have various health benefits such as high fibre, antianemia, antioxidant, antidiabetic and antimicrobial activity. Referring to the current status of research, the processing of kalakai as a food functional or nutraceutical ingredient for antioxidative function and controlling blood sugar are the most potential. However, clinical and safety trials still need to be carried out as part of the preparation for the use of kalakai as a functional and nutraceutical food ingredient in the near future.

Keywords: bioactive compounds; functional food; health benefits; kalakai; stenochlaena palustris

ABSTRAK

Kalakai (*Stenochlaena palustris*) merupakan tanaman yang umum di Kalimantan dan Sumatera yang banyak dikonsumsi secara lokal sebagai sayuran. Informasi tentang manfaat kalakai sebagai pangan fungsional telah tersebar luas di masyarakat. Namun, informasi ilmiah dan penelitian yang menyelidiki potensi kalakai masih langka, dan literatur ilmiah didominasi oleh jurnal lokal berbahasa Indonesia. Tinjauan ini bertujuan untuk mengeksplorasi dan menyusun potensi manfaat kesehatan dari kalakai berdasarkan kandungan nutrisi dan bioaktifnya. Kalakai dilaporkan memiliki berbagai manfaat kesehatan seperti serat tinggi, antianemia, antioksidan, antidiabetes dan aktivitas antimikroba. Mengacu pada status penelitian saat ini, pengolahan kalakai sebagai bahan pangan fungsional atau nutrasetikal dengan fungsi antioksidan dan pengendalian gula darah adalah yang paling potensial. Namun, uji klinis dan keamanan masih perlu dilakukan sebagai bagian dari persiapan penggunaan kalakai sebagai bahan pangan fungsional dan nutraceutical dalam waktu dekat.

Kata kunci: kalakai; kandungan bioaktif; manfaat kesehatan; pangan fungsional; stenochlaena palustris

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INTRODUCTION

Indonesia has the potential for biological wide-range plant biodiversity that has not been explored and utilized optimally. Based on the local wisdom approach, many traditional tribes have utilised plants and herbs to produce foods and medicine which have a positive effect on health (Falah and Hadiwibowo, 2017). One of the plants that have great potential as a functional food is Kalakai (*Stenochlaena palustris* (Burm.f.) Bedd.). Kalakai is also known locally by other names such as *lemidi*, *lemiding*, *ramiding*, and *paku hurang*, while in Bangka Belitung, this plant is known as *pucuk iding-iding* and *paku miding* (Roanisca et al., 2017). Kalakai or *kelakai* (Kalimantan Tengah/Selatan), *miding*, *lemiding* (Kalimantan Barat), *paku bang* (Jawa), *majamajang*, *bampesu*, *wewesu* (Sulawesi), *lemidi* (Sumatera) and *paku nyai* (Malaysia) (Wahab et al., 2015).

Kalakai *(Stenochlaena palustris)* is a common vegetable found in Kalimantan. Kalakai has been used by local residents of Kalimantan, for example, the Dayak tribe to be consumed as vegetables. This wild-growing vegetable is usually easily found in lush areas or forests, especially in Central and South Kalimantan. Kalakai can thrive on peat land due to the high-water intensity, so kalakai can regrow optimally even though it has been harvested several times as long as the water supply is still available in the soil (Shinta and Atyk, 2011). Besides being used as a source of food, the local people of Kalimantan have used kalakai for health and medicinal purposes for example to cure anemia, stimulate breastfeeding milk production for postpartum mother , antipyretic, skin infection and as a diarrhea medicine (Yulianthima, 2017; Mawaddah, 2019).

Currently, functional food and nutraceuticals market are experiencing growth. The health and wellness food market value is experiencing an increasing trend globally from 733 billion U.S. dollars in 2020 increased significantly to 1,000 billion U.S. dollars in 2026 also for the value of the global superfoods market of 137 billion U.S. dollars in 2020 increased significantly to 209 billion U.S. dollars in 2026 (Shahbandeh, 2021a; Shahbandeh, 2021b). This shows that there is an increasing public need for the importance of food products that have a positive effect on health. With its potential benefit to health and if optimally developed into a functional food and nutraceuticals product, kalakai has the potential to be accepted by the market in the future.

Information about the benefits of kalakai as a functional food has spread widely in the community. However, scientific information and research investigating the potency of kalakai are scarce, and the scientific literature are dominated by local Indonesian-language journals. Furthermore, not many scientific papers have reviewed the potential of kalakai for human health. This review aims to explore and compile the potential health benefits of kalakai based on the nutritional and bioactive content, therefore the potential applications to be a further functional food would be noticeable. With this review, hopefully, more people will be scientifically aware to utilise kalakai as food, medicine or a new food ingredient.

GENERAL INFORMATION ABOUT KALAKAI

Geography

Stenochlaena palustris, or more commonly known as kalakai originates from Palangkaraya area in Central Kalimantan (Indrayanti et al., 2016). This plant can grow almost everywhere, even in less fertile growth mediums. Because of this, it is very easily grown and has the potential to be planted anywhere. It is most commonly grown in nutritionally dense peats, but can also be grown in less fertile soil, such as quartz sand and alluvial soils (Thursina et al., 2010). It grows in tropical, subtropical, or Monsal climates (Royal Botanic Gardens Kew, 2022). Kalakai can be also found in several countries around Indonesia such as in Australia, Malaysia, Thailand, Papua New Guinea, and even India and China as presented in Figure 1.

Taxonomy & morphology

Kalakai is a plant of the *Stenochlaena* genus in Blechnaceae family and Pteridophyta Division. The complete taxonomic profile of kalakai are as follows:

Kingdom	: Plantae
Class	: Filicopsida
Order	: Filicales
Tribe	: Blechnaceae
Genus	: *Stenochlaena*
Species	: *Stenochlaena palutris*

Figure 1. Geographic Location of Kalakai Plants (Royal Botanic Gardens Kew, 2022)

Other scientific names of *Stenochlaena palustris* (Royal Botanic Gardens Kew, 2022):

- *Lomaria haenkeana* C.Presl
- *Lomaria juglandifolia* C.Presl
- *Lomaria scandens* Willd.
- *Onoclea scandens* Sw.
- *Stenochlaena blumeana* C.Presl
- *Stenochlaena fraxinifolia* C.Presl
- *Stenochlaena hainanensis* Ching & P.S.Chiu
- *Stenochlaena juglandifolia* C.Presl
- *Stenochlaena laurifolia* C.Presl
- *Lomariopsis scandens* Mett.
- *Olfersia scandens* C.Presl
- *Polypodium palustre* Burm.f.
- *Acrostichum laurifolium* Hook.
- *Acrostichum palustre* (Burm.f.) C.B.Clarke
- *Acrostichum scandens* Hook.
- *Chrysodium palustre* (Burm.f.) Luerss.
- *Pteris scandens* Roxb.

Kalakai (*Stenochlaena palustris*) is one of the pteridophyte plants that grow well during the rainy season. The plant height is approximately 50 cm while the length of its leaves is ranged from 7.5–10.2 cm. Through visual observation, there are two types of kalakai, for instance red kalakai and green kalakai, as shown in Figure 2. The differences in colour indicate the level of leaf ripeness. Red indicates that the leaves are still young, while green indicates that they are old or mature. Kalakai develops vegetatively with fairly high ability. There is a difference in growth rate between the dry season and the rainy season. In the dry season, the growth rate of kalakai is slower than in the rainy season. This is due to differences in the ability to produce biomass and the limited amount of water that can be utilized. This plant has a relatively short harvest period (4-6 days) meaning that within that period it can be harvested again and grows well in areas that have high humidity such as peatlands (Shinta and Atyk, 2011).

Figure 2. Kalakai Plant (*Stenochlaena palustris*) Has Both Red and Green Leaves (Hidayah, 2020)

General use

Kalakai can be easily obtained by the public without having to buy it in certain places such as market or shop. This plant is widely found in the neighbourhood where the Dayak people live. Even though it is highly nutritious, the use of kalakai is still very limited and basic, due to the lack of innovation in processing. Their main use is primarily just in dishes and traditional medicine. Kalakai is usually cooked and often eaten with rice. The entire plant is edible, but the leaves and stalks are the most common parts to be cooked, as shown in Figure 3. It is considered a very common ingredient in the cuisine of the people in Central Kalimantan. In addition, Kalakai leaves can also be used to make herbal drinks. To compensate for the lack of scent, ginger is usually added, due to its strong, powerful scent, and therapeutic benefits (Juliani et al., 2019). Specifically, the kalakai used by the Dayak tribe to treat anemia has not been studied, but it provides real empirical evidence (ethnobotany) (Yulianthima, 2017). In Dr. Duke's Phytochemical and Ethnobotanical Databases, *Stenochlaena palustris* has a function for the treatment of aperient and fever treatment (USDA, 2022).

Figure 3. Sayur Kalakai (Example of a Dish Using Kalakai)

Table 1. Energy and Macronutrient Composition of Kalakai per 100 g

Type of vegetables	Energy (kkal)	Water (g)	Carbohydrate (g)	Protein (g)	Total Fat (g)	Fibre (g)	References
Kalakai leaves	38	89.9	6.6	2.4	0.2	5.8	(Kementerian Kesehatan RI, 2018)
	-	91.6 ± 1.74	4.23 ± 0.1	0.24	0.3		(Wahab et al., 2015)
Kalakai leaves	-	9	-	-	2.6	2	(Maharani and Haidah, 2006)
	-	10.2	-	8.3	0.7	24	(Jaelani et al., 2019)
Kalakai stem	-	7	-	-	1.4	3.4	(Maharani and Haidah, 2006)
Kalakai flour	329	7.87	68.3	8.3	2.5	-	(Fahriza et al., 2021)

NUTRITION COMPOSITION OF KALAKAI

Table 1 presented the list of Energy and macronutrient composition of kalakai per 100 g. In general, kalakai has a high-water content, just like some common leafy vegetables. Flour kalakai tends to have a higher nutritional value because the water content has been removed. When compared to fresh spinach, kalakai has 2x higher in energy value, 2.5x higher in protein content and 8x higher in fibre (Kementerian Kesehatan RI, 2018). The fibre content in kalakai is considered the highest when compared to common leafy vegetables such as kale, cassava leaves and mustard greens. Kalakai stem has less fibre compared to kalakai leaves.

Vitamin C

Some people think that kalakai has a high level of vitamin C. Vitamin C or ascorbic acid is one of the vitamins that human beings need. It is a coenzyme. It is commonly used for collagen formation which hydroxylation proline and lysine into hydroxyproline. Vitamin C is also an antioxidant and an electron donor for the human body that had a moderate effect on reducing the severity and duration of cold symptoms (Padayatty and Levine, 2016). It can be seen in Table 2. that kalakai contains varying levels of vitamin C 8-47 mg per 100 g. Kalakai may have higher levels of Vitamin C than carrots, but less than spinach and sweet orange. The variation of nutrient contents including

vitamin C is more likely to happen due to variation in seasonal and temperature variability and precipitation variability (Phillips et al., 2018; Giulia et al., 2020).

Table 2. Vitamin C Content of Orange and Kalakai per 100 g

Vegetables	Vitamin C (mg)	References
Kalakai leaves	8	(Kementerian Kesehatan RI, 2018)
	33 ± 13.7	(Wahab et al., 2015)
Carrot	18	(Kementerian Kesehatan RI, 2018)
Spinach	41	(Kementerian Kesehatan RI, 2018)
Sweet orange	49	(Kementerian Kesehatan RI, 2018)

Iron (Fe)

Fe, known as iron, is an important element to form hemoglobin (Hb) and its function as a transporter, storage, and oxygen utilization. It aids in the formation of haemoglobin, myoglobin, and cytochrome (Qamariah and Yanti, 2018). As shown in Table 3, from several studies, the iron content of kalakai per 100 g varies greatly from 1 to 291 g. The difference in iron content might be explained due to the different methods of iron analysis in each study. Nevertheless, the content of fresh and red kalakai leaves tends to be higher when compared to fresh spinach.

Dietary iron comes in two forms, non-heme iron and heme iron, with heme iron being greater efficiently absorbed in the gut. The greater efficiency of absorption because of specific heme transporters which enable heme iron to pass directly across cell membranes and into the bloodstream, while non-heme iron's is unable to use these transporters, reduction of ferric iron to ferrous iron must take place before the absorption (Young et al., 2018). The bioavailability of heme iron is considered as high as the absorption percentage is 25-30% (Harvey et al., 2005) while the absorption of non-heme iron tends to vary between 1-10% (Beck et al., 2014; Asakura et al., 2017). A study reported that sources of iron derived from plant-based sources of non-heme iron, which tend to contain certain iron inhibitors such as phytic acid, calcium, and polyphenols, therefore the amount and level of bioavailability in foods are important to prevent or treat anemia (Skolmowska and Głąbska, 2019). The aforementioned study also suggested that people, whose diet is dominated by plant-based foods, to increase their vitamin C and other organic acids intake to improve the iron bioavailability from their meals.

Table 3. Comparison of Iron Content in Milk and Other Foods per 100g

	Iron (Fe) (mg)	References
Kalakai flour	39.43	(Fahriza et al., 2021)
Kalakai leaves	291,32	(Maharani and Haidah, 2006)
	1.1	(Kementerian Kesehatan RI, 2018)
Kalakai stem	221	(Maharani and Haidah, 2006)
Kalakai leaves	33.64	(Thursina et al., 2010)
	0.08	(Wahab et al., 2015)
Red Kalakai	4.153	(Irawan et al., 2006)
Spinach	3.5	(Kementerian Kesehatan RI, 2018)

Calcium (Ca)

The comparison of Calcium content per 100 g of foods is presented in Table 4. Most of the studies reported that the calcium in kalakai tends to be lower than in cow milk and spinach. On the other hand, only a study conducted by Maharani and Haidah, (2006) found the opposite finding. Another study reported that a high intake of calcium from plant-based foods can reduce the risk of osteoporosis and increase bone mineral density in postmenopausal Korean women therefore plant-based foods such as vegetables can be an important source of calcium and provide vitamins and minerals that have beneficial effects on bone (Park et al., 2011).

HEALTH BENEFITS

Anemia prevention

Not only for cooking, but kalakai are also often used as traditional medicinal plants that are beneficial, such as antiaging, and help increase Iron content. Many people believe that these leaves can help to increase Hb blood levels. This is mainly due to the high Iron (Fe) content that is known to be contained in kalakai. From table 5, several studies found that the administration of kalakai-based food to females with anemia can raise the Hb blood level. Consumption of kalakai will contribute to supplying non-heme iron content that will be absorbed by the body and increase blood levels, as indicated by an increasing amount of Haemoglobin levels (Petricka et al., 2018; Aden, 2019). They are also suitable for lactating mothers (Hadi, 2017; Fahruni et al., 2018; Mahdiyah, 2020) as iron content can affect breast milk production (Rahmadiliyani and Audita, 2017). It was also reported that anemia is very common for new mothers, as there is a correlation between breastfeeding and a decrease in iron content (Lakew et al., 2015). Therefore, it is due to these benefits that kalakai is often consumed by new mothers. However further research, such as the study with larger samples, is needed to confirm the validity of previous findings regarding the effects of kalakai consumption on blood Hb level. Additionally, the bioavailability of kalakai in the human digestive system through a specific absorption mechanism is also required.

Table 4. Comparison of Calcium Content in Milk and Other Foods per 100g

	Calcium (mg)	References
Kalakai leaves	18	(Kementerian Kesehatan RI, 2018)
Kalakai leaves	182	(Maharani and Haidah, 2006)
Kalakai leaves	69.7	(Thursina et al., 2010)
Kalakai leaves	6.76	(Wahab et al., 2015)
Kalakai stem	169	(Maharani and Haidah, 2006)
Cow Milk	143	(Kementerian Kesehatan RI, 2018)
Spinach	166	(Kementerian Kesehatan RI, 2018)

Having enough Iron is, in general, more important for females than males. This is especially important for menstruating females. It is recommended that adolescent (14-18 years old) females consume 15 mg/day, while males consume 11 mg/day. The difference is more significant in adults (>19 years old), as it is recommended that females consume 18 mg/day, while males consume 8 mg/day. In general, Iron consumption is also beneficial for health. Iron is used to produce myoglobin, which functions as an oxygen supplier for muscle tissues. Some hormones also require Iron, as they are beneficial in hormone production (Abbaspour et al., 2014).

Table 5. Several Research on the Effect of Kalakai Consumption to Hemoglobin Level

Research method		Results	References
Study design	Quasi Experiment	After administration, the Hb levels of female students who experienced anemia increased by 6.859 gr/dl.	(Mawaddah, 2019)
Sample	10 female students with anemia		
Variable	After administration of Kalakai syrup, Hb levels increased		
Instrument	Hemoglobin Testing System Quick Check		
Statistical	Paired T – Test		
Study design	Quasi Experiment	The result showed of significant increases on Hb levels (3.24 g/dl) after consuming Kelakai (*Stenochlaena palustris*) for a week ($p \leq 0.05$)	(Petricka et al., 2018)
Sample	66 anemic Midwifery students (8-11 g/dl)		

Variable	Kelakai (*Stenochlaena palustris*) (250 mg) and ferrous fumarate tablet (60 mg) were administrated daily for a week.		
Instrument	Hb testing system quick-check tool		
Statistical	Paired and independent t-test.		
Study design	Quasi Experiment	The effect of the combination of iron supplements (Fe tablets) and vegetable kalakai (*Stenochlaena palustris*) on the average Hb levels of adolescent girls after the intervention of a combination of Fe tablets and vegetable kalakai there increased by 1.106 gr/dl.	(Sibala, 2018)
Sample	34 females who are already menstruating		
Variable	The effect of the combination of iron and kalakai on Hb levels		
Instrument	Hemoglobin Digital (Hb meter)		
Statistical	Paired T – Test		
Study design	Quasi Experiment	Hb levels of pregnant women after consuming vegetable stew of kalakai compared to before consuming boiled vegetables of kalakai increased by a difference of 0.711 gr/dl.	(Aden, 2019)
Sample	29 Pregnant Women who experience a decrease in Hb		
Variable	Local food on Hb levels in pregnant women		
Instrument	Hemoglobin Digital		
Statistical	Paired T – Test		

Antioxidative properties

Kalakai has several bioactive substances that act as strong antioxidants and potential cytotoxic because of the flavonoids, phenols, saponins terpenoids, and content in the extract (Roanisca et al., 2017; Arullappan et al., 2017; Adawiyah and Rizki, 2018; Adawiyah et al., 2020). Figure 4 and 5 showed the basic structure of flavonoid and phenols. It was shown that Kalakai has the ability to become antioxidants and anticancer, which could help in preventing diseases caused by free radicals. These properties are mainly due to the phenolic and flavonoid content in kalakai**.** Table 6 showed total phenolic and flavonoid content, and antioxidative activity measured using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assays. A study showed that kalakai is to able keep the freshness for up to two days after harvesting before it wilts, while a moisture restriction, storing the fronds at a low temperature and covering the cut ends of the ferns with wet cotton then wrapped after harvesting enhances the degenerative process of senescence (Gunawan-Puteri et al., 2021).

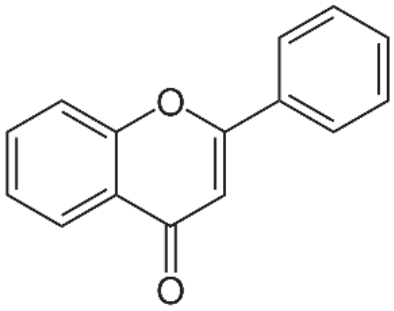

Figure 4. Basic Structure of Flavonoid

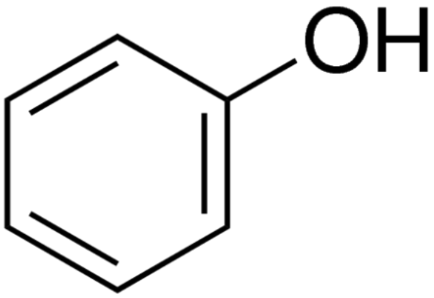

Figure 5. Basic Structure of Phenols

Table 6. Total Phenolic and Flavonoid Content, and Antioxidative Activity Measured Using DPPH Assays

Kalakai Extracts	Extraction	Phenolic Content (mg GAE/g)	Flavonoid Content (µg/mL of QE)	DPPH Assay	References
Leaves	Ethanol	3.80 ± 0.22	2.15 ± 0.005	24.24 ± 0.174 %	(Ndanusa et al., 2020)
Stem	Ethanol	2.30 ± 0.09	1.85 ± 0.030	89.96 ± 0.527 %	(Ndanusa et al., 2020)
Leaves	Ethanol	4.67	1.99	14.13 ppm (IC_{50})	(Kusmardiyani et al., 2016)
Kalakai root on peat soil	Ethanol	-	-	19,06 ppm (IC_{50})	(Adawiyah and Rizki, 2018)
Kalakai root on peat soil	Ethanol	-	-	24,40 ppm (IC_{50})	(Adawiyah and Rizki, 2018)
Leaves	Maceration.	14.5 ± 0.7	-	-	(Suhartono et al., 2012)
	Acetone	-	-	56,981 µg/mL	(Roanisca et al., 2017)
	Hexane	19.7 ± 0.8	12.4 ± 0.6	464.70 ± 20.75 (EC50)	(Chai et al., 2015)
	Chloroform	43.4 ± 2.4	1.7 ± 0.6	218.65 ± 9.57 (EC50)	(Chai et al., 2015)
	Ethyl acetate	133.0 ± 2.0	35.2 ± 1.3	49.68 ± 3.44 (EC50)	(Chai et al., 2015)
	Methanol	503.4 ± 22.8	6.9 ± 0.1	11.65 ± 0.46 (EC50)	(Chai et al., 2015)
	Water	319.5 ± 7.5	13.7 ± 0.1	19.30 ± 0.21 (EC50)	(Chai et al., 2015)
	Chloroform fraction	95.6 ± 0.9	–	134.80 ± 3.70 (EC50)	(Chai et al., 2015)
	Ethyl acetate fraction	415.2 ± 7.2	85.7 ± 5.4	15.03 ± 0.25 (EC50)	(Chai et al., 2015)
	N-butanol fraction	457.0 ± 9.5	58.3 ± 0.9	13.16 ± 0.22 (EC50)	(Chai et al., 2015)
	Water fraction	623.7 ± 5.1	7.5 ± 0.2	7.71 ± 0.11 (EC50)	(Chai et al., 2015)

The potent free radical-scavenging and antioxidant activity are highly dependent on the content and types of flavonoids contained. Flavonoids have been shown to exhibit antioxidant activity that has positive effects on human nutrition and health. The mechanism of flavonoid action is based on the scavenging or chelating process. A study conducted by (Suhartono et al., 2012). The purpose of this study was to determine the antioxidant activity contained in 75 µg/ml of kalakai leaf extract, and it was found that kalakai has a percentage of antioxidant activity of 27.64 ± 3.12, 16.60 ± 7.72, 60.10 ± 9.19 for ferrous ions, hydroxyl radical scavenging activity, hydrogen peroxide scavenging respectively. The aforementioned study suggested that kalakai has moderate antioxidant activity, so it has the potential to protect cells from H_2O_{2-} induced cytotoxicity with a decrease in the generation of Reactive oxygen species (ROS). Therefore, there is a potency of kalakai that exhibited higher cytotoxic effect against HeLa cells, and higher radical

scavenging activity to cure cancer in the future, but it obviously needs further isolation and purification, followed by structural elucidation, therefore, may reveal the potential pure compounds that exert its bioactivity (Arullappan et al., 2017).

Another study by Chai et al., (2015) found that Among the extracts, Methanol extraction had the highest total phenolic and total hydroxycinnamic acid contents. Among the fractions, the water fraction had the highest total phenolic and total hydroxycinnamic acid contents (Table 6). For the principal component isolation, water fraction is the best method to obtain the highest level of total hydroxycinnamic acid content with 154.3 ± 15.4 mg CAE/g. Another research also showed that due to the antioxidative properties, kalakai has anticholinesterase properties, which could aid in preventing cognitive diseases and disorders, such as Alzheimer's disease and dementia (Chear et al., 2016). In addition, a study by Adawiyah & Rizki (2018) found that the ethanolic extract from the roots of kalakai peat soil had a very strong antioxidant activity with high levels of flavonoids IC 50 of 19,06 ppm. Flavonoids as antioxidants have the potential to reduce levels of total cholesterol, triglycerides, low-density lipoprotein (LDL) and increase high-density lipoprotein (HDL). Flavonoids can prevent cell damage due to oxidative stress. Through in vivo testing, it was found that the administration of kalakai root extract with a dose of 400 mg/kg body weight, can reduce cholesterol and triglyceride levels in experimental rats whose results are close to simvastatin, a commonly prescribed medicine of lipid-lowering medication (Adawiyah et al., 2020). This opens up the possible application of kalakai as a functional food for hyperlipidaemia problems. In addition, a study found that phytosterols from kalakai may have a potential anti-breast cancer activity by inhibiting estrogenic receptor α and could be considered to prevent estrogen-dependent malignancies in humans, such as breast cancer (Marisa et al., 2021)

Antidiabetic

The alpha-glucosidase enzyme is known to be one of the main enzymes that break down carbohydrates into simple monosaccharides. It is an essential enzyme, as it helps convert complex carbohydrates into energy in the form of glucose. However, for those who have trouble regulating blood sugar levels, this enzyme activity may be detrimental. An example would be people who suffer from Diabetes, as an uncontrolled blood sugar level may lead to severe diseases (Domenichini and Ferri, 2022).

It is also known that *Stenochlaena palustris* exhibit alpha-glucosidase inhibitory (AGI) activity. AGIs are essential to diabetics, as they can halt the activity of the enzyme. This is done by preventing the breakdown of poly and oligosaccharides into monosaccharides. This delays the production of glucose and therefore delays the increase of blood glucose levels. However, most conventional AGIs are synthetic and may cause adverse side effects (Leng, 2016; Mahadika et al., 2017). Some research found that kalakai extract from young and mature have possessed potent, high natural alpha-glucosidase inhibitory (AGI) activity associated with hyperglycaemia treatment (Chai et al., 2015; Leng, 2016).

Methanol extract had better extraction capabilities when extracting the principal AGI components from *Stenochlaena palustris* (Chai et al., 2015; Gunawan-Puteri et al., 2021). For the alpha glucosidase inhibitors activity of S. palustris, water fraction showed the strongest alpha glucosidase inhibitors activity with EC50 2.92 ± 0.11 µg/ml (Chai et al., 2015) while different result was found by Gunawan-Puteri et al., (2021) that the ethyl acetate fraction had significantly higher alpha glucosidase inhibitory activity compared to the other fractions. Indeed, the smaller particle size of dried kalakai powder was shown to better facilitate the extraction of the principal AGI component. A study suggested that phenolic compounds, particularly hydroxycinnamic acids, and alkaloids may have contributed to the alpha glucosidase inhibitors and antioxidant activities detected in *Stenochlaena palustris* (Chai et al., 2015; Leng, 2016). However, the aforementioned study suggested that hydroxycinnamic acids is the most promising target for the isolation of alpha glucosidase inhibitors and antioxidants in *Stenochlaena palustris* while another study identified that astragalin or kaempferol 3-O-β-

glucopyranoside as the active compound which is responsible for the alpha glucosidase inhibitory activity in *palustris*(Gunawan-Puteri et al., 2021).

Antimicrobial properties

Research on the Antimicrobial properties effect of kalakai extract is still very limited. Extract of kalakai with various solvents showed a range of antimicrobial activity including gram-positive bacteria, gram-negative bacteria and fungi (Table 7). A study conducted by Budiarti et al., (2021) reported antimicrobial activity with methanol solvent of green kalakai (mature leaves) is higher than in red leaves (young frond). The study showed that the concentration of 90% of green kalakai had equivalent activity as ampicillin in *Staphylococcus aureus* while a concentration of 90% had equivalent activity as ketoconazole in *Candida albicans.*

When the zone of inhibition in *Staphylococcus aureus* is compared with the antibiotics standard zone of inhibition (susceptibility range set by CLSI), the antibiotic azithromycin had a standard zone of inhibition of 21-26 mm (Demissie et al., 2021), then the kalakai ethanol extract with concentrations of 60%, 75%, and 90% had equivalent inhibition zones, 23 mm, 25 mm, and 28 mm of each (Budiarti et al., 2021). This is similar to the antibiotics Amoxicillin and Ciprofloxacin for *Escherichia coli* which have standard inhibition zones of 15-22 mm and 30-40 mm respectively (Demissie et al., 2021), then the kalakai extract with 70%, 80% and 90% ethanol had an inhibition zone > 22 mm (Budiarti et al., 2021), exceeded the amoxicillin inhibition zone, however, it is still below the ciprofloxacin. For other studies that have been carried out (Table 7), there are no comparable studies with similar inhibition zone. Thus, kalakai ethanol extract may have potential as a natural antibiotic in the future.

The antimicrobial activity in table 7 showed the variation of the inhibitory activity due to the different mechanism of inhibition of the active compound contained in an extract. In addition, the possible explanation on why the low antimicrobial activity in kalakai is due to the compounds are still in a mixture of various compounds, so that the inhibition activity is not optimal (Roanisca et al., 2018).

USE OF KALAKAI AS A FUNCTIONAL FOOD

Several attempts have been made to use kalakai as raw material for the manufacture of functional food products but in rather limited quantities. Santoso et al., (2022) tried to formulate kalakai into coffee and tea drinks mixed with artificial sweetener and milk. Kalakai extract has a characteristic putrid aroma, which increases with the addition of the amount of kalakai extract into the drink. Coffee was able to mask the putrid aroma and milk helped to decrease the bitterness, astringency, and distinct aroma of the kalakai. The opposite occured in tea drinks, the Putrid aroma in tea drinks was still existed and tended to get stronger with the addition of the amount of kalakai extract in the drink, followed by a darker colour change. However, the leafy aroma in coffee was still detected at a higher concentration of kalakai. It appeared that the addition of Carboxymethylcellulose as the thickener in the beverage could reduce the flavour and aroma perception due to less free water available to transport those taste stimuli. It was found that the highest sucralose (0.02%) and CMC (0.15%) concentration was the most suitable for fixing the attributes of coffee with kalakai and milk. However, Through AGI assay, it was found that the AGI inhibition activity was very low (<20%), thus it can be concluded that the sample showed no inhibition activity. Another effort to utilise kalakai as a functional beverage product was initiated by Mahdiyah et al., (2021) to develop a fermented tea from the leaves of kalakai, with the process such as, drying the kalakai leaves, then the kalakai leaves was boiled and then mixed with a starter of microorganisms with addition sugar and fermented for a few days to produce fermented tea.

Fahriza et al., (2021) developing high-iron cookies products made from chickpea flour substituted with kalakai flour for anemic pregnant women. The purpose of this product is chickpea cookies substituted with kalakai flour to be an alternative snack to reduce the increase in anemia rates for pregnant women, especially iron anemia. There was an increase in the iron content of cookies along

with the increase in the number of substitutions of kalakai flour used. This also happened in a study on the manufacture and formulation of baby porridge flour made from cassava and kalakai, where the addition of kalakai flour to baby porridge flour, can increase the iron content in baby porridge flour. (Sholihah and Agustina, 2018).

However, sensory-wise, the more substitutions of kalakai flour in cookies, the lower the panelists' preference for the taste and aroma of cookie products. Another effect is by increasing the proportion of kalakai flour used, making the resulting cookies darker in color. For texture, the addition of kalakai flour still gives a crunchy and crunchy taste but is a little rough when consumed.

Table 7. Antimicrobial Activity on Kalakai

Microorganism	Solvent	Concentration	Inhibition zone (mm)	References
Gram Positive Bacteria				
Staphylococcus aureus	methanol	10%	7	(Erwin et al., 2016)
	methanol	15 %	7	(Erwin et al., 2016)
	methanol	20 %	8	(Erwin et al., 2016)
	methanol	25 %	9	(Erwin et al., 2016)
	methanol	30 %	10	(Erwin et al., 2016)
	Ethanol	10%	4,88	(Roanisca et al., 2018)
	Ethanol	45%	17	(Budiarti et al., 2021)
	Ethanol	60%	23	(Budiarti et al., 2021)
	Ethanol	75%	25	(Budiarti et al., 2021)
	Ethanol	90%	28	(Budiarti et al., 2021)
	n-hexane	30%	6.2	(Malik and Hendra, 2021)
	Ethyl Acetate	30%	7.1	(Malik and Hendra, 2021)
	Dichloromethane	30%	6.4	(Malik and Hendra, 2021)
Bacillus subtilis	Ethanol	5%	6	(Roanisca et al., 2018)
Gram negative Bacteria				
Escherichia coli	methanol	10%	7	(Erwin et al., 2016)
	methanol	15%	7	(Erwin et al., 2016)
	methanol	20%	7	(Erwin et al., 2016)
	methanol	25%	8	(Erwin et al., 2016)
	methanol	30%	12	(Erwin et al., 2016)
	Ethanol	5%	5,28	(Roanisca et al., 2018)
	Ethanol	10%	5,28	(Roanisca et al., 2018)
	Ethanol	50%	16	(Budiarti et al., 2021)
	Ethanol	60%	21	(Budiarti et al., 2021)
	Ethanol	70%	24	(Budiarti et al., 2021)
	Ethanol	80%	27	(Budiarti et al., 2021)
	Ethanol	90%	30	(Budiarti et al., 2021)
	n-hexane	30%	6.1	(Malik and Hendra, 2021)
	Ethyl Acetate	30%	6.1	(Malik and Hendra, 2021)
	Dichloromethane	30%	12.1	(Malik and Hendra, 2021)
Fungi				
Candida albicans	Ethanol	60%	13	(Budiarti et al., 2021)
		70%	17	(Budiarti et al., 2021)
		80%	21	(Budiarti et al., 2021)
		90%	24	(Budiarti et al., 2021)

One of the other food products made from kalakai is kalakai leaf chips (Indrayanti et al., 2016). In addition, the use of kalakai leaves formulated with ginger in herbal drinks has a significant effect on antioxidant activity and sensory properties with the formulation of kalakai leaves and ginger 50:50 is the best treatment, because the presence of ginger formulations can overcome the weakness of colour, aroma and taste of the leaves of kalakai which found in herbal drinks (Juliani et al., 2019).

CONCLUSION

To date, this study showed that kalakai has potential as an ingredient for functional and nutraceutical foods. The potential of the kalakai is also supported by several research findings such as high fibre content, iron content which showed it can increase Hb levels in the blood, antioxidative properties that can be useful for controlling free radicals, as a treatment for Alzheimer's treatment and also has the potential to reduce hyperlipidaemia. However, referring to the current status of research, the processing of kalakai as a food functional or nutraceutical ingredient for antioxidative function and controlling blood sugar are the most potential.

Research related to the health benefits of kalakai and its use as a functional food is still very limited. Further research is still needed to deeply understand the content of vitamins, minerals and bioactive components in red (young frond) and green (mature frond) kalakai and its potential health benefits. In addition, the potential for further research must cover the identification of the role of each bioactive component in kalakai on its certain functional effects, synergistic effects caused by the combination of kalakai with other ingredients, as well as clinical and safety tests that still need to be carried out as part of the preparation for the use of kalakai as a functional food ingredient. and nutraceuticals shortly.

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SENSORY AND BIOACTIVE PROPERTIES RESPONSE TO REFORMULATION AND PROCESSING OF JAVA-TEA-BASED FUNCTIONAL DRINK: A REVIEW

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ABSTRACT

Java-tea-based (*Orthosiphon aristatus* B. Miq) functional drink is reported to have privileges, mainly its physiological functions and bioactive properties such as antioxidant activity and antihyperglycemic ability. Java-tea-based functional drinks contain bioactive compounds from natural materials, including java-tea leaves, ginger, sappan-wood, curcuma, lime, and kaffir lime. Several innovations and challenges have been carried out during its development, affecting the drinks' quality and stability. The stability of the beverage, especially in the aspects of sensory and bioactive properties, is still considered inferior. This review focuses on the formulation and processing technology conducted from 2007 to 2019. In the case of the reformulation, it showed that the addition of kaffir lime extract, lime extract, and flavor enhancer could improve the sensory properties in terms of aroma and taste. Furthermore, the changes in the specific java-tea plants' variety as the main ingredient and replacing the sucrose with non-sucrose sweeteners increased the bioactive properties. Recently, the formula has been enriched with a red fruit oil emulsion to improve the drink's appearance due to contains abundant carotenoid pigments. However, the addition also created another hurdle, particularly in the flavor sensory aspect and emulsion stability. In the case of processing, applying microencapsulation and nanoencapsulation in java-tea drinks has increased the bioactive properties, especially antioxidant activity and antihyperglycemic, and also improved the product's stability which prolonged the shelf life. The development of java-tea-based functional drinks might become a milestone to reference other similar product development.

Keywords: antihyperglycemic; antioxidant; java-tea-based functional drink; reformulation; technology

ABSTRAK

Minuman fungsional berbasis kumis kucing *(Orthosiphon aristatus* B. Miq) memiliki keunggulan berupa fungsi fisiologis dan aktivitas biologis seperti aktivitas antioksidan dan antihiperglikemik. Minuman fungsional berbasis kumis kucing memiliki senyawa aktif yang berasal dari bahan baku alami meliputi daun kumis kucing, jahe gajah, kayu secang, temulawak, jeruk nipis, dan jeruk purut. Rangkaian inovasi dan tantangan dalam pengembangan produk memengaruhi kualitas dan stabilitas minuman fungsional berbasis kumis kucing. Kestabilan minuman dinilai masih rendah ditinjau dari aspek sensori dan aktivitas biologis. Review ini difokuskan pada pengembangan dari aspek formulasi dan teknologi pengolahannya pada periode 2007-2019. Pengembangan dari aspek reformulasi minuman fungsional berbasis kumis kucing menunjukkan bahwa penambahan ekstrak jeruk purut, ekstrak jeruk nipis, dan penguat rasa dapat meningkatkan sifat sensori dari aspek aroma dan rasa. Perubahan varietas tanaman kumis kucing sebagai bahan baku utama dan penggantian sukrosa menjadi pemanis non-sukrosa dapat meningkatkan aktivitas biologis. Penelitian terbaru, formula diperkaya dengan emulsi minyak buah merah untuk meningkatkan penampilan minuman karena mengandung pigmen karotenoid. Namun, penambahan tersebut juga menimbulkan kendala lain, terutama dalam aspek sensori rasa dan stabilitas emulsi. Pengembangan dari aspek pengolahan menunjukkan bahwa penerapan mikroenkapsulasi dan nanoenkapsulasi pada minuman fungsional berbasis kumis kucing meningkatkan aktivitas biologis, terutama aktivitas antioksidan, dan antihiperglikemik, serta meningkatkan stabilitas sehingga memperpanjang umur simpan produk. Pencapaian dalam pengembangan minuman fungsional berbasis kumis kucing berpeluang menjadi acuan bagi pengembangan produk sejenis lainnya.

Kata kunci: antioksidan; antihiperglikemik; minuman fungsional berbasis kumis kucing; reformulasi; teknologi

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INTRODUCTION

Food product development is a strategy to fulfill consumer needs and market segmentation (FAO 2006). Food product innovation and development is an ample opportunity to produce functional drinks along with the increasing market demand for food products that also provide health benefits (Bigliardi and Galati, 2013; Ogundele et al., 2016). According to Laura (2020), the market trend of functional drinks is estimated to increase by 8.08% Cumulative Annual Growth Rate (CAGR) by 2023.

The strategy of product innovation and development is conducted through reformulation and processing modification aimed at improving the characteristics of the final product to meet the customer specifications (Luo et al., 2019; Souza et al., 2019). The parameters of product development based on functional drinks have been done through the use of natural ingredients, the addition of prebiotics and probiotics, the reduction of sucrose through non-sucrose sweeteners substitution, processing technology modification, non-thermal application, and optimization of formulas and processing processes (Waziiroh, 2013; Corbo et al., 2014; Ogundele et al., 2016; Food review, 2019).

The java-tea-based functional drink is a non-alcoholic drink that gives physiological functions through its bioactive properties. It is made from spices and herbs with the initial ingredients including java-tea leaves, ginger, sappan wood, curcuma, and lemon (Wijaya et al., 2007). The drawback of java-tea drink has a slightly bitter aftertaste detected from the spices (Kordial, 2009; Afandi, 2011; Febriani, 2012). Based on sensory properties, java-tea drink has a hedonic preference score on the "slightly like" scale. The sensory properties with a lower level of preference for consumers are still unacceptable as a functional food, even though the products have health benefits (Wijaya, 2007).

Moreover, the stability of the java-tea ready to drink type is characterized by the decreasing of its physicochemical, sensory properties, antioxidant activity, and microbiological activity. Reformulation through substitution or addition of other ingredients might improve the product characteristics so it will prolong the shelf-life. Modifying the type of product by processing such as through nanoencapsulation and non-thermal technology can increase the product's shelf life and maintain the final product's stability and quality during storage (Hartyáni et al., 2011; Plaza et al., 2011).

The development of java-tea based on functional drinks has been implemented through reformulation and modification of processing technology to improve the quality of drinks and extend the product shelf life (Afandi, 2011; Indariani, 2011). This series of research results for 2007-2019 related to java-tea based on the functional drink has been used as a reference for food innovations and development (Wijaya, 2007; Michael 2019). Recently, the topic of the development of functional drinks is in great demand by scientists and industry practitioners to meet market trends. The scientific review discusses on the effect of reformulation and processing modification on java-tea based on a functional drink that can affect sensory and bioactive properties might enrich the inquiry.

MATERIALS AND METHOD

Writing the literature reviews involves brainstorming and exploring the literature related to the topic through collection, analysis, interpretation, and conclusions. The inclusion criteria of literature used to find the relevant topic discuss the characteristics of java-tea-based functional drinks and the effect of product development based on aspects of reformulation and processing supported by articles published in national and international scientific journals. Internet sites used to access primary information sources include the IPB Repository (www.repositoryipb.com), Mendeley Web (www.mendeley.com), Elsevier (www.elsevier.com), Wiley Online Library (onlinelibrary.wiley.com), Springer Link (www.springer.com), Google Scholar (www.scholar.google.com), and Science Direct (www.sciencedirect.com). Secondary sources of information used in writing scientific reviews are electronic books, scientific magazines, Google Books (www.books.google.com), News Letters-

IFT (www.ift.org), and Food Insight (www.foodinsight.org). There were 224 literature sources used as primary sources of information, comprising 15 undergraduate theses, five graduate thesis, three patents, 22 national journals, and 179 international journals. Also, 32 literature sources were used as secondary sources of information completed on scientific review. The scientific literature should fulfill the inclusion criteria, there is a thesis about "java-tea based functional drink" product developments, as the main source of literature conducted from 2007 to 2019. The journals were used as complementary information which must be published in the last ten years. The national and international journals had been validated through Sinta and Scimago. The literature was analyzed and interpreted to obtain the findings of a comprehensive scientific review. The type of data analysis carried out was in the form of comparative analysis and cause-effect analysis on each critical source of information. Data and information were interpreted and synthesized to answer the problem formulation and obtain a novel study. The scientific reviews were evaluated through the Turnitin website (www.turnitin.com) to check for plagiarism with the condition that the similarity percent is below 20%.

RESULTS AND DISCUSSIONS

JAVA-TEA-BASED FUNCTIONAL DRINK CHARACTERISTICS

The physicochemical and sensory properties of the java-tea-based functional drink are shown in Table 1. The functional drink is characterized as a low-calorie-based beverage with lower sugar content. The java-tea-based functional drink has a citrus flavor characterized by a citrus, fresh, fruity sensation. The java-tea-based functional drink has a reddish-yellow color to a yellow color based on color parameter through (Hue) method analysis. The yellow color of its beverage is derived from the sappan wood that contains brazilin pigment formed at a low pH after the addition of kaffir lime extract and lime extract, which also acts as an acidulant (Herold, 2007; Yemirta, 2010; Nirmal et al., 2015).

The java-tea-based functional drink has a total solids (TPT) of 14.52 °Brix, influenced by the concentration of each ingredient, especially the concentration of sucrose (Herold, 2007; Indariani, 2011). The java-tea-based functional drink has a low pH value due to lemon extract and contains bioactive compounds from spices dominated by the flavonoid group (Indariani, 2011). The extract of each natural ingredient for making the java-tea-based functional drink contains active compounds that are analyzed through high-performance liquid chromatography. Further information about the active compounds in each ingredient can be seen in Table 2.

Table 1. Physicochemical of Java-tea-based Functional Drink

Parameters	Value	Unit
Moisture content	98.88 ± 0.10	% b.b
Protein content	0.14 ± 0.01	% b.b
Fat content	0.60 ± 0.01	% b.b
Ash content	0.14±0.02	% b.b
Carbohydrate content	0.24 ± 0.14	% b.b
Calorie	6.92	kcal/100 g
Color	94.20	°hue
pH	3.7 ±0.1	-
Total phenol	426.73 ±37.81	ɒm GAE*/ml

Source: (Indariani, 2011)
*GAE: Gallic Acid Equivalent

Table 2. Bioactive Compounds Indicator in Each Ingredient

Ingredients	Bioactive compounds	Concentration (mg/mL)
Java-tea leaves	Sinensetin	0.024
Curcuma	Curcumin	0.35
	Desmetoksi curcumin	0.15
Ginger	6-gingerol	1.019
	8-gingerol	0.2
	6-shogaol	0.067
	10-gingerol	0.472
Sappan wood	Brazilin	1.32
Kaffir lime	Hesperidin	0.092
	Naringin	0.01
Lime	Hesperidin	0.026

Source: (Indariani, 2011)

BIOACTIVE PROPERTIES OF JAVA-TEA-BASED FUNCTIONAL DRINK

The java-tea-based functional drink has an antioxidant activity of 783.78 ± 9.08 moles Trolox/L, which was analyzed using the DPPH

method (2,2-diphenyl-1-picrylhydrazyl) with the principle of hydrogen donor from the hydroxyl component to stabilize free radicals in the form of DPPH. (de Souza et al., 2013; Liu et al., 2017; Michael et al., 2019). The antioxidant activity of the java-tea-based functional drink should be abundantly influenced by phytochemical compounds such as flavonoids, alkaloids, tannins, saponins, triterpenoids, steroids, and hydroquinones (Indariani, 2011). High antioxidant activity has the potential to have antihyperglycemic abilities (Naibaho, 2018). High antioxidant activity can inhibit the oxidation of peroxide compounds when blood glucose levels are high (Monroy and Cristina, 2013), therefore this java-tea drink has been reported for having antihyperglycemic activities.

The antihyperglycemic abilities of the java-tea based functional drink have been analyzed by *in vivo* and *in vitro* methods (Diana, 2010; Indariani, 2011). The antihyperglycemic ability worked through the inhibition of α-glucosidase and α-amylase enzymes with IC_{50} values of 217.12 and 217.41 mg/mL (*in vitro*), and increased glucose absorption by mice diaphragm cells by 37.48 g. glucose/g cells indicate that the absorbed glucose will be converted into an energy source in the cells *(in vivo*) (Diana, 2010). Furthermore, the java-tea-based functional drink ready-to-drink showed an antihyperglycemic activity of 65.83% at a concentration of 16 times the formula based on in vivo model, increased insulin sensitivity, and suppressed pancreatic cell damage (Indariani, 2011).

THE INGREDIENTS PROFILE OF JAVA-TEA DRINK

Java-tea leaves (*Orthosiphon aristatus* B. Miq)

The java-tea plant (*Orthosiphon aristatus* B. Miq) also populer called as “kumis kucing” plant, in terms of flavor, has a characteristic woody aroma which is often described as the smell of "wet wood" or "wet cardboard" derived from β-caryophyllene as the highest compound content (Omowaye et al., 2015; Zaidan and Djamil, 2015; Joshi, 2020). In case of the bioactive properties of the java-tea plant are containing the rosmarinic acid, eupatorium, and sinensetin which act as antioxidants (Yuliana et al., 2016; Michael et al., 2019). Rosmarinic acid in java-tea leaves has the highest antioxidant activity by inhibiting the Reactive Oxygen Species (ROS) formation (Yuliana et al., 2016). The java-tea’s compound is also reported to inhibit the activity of the α-glucosidase enzyme, thereby inhibiting the postprandial increase in glucose levels.

Yuliana et al., (2016) reported that the java-tea plant had conducted the metabolomic analysis resulting in the antihyperglycemic activity from the aspect of α-glucosidase enzyme inhibition. Kim et al., (2010) reported that metabolomic analysis aims to observe of secondary metabolite profile and biological activity of each secondary metabolite specifically. The java-tea plant has the bioactive compounds as antihyperglycemic activity indicator is sinensetin which can inhibit the activity of the α-enzyme and α-glucosidase. On the other hand, the active compounds-based java-tea plant have antihyperglycemic abilities namely betulinic acid, through reducing insulin resistance (Indariani, 2011; Yuliana et al., 2016; Ko et al., 2016).

Ginger (*Zingiber officinale Roscoe*)

Ginger (*Zingiber officinale Roscoe*) is containing active compounds in the form of nonvolatile and volatile components, which can be observed based on the flavor and bioactive properties. The nonvolatile components in ginger in the form of 6-gingerol and 6-shogaol compounds contribute to a pungent trigeminal. Volatile components derived from terpenoids such as zingiberene, β-sesquifelandrin, α-farnesin, ar-curcumin, β-bisabolene contribute to flavor profiles of warm, spicy, herbal, sweet-balsamic, and woody (Purnomo, 2010; Manuhara et al., 2018).

The antioxidant activity of ginger is contributed by several active compounds, including gingerol, shogaol, and zingerone which play a role by inhibiting the formation of oxidative stress. The shogaol compound in ginger contributes to antihyperglycemic activity by inhibiting the activity of the α-glucosidase enzyme, thereby reducing the impact of the postprandial increase in glucose levels by slowing glucose absorption in the

brush border membrane of the small intestine (Dugasani et al., 2016; Sampath et al., 2017; Mao et al., 2019).

Sappan wood (*Caesalpinia sappan* L)

Sappan wood contain brazilin and brazilein as the major compound which contribute as the colorant agents also have the bioactivity. The brazilin compound in the sappan wood contributes to the formation of color in food products. This compound is odorless and does not give specific flavor into the food products. The stability of the brazilin pigment is influenced by the pH value, around pH 2 to 5, to form a stable color from brazilin pigment because of acid condition. Meanwhile, pH 5 to 7 impacted the instability of brazilin pigment showed with changes of color from red to yellow, and the pH value of 8 is shown red and purplish-red color in the final product due to the alkaline condition. The compounds of brazilin and brazilein showed the stability of active compounds as bioactive properties can neutralize free radicals and inhibit lipid peroxidation. Quercetin in sappan wood also plays a role in α-glucosidase and α-amylase inhibition, suppressing postprandial blood sugar rises (Padmaningrum, 2012; Nirmal et al., 2015; Sakir and Kim, 2019).

Curcuma (*Curcuma xanthorrhiza* Roxb.)

Curcuma contains active compounds such as xanthorrhizol, which contribute to the bitter, spicy, and bitter taste. This compound is also reported to increase insulin sensitivity to suppress hyperglycemia. Other active compounds such as β-curcumin and ar-curcumin have a spicy-herbal aroma profile. (Jantan, 2012; Tiara et al., 2017). Dosoky and Setzer (2018) reported that curcuminoids such as curcumin, desmethoxycurcumin, and bisdemethoxycurcumin act as antioxidants by inhibiting nitric oxide (NO).

Kaffir lime (*Citrus hystrix* DC) and lime (*Citrus aurantifolia)*

Kaffir lime and lime-containing volatile compounds have unique flavors, there are limonin, naringin, and hesperidin. These compounds have been identified as major bioactive compounds and bioactivity through thin layer chromatography. Kaffir lime has a fruity, citrus, fresh, flowery, and floral taste. Meanwhile, the volatile compounds in lime are dominated by limonin compounds, with the other flavor being citrus, minty, orange, and lemon-like (Szczygiel et al., 2018a; Szczygiel et al., 2018b). These two citrus varieties act as flavor modifiers that can increase the palatability of the final product. Both oranges contain naringin as active compounds that play a role in increasing the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Oon et al., 2015; Warsito et al., 2018). This compound also plays a role in α-glucosidase and α-amylase inhibition (Keskes et al., 2015). Other compounds such as hesperidin can inhibit the activity of the α-glucosidase and α-amylase enzymes and can increase insulin production, affecting the decrease in blood sugar levels (Enejoh et al., 2015; Al-Aamri et al., 2018; Pangestu and Arifin, 2018).

JAVA-TEA-BASED FUNCTIONAL DRINK FORMULATION AND PROCESSING

The initial formula for a java-tea-based functional drink can be seen in Table 3. The main ingredients used in the initial formula of this drink are included java-tea leaves (*Orthosiphon aristatus* B. Miq), ginger (*Zingiber officinale* Roscoe), and sappan wood (*Caesalpinia sappan* L), curcuma (*Curcuma xanthorrhiza* Roxb.), and lemon (*Citrus*). medica var. Lemon). The supporting ingredients used are included sucrose and various food additives such Carboxymethyl Cellulose (CMC) as stabilizers and sodium benzoate as preservatives. Sucrose stock solution can improve the palatability of beverages, especially related to the taste and intensity of sweetness, as well as the mouthfeel and body characteristics in the final product (Winarno 2008; Alothman et al., 2018). Carboxymethyl Cellulose (CMC) stock solution is a stabilizer to ensure the stability of mixed extract and preserve the sediment in the final product. Meanwhile, the stock solution of sodium benzoate functions as a preservative capable of inhibiting the growth of molds and yeasts in the final product (Herold, 2007).

The processing steps of the java-tea-based functional drink are: formulation, preparation of solutions, extraction, mixing of each ingredient,

pasteurization, and packaging. The sugar stock solution was obtained from white granulated sugar and water (2:1). Ten grams of CMC was dissolved in 1000 mL of hot water at 65 °C. Meanwhile, a 5000 ppm sodium benzoate stock solution was obtained from 5 g of powdered sodium benzoate dissolved in 1000 mL of drinking water. The initial stage in processing java-tea-based functional drinks is the extraction of herbs and spices. The spice extract was vacuum filtered to remove the insoluble components that resulted in the formation of turbidity. The extract was pasteurized at 75 ºC for 30 minutes.

Table 3. Java-tea-based Functional Drink Formulation

Ingredients	Concentration
Mixture of extract ingredients	X g (X % b/v)
Lemon	Y g (Y % b/v)
Stock solution of sucrose (69-72 °Brix)	Z g (Z % b/v)
Sodium benzoate solution 5000 ppm	10 mL (final concentration 500 ppm)
Stock Solution of CMC* 1 %	10 mL (final concentration 0,1% v/v)
Water	Added till 100 mL

*CMC: Carboxymethyl cellulose
Source: (Herold, 2007)

The squeezed lemon has been prepared by using a juice extractor, then filtered with a filter cloth to separate the pulp and seeds. The next step is a mixture of spice and herb extracts, sugar stock solution and CMC stock solution, and sodium benzoate, which then were added to water and stirred until homogeneous. Lemon extract is added freshly at the final stage of mixing to avoid the loosing of aroma and prevent the enzymatic reactions (Ningrum, 2016). The final mixture solution, is immediately pasteurized at 75°C for 30 minutes, followed by a shock cooling process. The java-tea-based functional drink is ready for packaged in a dark-colored glass bottle.

PRODUCT DEVELOPMENT OF JAVA-TEA BASED FUNCTIONAL DRINK

The java-tea-based functional drink reported has advantages from the aspect of biological activity compared to commercial-based spice drinks. Herold (2007) reported that the antioxidant activity of java-tea-based functional drinks was higher than commercial ginger drink, commercial curcuma drink, commercial turmeric-tamarind drink, ginger-based fresh drink, lemon-flavored fresh drink, and orange-flavored fresh drink. However, this java-tea-based functional drink during storage in temperatures 30ºC and 55ºC was stable for 15 days, as indicated by the decrease in the characteristics of physicochemical properties, sensory properties, and biological activities (Herold, 2007; Indariani, 2011).

As reported by Indariani (2011), the shelf life of the java-tea-based functional drink was only 21 days at±4 °C due to the hydrolysis of sucrose into glucose or fructose by the activity of microorganisms. The changes reduced the sensory acceptability (Herold, 2007; Kordial, 2009; Farikha et al., 2013). The activity of microorganisms also causes a decrease of flavor and taste quality with its fermented characteristics and bitter taste. The pH value was also decreased due to the fermentation of glucose and fructose by anaerobic microbes (Herold, 2007; Kordial, 2009).

The decreased of antioxidant activities of the java-tea-based functional drinks predicted due to the oxidation process due to light and oxygen factors during storage (Indariani, 2011). The development of java-tea-based functional drinks referred to the review in two ways: reformulation and modification of processing. These were considered necessary to improve its functional drink based on sensory and bioactive properties.

REFORMULATION

As reported above, the initial formula for java-tea-based functional drink had a low sensory quality and a short shelf life. Reformulation was able to improve the beverage sensory quality through the addition of kaffir lime (Kordial, 2009). A combination of adding a mixture of lime and kaffir lime extracts, as well as flavor enhancers (Afandi, 2011), optimization of the concentration of non-sucrose sweeteners and citrus varieties (Febriani, 2012), optimization of the concentration of citrus varieties (Waziiroh, 2013), changing varieties of java-tea plants (Indariani, 2011), and adding red fruit oil emulsion (Sonatha, 2017; Michael, 2019)

had been conducted. Furthermore, the java-tea-based functional drink is also added with lemon flavor, salt, and substitution of CMC with xanthan gum. The reformulation of java-tea-based functional drinks causes changes in sensory properties in terms of improving the flavor and decreasing herbs taste, also the antioxidants and antihyperglycemic. Ogundele et al., (2016), Luo et al., (2019), and Astray et al., (2020) stated that reformulation could improve the characteristics of the final product from physicochemical and bioactivity, such as improved of stability of flavors, vitamins, colorants in beverages, reduced sugar intake, and improved antioxidant properties.

Java-tea harvesting time and the variety of java-tea plant

The variety of java-tea plants affected the bioactive properties of java-tea-based functional drinks. Indariani (2011) and Afandi (2011) reported that a variety of java-tea plant extract on a single component has a different total phenolic compound. The java-tea plant extract characterized by the variety aspect resulted in different total phenol content (Indariani, 2011). Afandi (2011) also reported that java-tea plants' harvesting age and variety affected the total phenol content. The extended harvest time of plants resulted the decreasing of the total phenol concentration due to the decreasing of water content (Setiawan et al., 2019). Furthermore, Afandi (2011) reported that the extract of white-flowered java-tea plant produces antioxidant activity of 570 ppm lower AEAC compared to purple-flowered java tea plant extract at 729 ppm AEAC. This finding has also been confirmed by Herold (2007), Kordial (2009), and Indariani (2011) who showed that the java-tea plant with white-flowered had higher antioxidant activity.

Lime and kaffir lime

The initial formula for java-tea-based functional drink used lemon extract, yet the addition of its ingredient showed "slightly like" in the final product. Kordial (2009), Afandi (2011), and Febriani (2012) reported that the addition of kaffir lime extract affects the taste and flavor of java-tea-based functional drink to improve the level of acceptance of its product. Based on sensory properties, kaffir lime contains more volatile compounds, 83% of which come from the terpene group. Kaffir lime is dominated by terpinene-4-ol and linalool compounds with a fruity, fresh, flowery aroma profile (Szczygieł et al., 2018a). Moreover, kaffir lime contains an aldehyde group (22%) which can improve the taste of the described aroma aspect fruity and fresh aroma profile. In comparison, the ketone (26%) and ester (46%) groups described a pleasant fruity aroma profile (Szczygeł et al., 2018b). Volatile components in kaffir lime extract act as "top notes" and can synergize with lime extract in improving the taste of a java-tea-based functional drink and increasing the level of acceptance of the product (Afandi, 2011).

Afandi (2011) and Febriani (2012) stated that lime extract added to a java-tea-based functional drink could improve the flavor and taste. The lime extract with its flavor characteristics, which is soft, refreshing sensation, will stand out when mixed into the functional drink formula of java-tea-based functional drink. The lime extract contains limonin as the most extensive compound, which has a citrus aroma profile, mint, and orange (Hausch et al., 2015). Carboxylic acids, such as malic and citric acids, also contribute to the sour taste (Ladaniya, 2008; Szczygeł et al., 2018b). Therefore, lime extract can act as a "base note" because it can give the impression of a long-lasting sour taste (long-lastingness) in the java-tea-based functional drink.

Based on the bioactive properties aspect, the reformulation with kaffir lime and lime can increase the antioxidant activity of java-tea-based functional drinks (Indariani, 2011). It contains components of polyphenols, flavans, flavones, and tannins (Szczygeł et al., 2018b). The active compounds which are belonging to the flavonoid group are naringin, and hesperidin can donate a hydrogen group to a radical compound (Indariani, 2011). Vitamin C in kaffir limes and limes acts as an antioxidant because of the structure of 2,3-enediol which facilitate the reducing of the free radical compounds.

Meanwhile, the reformulation carried out by Kordial (2009), Afandi (2011), and Febriani (2012)

reported that the addition of kaffir lime and lime extract mixture showed improved antioxidant activity actual comparing to the initial formula. It is influenced by the synergism between components bioactive in each ingredient. The results of the study conducted by Hyardin et al., (2012) showed that the synergism factor between the bioactive components of each ingredient has a significant effect on antioxidant activity. The antioxidant activity of its functional drink should adjust through *in vitro* digestion (INFOGEST) to maintain the stability of the bioactive compound in the gastric and intestinal phase so that the role of the functional drink as antioxidants can be uptake optimally by the body (Brodkorb et al., 2019).

Flavor enhancer (GMP:IMP)

The initial formula of the java-tea-based functional drink is having a typical spicy taste (jamu-type specific taste) and slightly bitter aftertaste sensation because characteristic of each ingredient. The specific flavor of this drink is challenging when it has to be formulated like a commercial “soft drink” product. The reformulation of the java tea-based functional drink by Afandi (2011) with the addition of a flavor enhancer guanosine monophosphate: inosine monophosphate (GMP: IMP) produces a higher level of preference than the initial formulas by Herold (2007) and Kordial (2009). The addition of a flavor enhancer (GMP: IMP) to the formula of a java-tea-based functional drink could strengthen the taste of oranges and suppress the bitter taste and aroma of herbs and spices which are less favored (Afandi, 2011). The combination of GMP and IMP flavor enhancers produces a better taste impact than a single component (Pszczola, 2010).

Flavor enhancers (GMP: IMP) can increase the value of palatability (richness and pleasantness) of a food product because of the excellent interaction between the taste components and the food matrix (Pszczola, 2010; Gaudette and Pickering, 2011; Asioli et al., 2017). Asioli et al., (2017) stated that adding glutamate to vegetables can improve the palatability of the food product with a preferred taste. The addition of a flavor enhancer (GMP: IMP) to a java-tea-based functional drink can be accepted and liked by panelists might be due its ability to strengthen the taste of orange characteristics.

Artificial sweeteners

Reformulation of a java-tea-based functional drink with the addition of acesulfame-K and sucralose as non-sucrose sweeteners caused changes in the sensory and bioactive properties (Febriani, 2012). Reformulation of a java-tea-based functional drink with the addition of non-sucrose sweeteners of sucralose and acesulfame-K has given the character of flavor profiles are"thin" and "watery," also the presence of a bitter aftertaste in java-tea based functional drink (Febriani, 2012; Burgos et al., 2016; Luo et al., 2019). The reduction of sucrose concentration of java-tea-based functional drink has induced the intensity of sweetness and mouthfeel with "bitter aftertaste", and "thin" characteristic; therefore, the level of consumer acceptance of its product was lower compared to java-tea-based functional drink formulated by Herold (2007) (Wijaya and Mulyono, 2010; de Souza et al., 2013; Burgos et al., 2016; Alothman et al., 2018). The taste profile of a reformulated java tea-based functional drink with non-sucrose sweeteners can be improved by adding xanthan gum. Xanthan gum has been able to contribute to the "body" (mouthfeel) of functional drinks, so there was no different level of acceptance among consumers toward the reformulated drink compared to the initial formula (Wijaya et al., 2018). The usage of non-sucrose sweeteners in java-tea-based functional drinks supports the antihyperglycemic activity of the antihyperglycemic functional drink (Febriani, 2012; Burgos et al., 2016). Java tea-based functional drink with sucralose and acesulfame-K showed higher α-glucosidase inhibition than the initial formula of its beverage (Febriani, 2012; Mardhiyah, 2012).

Capriles and Areas (2013), Gao et al., (2015), and Luo et al., (2019) also reported that the addition of non-sucrose sweeteners to bread can reduce the glycemic index, therefore it can suppress the increase of blood glucose levels postprandial. Non-sucrose sweeteners also can delay the diffusion of glucose and glucose absorption to suppress the increase in blood glucose levels postprandial. In

addition, the molecular structure of non-sucrose sweeteners cannot be metabolized by the body so that it inhibits the rise of blood sugar postprandial, as well as sweeteners sucralose and acesulfame-K (Handayani and Ayustaningwarno, 2014; Temizkan et al., 2015).

Enrichment of red fruit oil emulsion

The java-tea-based functional drink has a reddish-yellow color due to the lime extract in its formulation (Kordial, 2009; Waziiroh, 2013). Red fruit oil contains high carotenoids, and its red color has the potential as a natural coloring agent in java-tea drinks. Red fruit oil contains 4,090 - 7,723 ppm of carotenoids depending on the clones and ripening stages, dominated by β-carotene compounds of 23-27 ppm (Murtiningrum et al., 2019; Sarunggalo et al., 2014). Red fruit oil has to be added to the formulation of java-tea drink as an emulsion. The final reddish-orange product had higher viscosity which might be due to the emulsion state and also 5,28 ppm of carotenoids (Sonatha, 2017).

The increase in viscosity will increase the viscosity of red fruit oil emulsion because it used CMC as an emulsifier (Sarunggalo et al., 2014). The number of hydroxyl groups (-OH) of CMC can increase the affinity to bind water molecules through hydrogen bonds. The more bound water molecules can decrease water activity, increasing the final product's viscosity (Michael, 2019; Gossinger et al., 2019).

The addition of red fruit oil emulsion increased the total phenolic content by around 367.43 ± 1.95 mg GAE/L (Sonatha, 2017; Michael, 2019). A high total phenolic content has the opportunity to increase antioxidant activity because both are positively correlated (Khalil et al., 2012).

The usage of red fruit oil emulsion produced a reddish-orange java-tea drink, which increase the level of hedonic color preference compared to the original formulation. However, the higher the addition of emulsion concentration, the lower the level of preference level since the color became darker. The appearance of java-tea-based functional drink with and without the addition of emulsion shown in Figure 1 (Michael, 2019).

Figure 1. Java-tea-based Functional Drink (a) Without and (b) With Red Fruit Oil Emulsion (Michael, 2019)

It should be noted there is still challenging hurdle in the addition of the emulsion. The java-tea-based functional drink enriched with red fruit oil emulsion showed an unaccepted strange and pungent taste on the tongue and throat (Sonatha, 2017; Michael, 2019). It might be due to volatile components in red fruit oil such as 1,3-dimethyl-benzene (27.46 %), N-glycyl-L-alanine (17.36 %), trichloromethane (15.22 %), and ethane (11.43 %) which has a description of an orange-like aroma, woody, rancid, and phenolic sensation (Noviyanti, 2010; Rohman et al., 2012; Riduwan, 2019). High flavonoids in red fruit oil give a tart taste and bitter aftertaste to the final product (Rohman et al., 2012; Arumsari et al., 2013). In addition, the rancid flavor caused by the activity of proteolytic enzymes and hydrolysis reactions in red fruit oil also influenced the sensory performance of the new reformulation.

PROCESSING TECHNOLOGY MODIFICATION

The java-tea-based functional drinks were also modified through the processing technology. A java-tea-based functional drink in powder form was obtained by applying microencapsulation and nanoencapsulation technology (Afandi, 2014; Rekasih et al., 2021; Naibaho, 2018).

Microencapsulation

The java-tea-based functional drink processed by microencapsulation technique increased the stability, and also improve the beverage characteristics (Pramestia et al., 2015). According

to Afandi (2014), the microencapsulated java-tea drink has a size of 563.10 nm, while Naibaho (2018) has been able to produce the microencapsulation with a size of 1785 nm. Total solid content and inlet air temperature spray drying can affect particle diameter size (Tonon et al., 2011). The functional drink based java-tea with the application of microencapsulation has high solubility, shorter dissolving time, uniformly dispersed particle size, lower viscosity, and higher foam volume.

Microencapsulated java-tea based functional drink had lower L and a values, with higher b values compared with the ready to drink type. This phenomenon might due to a chemical reaction that oxidizes the active compound, therefore it turns the final product into more intense in yellow color. The microencapsulated drinks resulted in a higher sweetness intensity than ready-to-drink, while the bitter and sour taste intensity is the opposite (Afandi, 2014). It might occurred due to the small particle size, and also the coating suppressed the bitter taste of phenolic components by controlling the level release of the compound active (flavor release) (Syafii et al., 2016, Safithri et al., 2020). The effect of encapsulation can reduce the intensity of the sour taste of the lime extract and the bitter taste of the spice extract so that in the end it will increase the intensity of the sweetness (Affandi, 2014).

Microencapsulated drink showed also advantages such as increased the stability and biological activity compared to the ready-to-drink drinks because the encapsulation can protect the bioactive components from environmental damage such as oxidation (Sun-Waterhouse et al., 2011; Jatupornwipat et al., 2017; Erminawati et al., 2019). Microencapsulated drinks had lower antioxidant activity (72.237 ppm AEAC) than the ready-to-drink (221.368 ppm AEAC). Moreover, it has a higher antihyperglycemic activity which is 30.74% in α-glucosidase inhibition (*in vitro*) for microencapsulated drinks, compared with ready to drink about 25.90%. The low antioxidant activity in microencapsulated beverages might be due to the slow releases time of the active compounds since the conducted analysis was *in vitro* (Afandi, 2014). The low antioxidant activity in microencapsulated beverages is due to the slow release time of the active compounds since the conducted analysis was in vitro. The high temperature of the spray dryer also can affect the structure of bioactive compounds, causing the antioxidant activity reduced (Rigon and Zapata, 2016). In the case of antihyperglycemic activities, the usage of enzymes can support the breakdown of the encapsulation wall so the bioactive compounds can be released immediately. In the case of antihyperglycemic activities, the usage of enzyme can support the breakdown of the encapsulation wall so the bioactive compounds can be released immediately.

Nanoencapsulation

Nanoencapsulation is a coating technology for active components on nanoscale ranging from 1-100 nm, but for some reason, it can also exhibit up to 1000 nm (Ezhilarasi et al., 2012; FDA, 2014; Zhao et al., 2014). The application of nanoencapsulation can improve the stability of the product quality and shelf life of food products (Anandharamakrishnan, 2014; Jeong et al., 2020; Feridoni and Shurmasti, 2020; Estakhr et al., 2020). Nanoencapsulation has been widely used to improve the taste of a product by regulating retention of flavor release, masking unwanted flavors, and protecting aroma compounds (Oktaviana, 2010; McClement, 2012). The application of nanoencapsulation in food products has the potential to increase the solubility of nonpolar components, prevent oxidation of active components, and increase the bioavailability of active components (Ezhilarasi et al., 2013; Khaled et al., 2014; Mohammadi et al., 2016; Bagheri et al., 2013).

Nano encapsulated java-tea-based functional drink had 662.99 ppm GAE in total phenols, which is lower than the ready-to-drink type. The homogeneous system in the nano-encapsulated beverage causes the intermolecular hydrogen bonds to become stronger so that the release of the active components is slower (Rekasih et al., 2021). Therefore, the hydroxyl group in the nano encapsulated beverage only slightly reduced the phosphomolybdate and phosphowalframate components in the Folin Ciocalteu reagent resulted

a lower total phenol measured (Yoksan et al., 2010; Opalinski et al., 2016). Nano encapsulated drink has a higher brightness level than ready-to-drink and micro-encapsulated drink (Rekasih et al., 2021). Java-tea nano encapsulated drink has an average particle diameter of 217.17 - 537.80 nm. The particle size of the nano-encapsulated drink affects the product's color because the light that passes through the object is reflected more and is marked with a pale yellow color (Afandi, 2014; Rekasih, 2021). The ratio of the core material to the coating material reported affecting the characteristics of the final beverage product (Wijaya et al., 2017).

Similar to the microencapsulated java tea based functional drink, the nano-encapsulated drink also had a higher sweetness intensity. It might be due to the a decrease of the intensity of the sour taste and masking effect of the bitter taste, tart and bitter aftertaste (Afandi, 2014). Nanoencapsulation can control the slow release of flavor (flavor release), especially the phenolic components of spice and herb extracts which give a bitter taste and astringent sensation; this technology can protect the volatile components that form aromas due to the oxidation, evaporation, thermal, and hydrolysis (Oktaviana, 2010; Nedovic et al., 2011; Magnuson et al., 2011; Khaled et al., 2014; Assadpour and Jafari 2017; Suryanto et al., 2019). Nano encapsulated beverages increase consumer acceptance with the sweeter taste characteristic as also reported by Ratanasiriwat et al. (2013) and Ghorbanzade (2017).

The nano encapsulated beverage showed lower antioxidant activity and α-glucosidase inhibition (*in vitro*) compared to microencapsulation java-tea drink (Afandi, 2014). A similar phenomena in microencapsulated java tea functional drink compared to the ready to drink type, it might be due to the maltodextrin can inhibit bioactive components' release due to strong hydrogen bonds (da Silva et al., 2018). Meanwhile, *in vivo*, measurement, the nano-encapsulated beverage showed a higher antihyperglycemic activity (Rekasih et al., 2021; Naibaho, 2018). The active compounds in the nano-encapsulated drink are predicted to be easier to reach the target site, and the active compounds' release rate is more controlled (Ozturk et al., 2014; Murata et al., 2017; Sudirman et al., 2018. The nano-sized particles have a large surface area in this drink, resulting in the bioactive components being more easily absorbed into the folds of the intestinal wall (Lee et al., 2012; Gupta et al., 2012; Zanotto-Filho et al., 2013; Pereira et al., 2015).

The roadmap of the development of java-tea-based functional drink from the reformulation aspect showed in Figure 2.

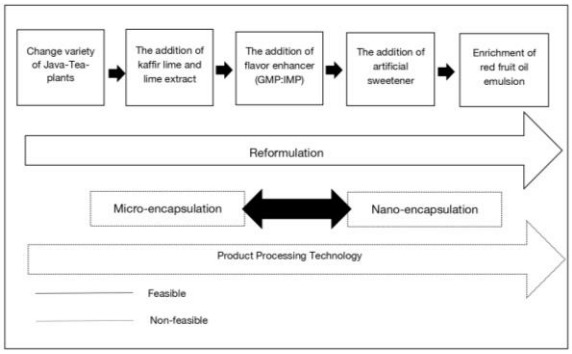

Figure 2. Flow Process of Product Development Java-tea-based Functional Drink in Terms of Reformulation and Processing Technology Modification

The mixture of kaffir lime and lime extracts combined with a flavor enhancer (GMP: IMP) increases the preference for the final product from the aspect of aroma and taste. Meanwhile, changes in the variety of the java-tea plant and the reduction in the concentration of sucrose replaced by sucralose and acesulfame-K sweeteners can increase the biological activity of the final product. The enrichment of red fruit oil emulsion has increased the sensory preference of color attributes. However, the product undergoes a separation of the water-soluble and oil-soluble phases, known as coalescence and flocculation phenomena. The product is also facing a touch hurdle for its flavor and mouthfeel. The reformulation aspect still has the opportunity to be carried out through the use of natural and functional ingredients. The application of microencapsulation in the java-tea-based functional drinks also still has drawbacks. It has a long dissolution time. Meanwhile, the application of nanoencapsulation beside it has unfavorable color, flavor, and stability, it also requires higher

production costs which affect the product's commercialization. Therefore, further product development through the application of technology should be done to improve the quality and shelf life of the product, especially in the form of ready-to-drink, which is more in demand by consumers due to its more practical.

CONCLUSION

The java-tea-based functional drink showed the antioxidant activity and antihyperglycemic ability due to the content of bioactive compounds of the identifiable contributed by each ingredient, especially the java-tea plant and sappan wood. However, the low stability of drink quality and weak sensory perform, it needed to be improved. Reformulation with the addition of lime and orange extracts, guanosine monophosphate (GMP): inosine monophosphate (IMP) flavor enhancer, changed the java-tea plant varieties, changed the non-sucrose sweeteners were able to improve sensory properties and bioactive properties, particularly the antioxidant capacity and the antihyperglycemic ability. The addition of red fruit oil emulsion increased the total phenolic content and color based on hedonic test. However, it might need further development due to unaccepted strange and pungent taste on the tongue and throat from java-tea drink enriched red fruit oil emulsion. The application of microencapsulation and nanoencapsulation technology produced the drink in a powder form which able to mask the inferior flavors, increase bioavailability, and extend the final product's shelf life. Further reformulation and processing modifications are still needed to increase the quality of its functional drink.

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Journal of Functional Food and Nutraceutical

INTELLIGENT PACKAGING AS A pH-INDICATOR BASED ON CASSAVA STARCH WITH ADDITION OF PURPLE SWEET POTATO EXTRACT *(IPOMOEA BATATAS L.)*

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ABSTRACT

Intelligent packaging is an indicator that has the capability to the condition of packaged foods and their environment through an indicator. This study aims to determine anthocyanin levels, pH sensitivity, solubility, swelling, and water content in intelligent packaging based on cassava starch with the addition of purple sweet potato extract. The method of this research is separated into two stages, which are the extraction of purple sweet potato and the development of indicator film. Furthermore, this research is conducted to analyze the physico-chemical properties of the film. This research is used Completely Randomized Design (CRD) with various extracts on indicator films that are 0%; 5%; 10%; 15% (v/v). The results showed that the indicator film with 15% extract has the best pH sensitivity with the color result 76.07 (L), 23.56 (a), 7.88 (b), solubility 52.15%, swelling power 133.90%, and it has 15.46% water content. The addition of purple sweet potato extracts significantly affected the total anthocyanin of the film indicator and pH sensitivity. The more extracts added to the film, the more obvious the color changes, the higher the water content and solubility but the lower the swelling properties.

Keywords: anthocyanin; cassava starch; intelligent packaging; pH indicator; purple sweet potato

ABSTRAK

Kemasan cerdas merupakan indikator yang memiliki kemampuan terhadap kondisi pangan yang dikemas dan lingkungannya melalui suatu indikator tersebut. Penelitian ini bertujuan untuk mengetahui kadar antosianin, sensitivitas pH, kelarutan, daya pengembangan, dan kadar air dalam kemasan cerdas berbasis pati singkong dengan penambahan ekstrak ubi jalar ungu. Metode penelitian ini dibagi menjadi dua tahap, yaitu ekstraksi ubi jalar ungu dan pembuatan indicator film. Selanjutnya, penelitian ini dilakukan untuk menganalisis sifat fisiko-kimia film. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) dengan variasi ekstrak pada indikator film yaitu 0%; 5%; 10%; 15% (v/v). Hasil penelitian menunjukkan bahwa indikator film dengan ekstrak 15% memiliki sensitivitas pH terbaik dengan hasil nilai warna 76,07 (L), 23,56 (a), 7,88 (b), kelarutan 52,15%, daya pengembangan 133,90%, dan memiliki kandungan air 15,46%. Penambahan ekstrak ubi jalar ungu berpengaruh nyata terhadap total antosianin dan sensitivitas pH pada indikator film. Semakin banyak ekstrak yang ditambahkan ke dalam film, semakin jelas perubahan warnanya, semakin tinggi kadar air dan kelarutannya, tetapi semakin rendah sifat pengembangannya.

Kata kunci: antosianin; indikator pH; kemasan cerdas; tepung singkong; ubi ungu

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INTRODUCTION

Food safety and quality are two factors affecting consumers in food selection. A factor impacting food safety and quality is food packaging. Food packaging aims to wrap and afford protection to food materials from external environmental conditions, e.g., heat, light, water, air, and microorganisms (Marsh & Bugusu, 2007). Changes in consumer preference for food safety breed innovations in food packaging technology, one of which is intelligent packaging (Biji et al., 2015).

Intelligent packaging is one of the developments of packaging concepts. It can monitor the condition and environment of the food using an indicator (Sitanggang et al., 2020). Intelligent packaging is an interactive indicator, the component of which is natural dye-based which can assess product quality from its chemical components. Freshness indicator was used to monitor fishes based on the number of total volatile basic nitrogen (TVB-N). Moreover, the detection of cod flesh was also observed using food freshness indicators made from natural sources such as curcumin, grape peel, and beetroot extract. The result shown that the applications of intelligent packaging which was incorporated with natural bio-based extract can effectively show freshness level based on color difference (Tichoniuk et al., 2017).

One of the packaging indicators is acid-based. This indicator is called a pH indicator. The color of the indicator, which is pH-based, is influenced by the environment where it is applied (Moradi et al., 2019). Plant-based dyes contain natural compounds at large, e.g., anthocyanin, β-carotene, curcumin, and chlorophyll. Some of these compounds are sensitive to pH and will exhibit a clear color spectrum when applied in two different environments, i.e., acid and base (Alizadeh-Sani et al., 2020). One of the natural dyes in a packaging indicator is anthocyanin.

As having a dominant hydroxy cluster, anthocyanin is blue in color and relatively unstable (a high pH), whereas if having a dominant methoxy cluster, it will be red in color and relatively stable (a low pH). Therefore, anthocyanin is suitable for being a bio-indicator and give a color change effect. Anthocyanin stability in water or polar solvent, which is neutral or basic, can be augmented with acid addition (Sipahli et al., 2017). A proper combination of polar solvent and an organic acid which breeds a very acidic pH condition (a pH of 1-2) can elevate anthocyanin stability in the form of red flavylium cation (Pedro et al., 2016). Accordingly, the process of anthocyanin extraction deploys an ethanol solvent and citric acid.

One of the raw materials containing anthocyanin is purple sweet potato. Anthocyanin contained in a purple sweet potato is an acyl chain in form. Acylation in anthocyanin of a purple sweet potato can indicate unique properties, e.g., pH stability, heat resistance, antioxidant capability, and anti-inflammation (Yaningsih et al., 2016). A purple sweet potato contains 19.61% of carbohydrates, 1.04% of protein, and 0.33% of fat. Additionally, it also contains 93.64 mg/100 g of anthocyanin (Chen et al., 2019).

Tapioca starch is extracted from cassava. Among polysaccharide products, tapioca starch affords many benefits, such as being affordable, reachable, colorless, odorless, non-toxic, and eco-friendly. Tapioca contained 96.74% starch and 37.43% amylose which is higher than corn starch and purple sweet potato starch (Lopattananon et al., 2012; Palacios-Fonseca et al., 2013; H. Wang et al., 2020). Amylose plays an important role in the crystallization of starch to form a film in the starch gelatinization process (Wang et al., 2022).

However, a starch-based film has a poor and inflexible mechanic property (Silva et al., 2019). So, the addition of plasticizer is an interesting formulation to improve film mechanical properties. The mixture of plasticizer and starch are tends to decrease the interaction between starch chains and increase their mobility (Yang et al., 2017).

The focal points of this research are total anthocyanin contents, pH sensitivity, solubility, swelling capacity, and water content in intelligent packaging in the form of a pH indicator. The indicator is made from a tapioca starch-based film layer added with the extract of purple sweet potato.

MATERIALS AND METHOD

Materials

Tools used were a hot plate stirrer (Thermo Scientific Cimarec, USA), magnetic stirrer, water bath shaker (B-ONE SWB 30), blender (Philips HR2115, Indonesia), oven (Memmert, Germany), glass mold, analytical balance (Ohaus, Indonesia), desiccator, UV-vis spectrophotometer (Thermo Scientific Genesys 15), digital pH meter (Nutron Tech), and chromameter (Konika Minolta CR-400).

The raw materials used were a Gunung Kawi purple sweet potato (CV Sarana Meraih Berkah, Indonesia), tapioca starch (PT Budi Starch, Indonesia), aquadest, glycerol, 96% of ethanol, HCl-KCl buffer (pH of 1), acetate buffer (pH of 4.5), 60% of citric acid, and whatman paper no. 41.

Methods

Extraction of anthocyanin from a sweet purple potato

The extraction method referred to (Chen et al., 2019) with a modification. The purple sweet potato was washed and cut into small cubes. The cubes were smashed using a blender until reaching a porridge-like texture and weighed as much as 20 g. Purple sweet potato was added with a mixture of ethanol 96% and citric acid 60% with a ratio of purple sweet potato and solvent 1:5. Then the mixture was extracted using a water bath shaker at 60℃ for 60 minutes. The extract was filtered using a piece of Whatman paper no. 41. Supernatants yielded were stored in a dark bottle at 4℃.

Preparation of indicator films from a sweet purple potato

In making the film, a casting method by (Piñeros-Hernandez et al., 2017) was applied with a modification. A 7.5g of tapioca starch was mixed with 1.5 g of glycerol and 91 ml aquadest. The mixture was stirred on a hot plate stirrer for 60 minutes at 90℃. The extract of purple potato (5, 10, 15% w/v) was then added and stirred for ten minutes at 50℃. The mixture was cast using a glass mold and left to dry at 50℃ for 15 minutes using an oven.

Total anthocyanin analysis

A pH differential method was employed to calculate the total anthocyanins content in indicator films containing the extract of purple sweet potato (Lee et al., 2005). The absorbance of the sample and total content of anthocyanins in the sample were determined using the following equation.

$A = (A_{max} - A_{700})\ pH = 1 - (A_{max} - A_{700})\ pH = 4.5$ (1)

$$\text{Total anthocyanin (mg/L)} = \frac{A \times BM \times FP \times 1000}{\varepsilon \times b} \quad (2)$$

Descriptions:

A: Absorbance

A_{max}: Maximum absorbance

A_{700}: Absorbance at 700 wave length

BM: Molecular weight (449,2 g/mol)

FP: Dilution factor

ε : Molar absorptivity (26900L/mol.cm)

b: Thick of cuvette

Color measurement

The color test on the indicator film contained the extract of sweet purple potato (5 x 5 cm^2) was carried out using a chromameter (Konika Minolta CR-400) at a CIE color system (Sitanggang et al., 2020). The film was put on a plate reader which was on a white table. Then the plate reader was shot using a chromameter. The measured value on the screen as L^* (lightness), a^* (appearance), b^* (blueness).

pH sensitivity

The test for the extract's pH sensitivity was conducted by preparing 18 ml of buffer solvent with a pH of 2-11. A 2 mL of anthocyanin extract was added and stirred using a magnetic stirrer for 30 minutes. Conditioning the pH value from acid to basic was critical to examine levels of color changes of anthocyanin extracts at each pH value.

The film was cut into a size of 2 x 2 cm and soaked in a mold containing a buffered solvent with a pH of 2-11 for five minutes. The acid-basic indicator film was measured for its pH using a pH meter (Moradi et al., 2019).

Solubility

The film was cut into a size of 1x 2 cm and put on a cup prior to put into an oven at 100℃ for an hour. The film was weighed for its initial dry weight (w_0) and soaked for 24 hours. An insoluble film was put into an oven for an hour at 100℃, leaving it to dry. It was then stored in a desiccator for 10 minutes and weighed for its post-soaking dry weight (w_1) (Unsa & Paramastri, 2018).

Swelling properties

The indicator film was cut by 1 x 2 cm and aerated for 30 minutes and weighed (w_1). It was then soaked in 30 ml of aquadest (25℃) for 60 minutes. The wet sample was filtered to remove its solvent and weighed (w2) (Popović et al., 2011).

Water content analysis

Water content was measured using a percentage of weight loss of the sample of anthocyanin indicator films after the sample was dried using an oven at 105℃ for 24 hours until a constant weight of the sample was reached (Sitanggang et al., 2020).

RESULTS AND DISCUSSION

Total anthocyanin content

Extracting a purple sweet potato using a water bath shaker for 60 minutes at 60℃ with a mixture of ethanol and citric acid as solvent resulted in an extract of purple sweet potato which was red in color and 42.74% of yields. After extraction, the total anthocyanin was measured, yielding a mass of 118.17 mg/100 g. Table 1 points out the total anthocyanins of the indicator film of purple sweet potato.

The highest total anthocyanin was showcased by the indicator film F3 as a result of a 15% of extract addition. Meanwhile, the lowest one was presented by the indicator film F1 because of the least extract addition of 5%. The indicator film F0 did not contain anthocyanins. The absence was marked by the measurement result of < 1 by virtue of no anthocyanins contained in the materials added to the film. Anthocyanin content of the extract of sweet purple potato, which was initially 118.17 mg/100 g, declined after being added to indicator films. The decline came about due to anthocyanin degradation after the heating process since one of the anthocyanin natures was heat-unresistant (Husna et al., 2013). In other research, anthocyanin from Hibiscus sabdariffa was extracted from four different solutions then treated to 80 and 50ºC for 6 hours. That extract declined generally in the pigmen retention. The number of anthocyanin degradation rose following the increase of heat temperature (Sipahli et al., 2017). In addition, the hydrogen peroxide which formed through oxidation of ascorbic acid oxidized anthocyanin pigments (Mercali et al., 2013).

Table 1. Total Anthocyanins of Film Indicators

Sample	Total Anthocyanin (mg/100 g)
F0	0.78 ± 0.09^{a}
F1	21.82 ± 0.10^{b}
F2	43.42 ± 0.17^{c}
F3	69.97 ± 0.17^{d}

The different superscripted letters show a significant difference ($p < 0.05$). F0 = without-extract film, F1 = film with 5% of extract, F2 = film with 10% of extract, F3 = film with 15% of extract.

The extract of sweet purple potato with a combination of ethanol solvent and citric acid yielded better than that with a water solvent and a total anthocyanin of 17.06 mg/100 g (Chen et al., 2019). A low anthocyanin level might be brought about by the sweet purple potato's plant tissue which could not be destroyed and penetrated by water. This brought on poor extraction of chemical compounds. Alcohol and citric acid as solvents could enhance the lysis ability of vacuole membranes, escalate osmosis abilities, and produce anthocyanins in a stable condition in the form of flavylium ions (pH of 1-2) (Li et al., 2013). In the research of (Chen et al., 2019), the difference in total anthocyanins extracted using different solvents was identified using water and ethanol, water and citric acid, and ethanol and citric acid as

the solvents. The highest anthocyanin was acquired with a combination of ethanol and citric acid solvents of 93.64 mg/100 g at an extraction temperature of 80℃ for 40 minutes. Previous Chen's research results were comparably different from ours owing to different variants of the sweet purple potato used and different extraction temperatures. Chen et al., (2019) used a TN73 sweet purple potato from Taiwan, while we used a Gunung Kawi one. In addition, Chen exerted an extraction temperature of 78.9℃ which exceeded the boiling point of ethanol. This caused several chemical compounds to evaporate during the extraction process.

Previous research demonstrated as the best result for sweet purple potato using a concentrated solvent extraction method (Cai et al., 2016). Anthocyanin content derived from the extraction was 215.29 mg/100 g at 70℃ for 90 minutes using ethanol and HCl as the solvents. Chai's research results were different from the current ones considering different variants of the sweet purple potato used. Besides, Chai centrifuged the extract acquired and evaporated the solvents using a rotary evaporator. This method yielded a more concentrated extract, which affected anthocyanin resulted, the amount of which increased. Nevertheless, the anthocyanin was heat labile because the extraction process was conducted in both high temperature and short time (accelerated-solvent extraction method), while the anthocyanin that was extracted using conventional extraction and ultrasound-assisted extraction generate high impurities extract.

Color characteristics of indicator films

Figure 1 presents indicator films of sweet purple potato in all formulations. The indicator film F0 was white in color and slightly transparent. Moreover, indicator films with an addition of sweet purple potato extracts were pink in color. The more the extract of sweet purple potato added, the brighter the red color. The pink color of an indicator film was engendered by the addition of sweet purple potato extract, which contained anthocyanins in the form of flavylium cation from the extraction process using acid (Khoo et al., 2017).

Table 2 exhibits the results of color analysis on indicator films made. The indicator film without the extract of sweet purple potato indicated the brightest color. Meanwhile, the addition of sweet purple potato extract might decrease film brightness but promote the red color as well as add a slightly-yellowish color. The yellowish color was on account of an addition of glycerol, which was then oxidated during the process of film molding. The pinker the indicator film, the more apparent the color change.

Table 2. Color Characteristics of Indicator Films

Sample	L^*	a^*	b^*
F0	85.27 ± 0.03^a	0.50 ± 0.03^a	3.47 ± 0.03^a
F1	82.94 ± 0.01^b	9.64 ± 0.05^b	5.82 ± 0.00^b
F2	76.69 ± 0.01^c	21.31 ± 0.03^c	8.12 ± 0.06^d
F3	76.07 ± 0.15^d	23.56 ± 0.44^d	7.88 ± 0.08^c

The different superscripted letters show a significant difference ($p < 0.05$). F0 = without-extract film, F1 = film with 5% of extract, F2 = film with 10% of extract, F3 = film with 15% of extract.

Previous research figured out that a chitosan-based indicator film incorporated with alizarin extracts declined in terms of brightness level after extract addition (Ezati & Rhim, 2020). The film, which was initially colorless and transparent, turned its color after the alizarin addition. Alizarin addition to a chitosan-based film could decrease its brightness but heighten its red and yellowish colors.

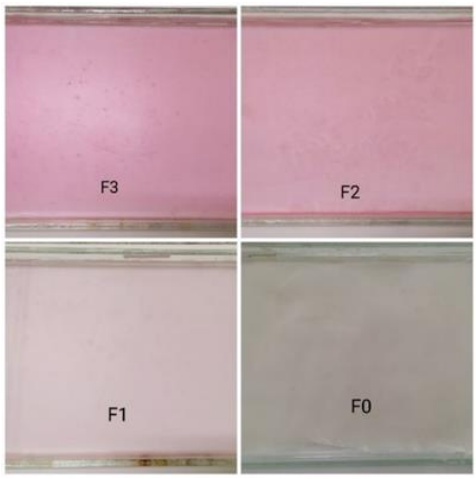

Figure 1. Colors of Indicator Films. *F0 = without-extract film, F1 = film with 5% of extract, F2 = film with 10% of extract, F3 = film with 15% of extract.*

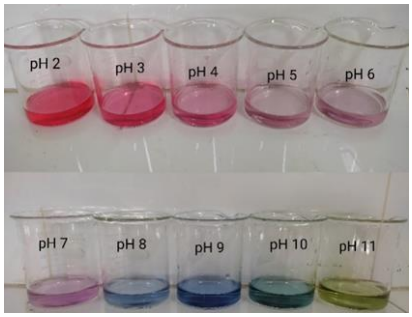

Figure 2. pH Sensitivity of Sweet Purple Potato extracts. *F0 = without-extract film, F1 = fifilm with 5% of extract, F2 = film with 10% of extract, F3 = film with 15% of extract.*

Figure 3. pH Sensitivity of Indicator Films. *F0 = without-extract film, F1 = film with 5% of extract, F2 = film with 10% of extract, F3 = film with 15% of extract.*

pH sensitivity of the extract and indicator films

Figure 2 shows color changes in the extract of sweet purple potato at a pH of 2-12. The extract color at an acid pH of < 5 was reddish, whereas that at a pH of 5-7 was purplish pink. Furthermore, the extract colors at basic pH of 8-9 and 10-11 were blue and yellowish-green, respectively.

Anthocyanin solution with the extract of sweet purple potato at different pH conditions (2-12) showcased a decreased value from +23.09 to -1.10, and the color parameter *a* showed off a color change from red to green (Chen et al., 2020). Moreover, with an increased value from +4.24 to +24.95, the color parameter *b* demonstrated a change in its yellow color on account of an increased pH. Hence, the extract of sweet purple potato was sensitive to pH changes and thereby effective for being used as a natural pH indicator.

Figure 3 exhibits color changes in indicator films. The test of pH sensitivity on the indicator film F0 did not indicate any color changes in different pH conditions. Meanwhile, indicator films with an extract addition changed in color in different pH conditions. The more significant the color change, the more the extract concentration added. The indicator film F3 pointed out the most significant color change. It was red in color at a pH of 2 but turned to pink on grounds of gradual pH increases to 5. At a pH of 6-9, its color was purple to blue, and at a pH of 10-11, the color was green. Similarly, Sitanggang et al., (2020) presented research results which stated that films made from gelatin with the extract of sweet purple potato were red in color at a pH of 2 and gradually turned their color when the pH was gradually increased to 5. At pH of 6-9 and 10, they turned to blue and green, respectively. Film that was incorporated with oxalic acid and anthocyanin extract was chosen because it can show a clear color change in pH 2-11.

Solubility of indicator films

The result of the test for the solubility of indicator films is shown in Table 3. The lowest and highest solubility was reached by indicator films F0 and F3, respectively. It attested that the more the amount of extract added, the higher the indicator film's solubility. An indicator film's solubility showcased that each formulation resulted in significantly different solubility in water. A test for solubility was crucial for film-based food packaging to identify film resistance against water (Mustafa et al., 2020).

The high solubility of an indicator film might be owing to the addition of sweet purple potato, which

contained anthocyanins. Anthocyanin increased the number of hydroxyl clusters which could promote affinity for water. High solubility was a result of the weak bond of starch molecules and an increased number of hydrophilic clusters which could absorb water after a starch-anthocyanin interaction. In a sweet purple potato starch-based film with an addition of sweet purple potato extract, the more the extract added, the higher the solubility (Sohany et al., 2021; Zhang et al., 2020).

Swelling capacity of indicator films

A test for swelling capacity was undertaken to measure the capacity of film swelling when the film was in a water environment. Table 3 demonstrates the result of the test for the swelling capacity of indicator films. The highest and lowest swelling capacities were reached by F0 and F3, respectively. In addition, the more the extract added, the lower the capacity of indicator film swelling. The swelling capacity of an indicator film with an addition of sweet purple potato extract was lower than that of swelling in an indicator film without the addition of the same extract. It was on the grounds that the film decreased in mass because of high solubility. It was proven by the result of the test for solubility, that the more the extract of sweet purple potato added, the higher the solubility.

A starch-based film could absorb more water since starch was a hydrophilic polymer which could strongly bind with water (Qin et al., 2020). A decreased capacity of swelling in a film could break out because of the hydrogen bond interaction between polyphenol compounds in the extract and film matrix. A high extract level might generate a potent interaction between starch molecules. Starch molecules would bind more densely, preventing them from floating when in water.

Nevertheless, (Sohany et al., 2021) demonstrated a higher capacity of swelling in a film based sweet potato starch powder/sweet potato peel with an addition of 2% commercial sweet purple potato anthocyanin, while the sweet potato starch film with 0% commercial sweet purple potato anthocyanin showed lower value. The higher capacity was by virtue of the addition of sweet purple potato skin which contain fiber, to the film. The latter addition would expand the surface, surface porous, and channels which could augment water absorption and capacity of film swelling. The higher the swelling capacity, the higher the water-resistance capability of a film (Cornejo-Ramírez et al., 2018).

Table 3. Water Content, Solubility, and Swelling Capacity of the Film Indicators

Sample	Solubility (%)	Swelling Capacity (%)	Water Content (%)
F0	15.40 ± 0.16^{a}	173.80 ± 1.55^{c}	14.77 ± 0.27^{b}
F1	27.06 ± 0.46^{b}	142.20 ± 1.78^{b}	14.13 ± 0.13^{a}
F2	37.95 ± 0.79^{c}	141.55 ± 1.36^{b}	15.11 ± 0.25^{b}
F3	52.15 ± 0.52^{d}	133.90 ± 1.55^{a}	15.46 ± 0.26^{c}

The different superscripted letters show a significant difference ($p < 0.05$). F0 = without-extract film, F1 = film with 5% of extract, F2 = film with 10% of extract, F3 = film with 15% of extract.

Water content of indicator films

Water content, solubility, and swelling capacity were investigated to observe how extract addition influenced the water resistance capability of indicator films. The results of the investigation are exhibited in Table 3. The lowest and highest water content (14.13% and 15.46%, respectively) was reached by indicator films F1 and F3, respectively.

The results of the test for water content indicated that the addition of the extract of ≥ 10% could elevate water content in indicator films. Water content in the indicator film F1 was lower than that in the indicator film F0 due to an extract addition of 5% and decreased amount of aquadest. Therefore, when indicator films were left to dry, more film solvents evaporated as the extract was in ethanol whose boiling point was lower than that of water. Besides, an extract addition at a certain concentration into indicator films would likely form a hydrogen bond which could decrease hydrophilic compounds for the bond of hydroxyl clusters and amino clusters which could induce a restrained binding capability of starch and water (Nguyen et al., 2020). Previous research pointed out that the addition of oleic acid and anthocyanin extract at a certain concentration could decrease water content. This was a result of interactions between protein and water and between protein and lipid. Additionally, it was also possible because

hydrogen bonds created on account of the interaction between anthocyanins and protein could decrease the water content of a film. Hydrogen bonds between film molecules could decrease free water content in that the extract still contained total solids which could affect the water content of the film (Sitanggang et al., 2020).

This section can either be separated (Results and Discussion in different section) or mixed together as one section (Results/Discussion). The results section provides the scientific findings instead of presenting whole/raw data. The discussion should focus on exploring the significance of the work. In general, those sections should describe the results of the experiments, the interpretation of the scientific results, and the conclusions that can be generated. The author may state the weakness of the study here. All tables and figures must be in separated page after the references page.

CONCLUSION

The conclusion that can be drawn from this study is that the addition of purple sweet potato extract had a significant effect on the total film indicator anthocyanins. The film indicator has a pH sensitivity which is indicated by a change in color at different pH conditions. The best formula is F3 with higher anthocyanin extract. The more extract added to the film, the more obvious the color change will be. The more extracts added, the higher the water content and solubility but the lower the swelling strength.

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HYPOGLYCEMIC AND ANTIOXIDATIVE EFFECTS OF CHROMIUM, MAGNESIUM, AND CINNAMON FORTIFIED PARBOILED RICE ON DIABETIC RATS

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ABSTRACT

One of the strategies to manage diabetic blood glucose is through the consumption of foods with a low glycemic index (GI) such as parboiled rice with adequate chromium and magnesium. In diabetes, insulin resistance leads to impaired glucose metabolism and oxidative stress. This oxidative rate can be prevented by adding to the intake of antioxidants from cinnamon. Therefore, this research aimed to determine the effect of chromium, magnesium, and cinnamon fortified parboiled rice on hypoglycemic and antioxidant activities in diabetic rats. The clinical trial treatments on rats with dietary intake were given for 28 days in 6 groups, namely healthy rats with standard feed and diabetic rats with standard feed milled or non-parboiled rice, non-fortified parboiled rice Cr and Mg fortified parboiled rice as well as Cr, Mg, and cinnamon fortified parboiled rice (DRFCP). The analysis carried out included glucose, insulin, malondialdehyde (MDA) levels, and statistical analysis using One-Way Annova. The results showed that in the DRFCP group, glucose and MDA levels decreased significantly ($p < 0.05$) from 258.63 mg/dL to 111.19 mg/dL (57%) and 9.28 ng/mL to 1.96 ng/mL (78.87%), while the insulin levels increased significantly ($p < 0.05$) from 413.97 ug/dL to 540.65 ug/dL (30.60%). This type of feed (DRFCP) can be used as a diet for diabetes because it can reduce blood glucose and malondialdehyde levels.

Keywords: antioxidative; diabetic; hypoglycemic; malondialdehyde; parboiled rice

ABSTRAK

Salah satu strategi untuk mengelola gula darah diabetesi ialah mengonsumsi makanan berindeks glikemik (IG) rendah seperti beras *parboiled*. Selain itu, diabetesi perlu kecukupan kromium dan magnesium. Resistensi insulin pada diabetesi mengakibatkan gangguan metabolisme gula darah, dan *stress oxidative*. Untuk mencegah atau menahan laju oksidatif tersebut dapat ditambahkan asupan antioksidan dari kayu manis. Tujuan penelitian ini untuk mengetahui efek pemberian beras *parboiled* terfortifikasi kromium, magnesium dan kayu manis terhadap efek hipoglikemik dan antioksidatif pada tikus diabetes. Perlakuan uji klinis pada tikus dengan asupan diet diberikan selama 28 hari pada 6 kelompok, yaitu tikus sehat dengan pakan standar, tikus diabetes dengan pakan standar, tikus diabetes dengan beras giling atau *non parboiled*, tikus diabetes dengan beras *parboiled* non-fortifikasi, tikus diabetes dengan beras *parboiled* terfortifikasi Cr, Mg, dan tikus diabetes dengan beras *parboiled* fortifikasi Cr, Mg, kayu manis (DRFCP). Analisis yang dilakukan meliputi kadar glukosa, insulin dan Malondialdehid (MDA). Analisis statistik data menggunakan *One Way Anova*. Hasil penelitian menunjukkan bahwa pada kelompok DRFCP kadar glukosa dan MDA berturut-turut menurun secara nyata ($p < 0,05$) dari 258,63 mg/dL menjadi 111,19 mg/dL (57%) dan 9,28 ng/mL menjadi 1,96 ng/mL (78,87%), sedangkan kadar insulin meningkat secara nyata ($p < 0,05$) dari 413,97 ug/dL menjadi 540,65 ug/dL (30,60%). Pemberian asupan DRFCP dapat menurunkan kadar glukosa darah, dan malondialdehid. Jenis pakan ini dapat digunakan untuk diet penderita diabetes.

Kata kunci: antioksidatif; beras *parboiled*; diabetes; hipoglikemik; malondialdehid

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INTRODUCTION

According to the International Diabetes Federation Diabetes Atlas 2021, the number of people with diabetes in Indonesia in 2021 will reach 19.465 million or 10.8% of the total population and in 2045 it is estimated to reach 28.6 million (IDF, 2021). Due to this large number of patients, there is a need for urgent treatment. One of the strategies to manage diabetes blood sugar is through the consumption of foods with a low glycemic index (GI) (GI < 55) such as rice. Although rice is a staple food with a high GI, its components can be modified to have lower GI by applying several processing methods. These include the parboiling process accompanied by cooling, which can increase the levels of resistant starch (RS). Yulianto et al. (2018) stated that parboiled rice processed by cooling treatment contained RS of 3.75-10.56%. According to Anderson (2008) diabetics are deficient in chromium and magnesium (Dong et al., 2011). Therefore, the availability of these micronutrients is needed to control blood glucose levels and increase insulin action by activating the receptors (Dubey et al., 2020).

The condition of hyperglycemia in diabetes mellitus (DM) patients is indicated by the accumulation of free radicals in the body. This can cause insulin resistance, leading to oxidative stress due to an increase in reactive oxygen species (ROS) with an impact on disrupting the balance of oxidation-reduction reactions (redox), which decreases antioxidants in the body. Therefore, it is necessary to improve glycemic control and antioxidants to inhibit oxidation reactions by free radicals (Réus et al., 2019). One source of high antioxidant food is cinnamon with several main flavonoid contents which are antioxidants, namely cinnamic aldehyde, cinnamyl acetate, and eucalyptol. There are also other chemical compounds in form of phenols, terpenoids, saponins, polyphenols, cinnamaldehyde, and flavonoids as good sources of antioxidants to control blood glucose levels (Talaei et al., 2017).

Yulianto et al (2018) reported that Cr, Mg, and cinnamon fortified parboiled rice soaking at 65°C for 2.5 hours and cooling at 2°C for 12 hours had the lowest GI of 20.03 and the highest RS content of 23.99%. The RS was shown to be negatively correlated with the glycemic index. This indicates the higher the RS in food, the lower the glycemic index. According to Sun et al. (2018), the administration of a high RS type 2 diet, namely a 15 g/kg BW dose can reduce blood glucose levels by 59.65% in STZ-induced rats. Zhu et al. (2017) stated that the cinnamaldehyde in cinnamon reduced glucose and lipid levels, while the presence of polyphenol compounds can prevent insulin resistance in diabetic rats. Therefore, there is a need to investigate the effects of hypoglycemia and antioxidant on diabetic rats fed with chromium, magnesium, and cinnamon fortified parboiled rice.

MATERIALS AND METHOD

Materials and tools.

The main ingredients used to make parboiled rice are prime varieties of Ciherang rice obtained from agricultural shop in Sleman, Yogyakarta, $CrCl_3$, magnesium acetate (Sigma Aldrich), and cinnamon powder (Beringharjo market, Yogyakarta). The materials for the treatment of experimental animals include male Wistar rats aged 2 months, weighing 150-200 g, and are obtained from the laboratory of the Center for Food and Nutrition Studies, Gadjah Mada University Yogyakarta (CFNS UGM) for *in vivo* biological testings. Streptozotocin (STZ) and Nicotinamide (NA) were used for diabetes induction. The AIN-93 standard feed formula includes aquadest, cornstarch, casein, sucrose, soybean oil, mineral mix, vitamin mix, L-cystine, and choline bitartrate (Reeves et al., 1993) This research also used GOD-PAP, CHOD-PAP, and Malondialdehyde (MDA) test reagents including phosphoric acid, while standard TEP (Tetraethoxypropane) and TBA (Thiobarbituric acid) were obtained from CFNS UGM. The tools used include glucose, insulin, and cholesterol kits (DiaSys diagnostic system GmbH, Alte Strasse 9 Holzheim, Germany).

Production of chromium (Cr), magnesium (Mg), and cinnamon extract fortified parboiled rice.

The process of making parboiled rice refers to the method of Yulianto et al. (2018) with modified addition of $CrCl_3$ 5.39 mg/L, Mg acetate 1.75 g/L,

and 10% cinnamon extract. The extraction process was carried out using 400 g of cinnamon powder with 1 L ml of water and blended for 10 minutes. The solution was filtered using a calico cloth and 40% cinnamon extract was stored in a refrigerator at 4°C. The grain to be used was washed 2 times with plain water and 1 time using distilled water with a 1:1.5 ratio of grain to water. Subsequently, 5 kg of grain was sorted and immersed in 3.75 L of a solution containing 20 mg $CrCl_3$, 6.56 g Mg acetate, and 10% cinnamon extract at a temperature of 65°C for 150 minutes. The fortified grain was cooked (boiled) for 20 minutes at a temperature of 100°C. After cooking, the grain was cooled at 0°C for 12 hours and was dried using a cabinet dryer at a temperature of 50°C until the moisture content of the fortified grain reached 13-14%. The dry grain is ground to produce parboiled rice fortified with Cr, Mg, and cinnamon extract.

Testing parboiled rice fortified Cr, Mg, and cinnamon extract on rats.

The use of experimental animals in this research has received ethical approval from the Health Research Ethics Committee, Universitas Respati Yogyakarta No: 355.3/FIKES/PL/X/2019. The animals used are 24 male Wistar rats aged 2 months, weighing 150-200 g. They were divided into 6 treatment groups, namely healthy rats with standard feed (HRSF), and DM rats with standard feed (DRSF), DM rats with milled or non-parboiled rice (DRNP), DM rats with non-fortified parboiled rice (DRNFP), DM rats with Cr, Mg fortified-parboiled rice (DRFP), and DM rats with Cr, Mg, cinnamon fortified-parboiled rice (DRFCP). Before treatment, the rats were adapted for 7 days by administering standard AIN-93 feed and drinking ad libitum. On the 8th day, STZ injection at a dose of 45 mg/kg BW and NA at 110 mg/kg BW dose was administered intraperitoneally, which had been dissolved in 0.1 mol/L sodium nitrate with a pH of 4.5. The glucose, insulin, and MDA were analyzed on the 8th day as initial, the 11th day as pre-treatment, and the 40th day as treatment data

The standard AIN-93 and treatment feed were given, with approximately 15 g/head/treatment group from the 12th to 40th day, which is the 1st to 28th day of treatment. The composition of standard and treatment feed is shown in Table 1.

Measurement of glucose and insulin analysis.

The glucose levels were analyzed using the *Glucose-Oxidase Peroxidase-Aminoantipyrine-Phenol* (GOD-PAP) method with a glucose kit (Diasys diagnostic systems GmbH, Alte-Strasse 9 Holzheim, Germany). The blood sample was taken from blood vessels around the eyes using microhematocrit and centrifuged at 2600 rpm for 20 minutes to obtain serum. Analysis of serum glucose levels was carried out using a kit consisting of standard solutions and reagents. A total of 10 µl sample was mixed with 1000 µl of reagent and 10 µl of aquadest. Subsequently, the solution was incubated at 37^{o}C for 10 minutes and measured at a wavelength of 500 nm absorbance. Measurement of insulin was carried out using the enzyme-linked immunosorbent assay (ELISA) method, which was reacted with monoclonal anti-mouse insulin in the ELISA kit and measured with a microplate reader at a wavelength of 450 nm.

Malondialdehyde (MDA) measurement.

The MDA analysis procedure used the thiobarbituric acid reactive substance (TBARs) method. The blood sample was taken from the blood vessels around the eyes using a microhematocrit and centrifuged at 2600 rpm for 20 minutes to obtain serum for analysis. Approximately 0.1 mL of serum was taken and put into a tube containing EDTA (Ethylene diamine tetraacetic acid). A total of 750 µL of phosphoric acid was put into a 13 mL polypropylene tube and 50 µL of plasma sample was added. The mixture was shaken until homogeneous and 250 µL of 40 mM TBA solution was added. Furthermore, 450 µL of distilled water was added to the tube and tightly closed. The mixture is brought to a boil, cooled in an ice bath, and the sample was applied to an 18-column C Package. The absorbance was measured with a spectrophotometer at a wavelength of 532 nm.

Data analysis.

The statistical analysis used was analysis of

variance (Annova) at the 95% confidence level ($p < 0.05$) to determine the difference between the treatment and the control group (standard feed). When the results are significantly different, the test was continued with the Duncan Multiple Range Test. Paired T-Test was also conducted to determine the difference before and after treatment. Subsequently, the statistical analysis was carried out using SPSS version 25.0 software.

Table 1. Diet Formulation

Ingredient	Treatment Group					
	HRSF	DRSF	DRNP	DRNFP	DRFP	DRFCP
Cornstarch (g)	620.7	620.7	-	163	218	215
Ciherang rice (g)	-	-	899.28	-	-	-
Parboiled rice (g)	-	-	-	656.16	-	-
Cr, Mg – parboiled rice (g)	-	-	-	-	587.54	-
Cr, Mg, cinnamon-parboiled rice (g)	-	-	-	-	-	559.28
Casein (85% protein) (g)	140	140	55.38	70.19	74.02	75.35
Sucrose (g)	100	100	30	50	50	100
Soybean oil (g)	40	40	31.59	33.3	31.59	30.50
Fibre (g)	50	50	0	0	0	0
Mineral mix (g)	35	35	35	35	35	35
Vitamin mix (g)	10	10	10	10	10	10
L-cysteine (g)	1.8	1,8	1,8	1,8	1,8	1.8
Choline bitartrate (g)	2.5	2.5	2.5	2.5	2.5	2.5
Total (g)	1000	1000	1066.04	1022.67	1010.45	1029.47
Energy (kcal)	3603.63	3603.63	3756.69	3604.58	3603.65	3602.36

The healthy rats with standard feed (HRSF), and DM rats with standard feed (DRSF), DM rats with milled or non-parboiled rice (DRNP), DM rats with non-fortified parboiled rice (DRNFP), DM rats with Cr, Mg fortified parboiled rice (DRFP), and DM rats with Cr, Mg, cinnamon fortified-parboiled rice (DRFCP)

RESULTS AND DISCUSSION

Hypoglycemic effect.

In this study, different amounts of cornstarch and sucrose were added to meet the required number of calories, which was equivalent to AIN-93 (3,603.63 kcal). Therefore, the differences in dietary components can be ignored and the study is aimed at how the effect of the type of diet group on the glucose and insulin levels of rats. The glucose levels of rats in all groups before STZ injection were in the normal range, namely <200 mg/dL. Meanwhile, 3 days after the injection, glucose levels were analyzed, and the results obtained are presented in Table 2. The table shows that in the STZ-injected group, blood glucose levels increased significantly to a hyperglycemic state (glucose >200 mg/dL). Based on the analysis of glucose levels in diabetics rate after 28 days of treatment as shown in Table 2, there are significant differences in glucose and insulin levels (Table 3) between the treatment and the control groups ($p<0.05$). The decrease in glucose levels with the highest percentage was in DRFCP at 57.00%, followed by DRFP, DRNFP, and DRNP at 50%, 46.23%, and 32.80%, respectively (Table 2). The largest increase in insulin levels was in DRFCP, which was 30.60%, followed by DRFP, DRNFP, and DRNP at 25.82%, 16.47%, and 11.62%, respectively (Table 3). These results showed the roles of parboiled rice, magnesium, and cinnamon bioactive components. This is in line with the research conducted by Hamad et al (2017), which stated that the administration of parboiled rice can reduce post-prandial blood glucose levels in type 2

Table 2. Glucose Levels Before and After Treatment

Glucose (mg/dL)					
Group	Before STZ Injection	3 Days Post STZ Injection/Before Treatment	Changes After STZ Injection (%)	28 Days of Treatment	Changes After Treatment (%)
HRSF	70.71 ± 5.47	74.70 ± 2.47^{a}	+5.6	77.22 ± 1.56^{aA}	+3.37
DRSF	68.56 ± 4.28	263.19 ± 7.00^{b}	+183.88	269.76 ± 5.23^{fF}	+2.49
DRNP	73.39 ± 2.21	258.03 ± 1.34^{b}	+251.58	139.31 ± 1.49^{dD}	-46.23
DRNFP	71.24 ± 1.56	259.13 ± 5.27^{b}	+263.74	130.54 ± 4.13^{cC}	-50.00
DRFP	73.60 ± 4.18	261.11 ± 2.80^{b}	+254.76	139.31 ± 1.49^{dD}	-46.23
DRFCP	72.85 ± 2.41	258.63 ± 3.61^{b}	+255.01	111.19 ± 4.09^{bB}	-57.00

Notation with different lowercase letters in the same row shows significant differences between treatment groups (One Way Annova, p<0.05). Notation with different capital letters on the same column shows a significant difference before and after treatment (Paired T-Test, p<0.05). The healthy rats with standard feed (HRSF), and DM rats with standard feed (DRSF), DM rats with milled or non-parboiled rice (DRNP), DM rats with non-fortified parboiled rice (DRNFP), DM rats with Cr, Mg fortified parboiled rice (DRFP), and DM rats with Cr, Mg, cinnamon fortified-parboiled rice (DRFCP)

Table 3. Insulin Levels Before and After Treatment

Insulin (ug/dL)					
Group	Before STZ Injection	3 Days Post STZ Injection/Before Treatment	Changes After STZ Injection (%)	28 Days of Treatment	Changes After Treatment (%)
HRSF	566.80 ± 7.81^{a}	563.39 ± 7.73^{b}	-0.60	552.86 ± 8.20^{fF}	-1.86
DRSF	566.80 ± 7.81^{a}	422.11 ± 11.77^{a}	-26.24	405.51 ± 7.98^{aA}	-3.93
DRNP	566.28 ± 2.18^{a}	419.75 ± 6.35^{a}	-2587	484.91 ± 3.04^{cC}	+16.47
DRNFP	577.31 ± 2.71^{b}	415.81 ± 2.32^{a}	-27.97	517.24 ± 6.77^{cC}	+25.82
DRFP	571.01 ± 6.18^{ab}	411.08 ± 4.23^{a}	-28.00	484.91 ± 3.04^{cC}	+16.47
DRFCP	573.89 ± 2.71^{ab}	413.97 ± 7.50^{a}	-27.86	540.65 ± 2.69^{dD}	+30.60

Notation with different lowercase letters in the same row shows significant differences between treatment groups (One Way Anova, p<0.05). Notation with different capital letters on the same column shows a significant difference before and after treatment (Paired T-Test, p<0.05). The healthy rats with standard feed (HRSF), and DM rats with standard feed (DRSF), DM rats with milled or non-parboiled rice (DRNP), DM rats with non-fortified parboiled rice (DRNFP), DM rats with Cr, Mg fortified parboiled rice (DRFP), and DM rats with Cr, Mg, cinnamon fortified-parboiled rice (DRFCP)

DM patients (p <0.05). It has a high RS content and can slow down carbohydrate digestion, thereby increasing satiety and inhibiting motility in the body's gastrointestinal tract. Rouhi et al (2017) discovered that giving magnesium supplements for 4 weeks in DM rats improved insulin resistance, sensitivity, as well as glucose metabolism and also prevent lipid disorders due to diabetes. A previous investigation identified the role of magnesium in insulin receptor phosphorylation and translocation of GLUT-4 from intracellular to plasma membrane. This indicated that magnesium can control blood glucose and has a direct effect on insulin receptors (Morakinyo et al., 2018). Zhu et al. (2017) stated that cinnamaldehyde and polyphenol compounds in cinnamon have the potential to reduce glucose levels and prevent insulin resistance in diabetic rats. Meanwhile, Talaei et al. (2017) reported that giving a cinnamon diet at a dose of 3 g/day for 3 weeks can reduce blood glucose and malondialdehyde and increase insulin levels by activating receptor proteins on cells. This process increases insulin sensitivity and lowers blood glucose levels to near normal. Foods that have dietary fiber and are rich in antioxidants play a role in reducing insulin resistance by capturing free

radicals and reducing inflammation. Therefore, -4 expression increases and causes a GLUT reduction in blood glucose levels (Beji et al., 2018).

Antioxidative effect.

Table 4 showed that there is a significant difference ($p<0.05$) in MDA levels between the treatment group and the control group. The highest decrease in MDA levels was discovered in DRFCP at 78.87%. This is because, in the DRFCP group treated with fortified parboiled rice with chromium, magnesium, and cinnamon, there are phenolic compounds obtained from the addition of 10% cinnamon extract. These results are in line with the research of Tuzcu et al. (2017), where the administration of polyphenols from cinnamon had a significant effect on MDA levels ($p < 0.05$) in rats. The cinnamon extract contains polyphenols that can stimulate antioxidant enzyme activity, thereby preventing oxidative stress and increasing nuclear factor-erythroid-2 related factor 2 (NrF2). Higher activity of NrF2 can also improve the production of antioxidants. Polyphenols have the potential to activate extracellular signal regulatory kinase (ERK) and NrF2, leading to an increase in endogenous antioxidant gene expression (Beji et al., 2018).

Table 4. MDA Levels Before and After Treatment

	MDA (ng/mL)				
Group	Before STZ Injection	3 Days Post STZ Injection/Before Treatment	Changes After STZ Injection (%)	28 Days of Treatment	Changes After Treatment (%)
HRSF	1.09 ± 0.16^{ab}	1.27 ± 0.13^{a}	+16.5	1.64 ± 0.25^{aA}	+29.13
DRSF	1.00 ± 0.34^{a}	8.76 ± 0.42^{b}	+776	9.28 ± 0.36^{dD}	+5.93
DRNP	1.54 ± 0.24^{c}	9.22 ± 0.54^{b}	+498.70	4.91 ± 0.17^{Cc}	-46.74
DRNFP	1.27 ± 0.23^{abc}	9.10 ± 0.51^{b}	+616.53	2.57 ± 0.27^{bB}	-71.75
DRFP	1.23 ± 0.21^{abc}	8.92 ± 0.20^{b}	+625.20	2.73 ± 0.15^{bB}	-69.39
DRFCP	1.41 ± 0.25^{bc}	9.28 ± 0.33^{b}	+558.15	1.96 ± 0.13^{aA}	-78.87

Notation with different lowercase letters in the same row shows significant differences between treatment groups (One Way Anova, $p<0.05$). Notation with different capital letters on the same column shows a significant difference before and after treatment (Paired T-Test, $p<0.05$). The healthy rats with standard feed (HRSF), and DM rats with standard feed (DRSF), DM rats with milled or non-parboiled rice (DRNP), DM rats with non-fortified parboiled rice (DRNFP), DM rats with Cr, Mg fortified parboiled rice (DRFP), and DM rats with Cr, Mg, cinnamon fortified-parboiled rice (DRFCP)

CONCLUSION

Dietary intake of parboiled rice fortified with chromium, magnesium, and cinnamon fortified-parboiled rice (DRFCP) significantly affected glucose, insulin, and malondialdehyde levels of rats. It shows that the administration of the feed to DM rats was best for lowering glucose and MDA levels, and increasing insulin levels. This type of food is suitable as a diet for diabetics.

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THE POTENTIAL OF GLUTINOUS RICE TAPE ADDED WITH *LACTOBACILLUS PLANTARUM* DAD-13 AND *SACCHAROMYCES BOULARDII* AS A PROBIOTIC FOOD

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ABSTRACT

Glutinous rice tape has lactic acid bacteria (LAB) as the dominant microorganism apart from mold and yeast. However, adding *Lactobacillus plantarum* Dad-13 and *Saccharomyces boulardii* can increase its potential as a probiotic food. This study is aimed to determine the effect of using glutinous rice varieties and the addition of probiotic cells on the number of BAL and yeast, levels of anthocyanins, antioxidant activity, as well as the preference level of glutinous rice tape produced. A completely randomized design with a factorial pattern was used in this study. The first factor was glutinous rice varieties (white and black), while the second was the probiotic inoculum type *(Lactobacillus plantarum* Dad 13 and *Saccharomyces boulardii)* which were added simultaneously or individually. The result show that adding the probiotic cells simultaneously or individually in the production of white and black glutinous rice tape increased the LAB amount by 2 log cycles and yeast by 1 log cycle. The addition of *Saccharomyces boulardii* together *with Lactobacillus plantarum*, or individually, resulted in higher anthocyanin levels in black glutinous rice tape than white glutinous tape. The antioxidant activity of white glutinous rice tape was improved by the addition of probiotic cells. For the black glutinous rice tape, the antioxidant activity (87.57-88.61 %RSA) was higher than the activity of white glutinous rice tape (15.58-51.22 %RSA). Furthermore, adding *Lactobacillus plantarum* simultaneously with *Saccharomyces boulardii* increased the preference level on aroma, color, taste, texture, and overall, and produced the white glutinous rice tape that the panelists most favored.

Keywords: black glutinous rice; *Lactobacillus plantarum* Dad-13*;* probiotic glutinous rice tape; *Saccharomyces boulardii*; white glutinous rice

ABSTRAK

Tape ketan yang beredar di masyarakat pada umumnya belum mengandung sel probiotik. Penambahan *Lactobacillus plantarum* Dad-13 dan *Saccharomyces boulardii* dapat meningkatkan potensi tape ketan sebagai pangan probiotik. Tujuan penelitian mengetahui pengaruh penggunaan varietas beras ketan dan penambahan *Lactobacillus plantarum* Dad-13 dan *Saccharomyces boulardii* terhadap jumlah bakteri asam laktat (BAL) dan yeast, kadar antosianin dan aktivitas antioksidan, serta tingkat kesukaan oleh panelis pada tape ketan probiotik yang dihasilkan. Penelitian ini menggunakan rancangan acak lengkap pola faktorial. Faktor pertama ialah varietas beras ketan (putih dan hitam) dan faktor kedua ialah jenis inokulum probiotik (*Lactobacillus plantarum* Dad 13 dan *Saccharomyces boulardii*) yang ditambahkan secara bersamaan maupun individual. Hasilnya menunjukkan bahwa penambahan *Lactobacillus plantarum* Dad-13 secara bersamaan dengan *Saccharomyces boulardii,* atau sendiri pada pembuatan tape ketan putih dan ketan hitam meningkatkan jumlah BAL sebesar 2 log *cycles*, sedangkan penambahan *Saccharomyces boulardii* secara bersamaan dengan *Lactobacillus plantarum* Dad-13, atau individual meningkatkan jumlah yeast sebesar 1 log *cycle*. Penambahan *Saccharomyces boulardii* secara bersamaan dengan *Lactobacillus plantarum* Dad-13, atau individual menghasilkan kadar antosianin pada tape ketan hitam. Penambahan sel probiotik meningkatan aktivitas antioksidan pada tape ketan putih. Aktivitas antioksidan tape ketan hitam (87,57-88,61 %RSA) jauh lebih tinggi daripada aktivitas tape ketan putih (15,58-51,22 %RSA). Penambahan *Lactobacillus plantarum* Dad-13 secara bersamaan dengan *Saccharomyces boulardii* meningkatkan tingkat kesukaan terhadap aroma, warna, rasa, tekstur, dan keseluruhan, dan menghasilkan tape ketan putih maupun ketan yang paling disukai oleh panelis.

Kata kunci: ketan hitam; ketan putih; *Lactobacillus plantarum* Dad-13; *Saccharomyces boularii*; tape ketan probiotik

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INTRODUCTION

Probiotic foods are known to have beneficial effects on consumers. WHO/FAO (2006) defined probiotics as live microorganisms that will offer health benefits to users, provided they are consumed in sufficient quantities (> 6-7 log CFU/g). To ensure the beneficial effects on consumers, the current criteria for probiotics must have the following characteristics: resistance to gastric acidity, bile acid resistance, adhesion to mucus and epithelial cells, antimicrobial activity against pathogenic microorganisms, co-aggregates with pathogens, and bile salt hydrolase activity to fight (resistant) digestive tract complications (Kassaa, 2017).

Based on various strains of lactic acid bacteria (LAB), several *Lactobacillus*, *Bifidobacterium*, and only one yeast species, *Saccharomyces boulardii* are recognized as the main probiotic microorganisms (Kitazawa et al., 2014). Furthermore, bacterial groups, especially *Lactobacillus plantarum* Dad-13 isolated from curd, have shown their potential as probiotics. This includes providing preventive effects such as antidiarrheal and immunomodulatory agents in male rats treated with enteropathogenic treatment (Tari et al., 2016) and reducing the population of *Escherichia coli* and non-*E. coli* coliform bacteria in school-aged children in Indonesia (Rahayu et al., 2021), as well as improving the metabolic syndrome in diabetic rats (Wulandari, 2021). Furthermore, the LAB can produce high folic acid, an essential element in preventing infant defects (Purwandhani et al., 2017). Meanwhile, the interaction of *Saccharomyces boulardii* with the innate immune system has recently opened up new therapeutic potential in cases of intestinal infections and other pathologies associated with dysbiosis, such as inflammatory diseases (Czerucka and Rampal, 2019). In fact, during the Covid-19 pandemic, *Saccharomyces boulardii* has been reported to have a positive (healthy) effect on the host (Pourhossein and Moravejolahkami, 2020).

However, application efforts, especially probiotic yeasts in Indonesia, are still limited. This country is known for having fermented food products that are used as a vehicle for the growth and development of probiotic cells, including glutinous rice tape, both white and black. The glutinous rice tape was selected as a probiotic cell carrier agent because it can be easily made, favored by the public, and consumed daily. Black glutinous rice tape is known to have functional food potential because it is rich in phytochemical compounds in the form of anthocyanins as antioxidant agents. Consumption of black glutinous rice tape up to a certain amount can prevent metabolic syndrome. Respondents who consumed ≤ 11.5 grams of black glutinous rice tape daily had a higher proportion of metabolic syndrome (82.1%). The proportion of the non-metabolic syndrome was greater in respondents who consumed black glutinous rice tape >11.5 grams per day (77.2%) (Fauziyah et al., 2018). Therefore, the probiotic cells of lactic acid bacteria and *Saccharomyces boulardii* are a required ingredient during the manufacture of tape. This study aimed to determine the effect of adding *Lactobacillus plantarum* Dad-13 and *Saccharomyces boulardii* on the amount of LAB and yeast, anthocyanin levels, antioxidant activity of glutinous rice tape, and their preference level by the panelists.

MATERIALS AND METHOD

Materials and tools.

Black and white glutinous rice obtained from Mirota Supermarket in Sleman, Yogyakarta, was used in this study. The lactic acid bacteria used as a source of probiotics were *Lactobacillus plantarum* Dad-13 obtained from the Center for Food and Nutrition Studies (CNFS), Gadjah Mada University, Yogyakarta, Indonesia. Meanwhile, *Saccharomyces boulardii* with the brand Now foods ORI USA were purchased through the online shop Tokopedia. MRS Agar was used as a medium for calculating LAB and PDA as a medium for counting the number of yeast cells. This study also used KCl buffer 0.025 pH 1, sodium acetate buffer 0.4 M pH 4.5, pure methanol, HCL for anthocyanin analysis, pure methanol liquid, and DPPH methanol (0.2M) for antioxidant activity analysis. The main tools used were UV–Vis spectrophotometry (Shimadzu UV mini1240), laminar airflow, Petri dishes, vortex (Maxi Mix II type 37600), and glassware.

Inoculum preparation.

Yeast inoculum was prepared by crushing 4 grains of NKL yeast (10 g) and filtering. Fine yeast was added to 15 g of finely roasted rice flour, and 1.6 g of yeast was used for every 100 g of cooked glutinous rice. The preparation of *Lactobacillus plantarum* Dad-13 was performed using 1 g of its freeze-drying granules (2.10^9 cells) and 19 g of roasted rice flour. Also, 1 g of its starter was used for 100 g of cooked glutinous rice. The yeast was prepared by mixing 1 capsule of (0.28 g containing 5.10^9 cells) *S. boulardii* with 27.72 g of roasted rice flour. Furthermore, 1 g of yeast starter was used for every 100 g of cooked glutinous rice.

The process of making probiotic glutinous rice tape.

The method begins with sorting glutinous rice (1 kg), washing it seven times, and soaking it in well water (1.3 L) for 7 hours. It was rewashed, cooked for 25 minutes using a rice cooker, and produced about 1,400 g of glutinous rice. After cooling of 1-2 hours, 100 g of cooked glutinous rice were added to 6 g of sugar and inoculated with 1 g of NKL yeast, 1 g of *Lactobacillus plantarum* Dad-13, and 1 g of *Saccharomyces boulardii.* Starter inoculation or inoculum according to the experimental design, namely control (only NKL yeast), NKL + *Lactobacillus plantarum* Dad-13, NKL + *Saccharomyces boulardii*, and NKL + a combination of *Lactobacillus plantarum* Dad-13 with *Saccharomyces boulardii*, were used both for cooked white and black glutinous rice. Fermentation was performed for 2 days for white glutinous rice tape and 3 days for black glutinous rice tape (from results of the preliminary study).

Analysis.

Analysis of the research conducted on this probiotic glutinous rice tape includes the number of lactic acid bacteria and yeast (Niamah, 2017), anthocyanin levels (Lee et al., 2005), antioxidant activity determined by the DPPH method (Li et al., 2007), and preference level (Kartika, et al., 1987). The test of preference level includes aroma, color, taste, texture, and overall. This preference level test was conducted with the participation of 30 untrained panelists (laboratory technicians and students of the Agricultural Product Technology Study Program, Mercu Buana University Yogyakarta). The organoleptic test of this glutinous rice tape uses 5 levels of preference scale, namely 1 = Very dislike, 2 = dislike, 3 = slightly like, 4 = like, and 5 = very like.

Experimental design and data analysis.

This study employed a factorial, completely randomized design (CRD) with two treatment factors and two replications. The first factor is the type of glutinous rice (white and black), and the second factor is the addition of inoculum types (NKL yeast, NKL yeast + *Lactobacillus plantarum* Dad-13, NKL yeast + *Saccharomyces boulardii*, NKL yeast + *L. plantarum* Dad-13 + *S. boulardii)*. The data obtained were evaluated using the ANOVA statistical method with SPSS software version 22.0, and if there was a significant difference between treatments, Duncan's Multiple Range Test ($p < 0.05$) was used.

RESULTS AND DISCUSSION

The number of lactic acid bacteria and yeast.

Table 1 shows the results of calculating the number of lactic acid bacteria and yeast on white glutinous rice tape after two days of fermentation and on black glutinous rice tape after three days. Tape quality testing is carried out when the two products reach their optimal maturity. According to the data, adding LAB *(Lactobacillus plantarum* Dad-13*)* and yeast *(S. boulardii)* can increase the total amount of LAB and yeast at the end of the fermentation of white and black glutinous rice tape.

The use of NKL yeast inoculum resulted in the amount of LAB and yeast on white and black glutinous rice tape, 4.4 - 4.5 x 10^6 CFU/g and 3.7 - 3.9 x 10^5 CFU/g, respectively. After adding the LAB and yeast probiotic cells, the amount of LAB and yeast either separately or simultaneously on white and black glutinous rice tapes ranged from 4.7 - 8.04 x 10^8 CFU/g for LAB and 4.9 - 7.4×10^7 for yeast. Comparing NKL alone, the inclusion of LAB and yeast could increase 100 times or 2 log cycles from 10^6 to 10^8 CFU/g for LAB and from

10^5 to 10^7 CFU/g for yeast. The two types of raw materials, white and black glutinous rice used, show almost the same population. Therefore, these materials can be used to make probiotic tape once combined with LAB *(Lactobacillus plantarum* Dad-13*)* and yeast *(S. boulardii)*. The table also reveals that the maximal amounts of LAB and yeast were produced by this combination. These findings also point to synergistic growth between the two added probiotic cells. In general, the amount of LAB at the end of fermentation qualifies as a probiotic food with a minimum LAB content of 10^6-10^7 CFU/g (WHO/FAO, 2006).

Table 1. The Number of LAB and Yeast of Glutinous Rice Tape Type with the Addition of Probiotic Cells

Type of probiotic addition	Amount of BAL (CFU/g)		Amount of yeast (CFU/g)	
	Type of glutinous rice			
	White	Black	White	Black
NKL	4.4×10^6	4.5×10^6	3.9×10^5	3.7×10^5
NKL + *Saccharomyces boulardii*	5.5×10^6	6.8×10^6	4.9×10^7	5.3×10^7
NKL + *Lactobacillus plantarum* Dad-13	4.7×10^8	7.2×10^8	4.8×10^7	4.5×10^7
NKL + *Saccharomyces boulardii* + *Lactobacillus plantarum* Dad-13	6.24×10^8	8.0×10^8	7.1×10^7	7.4×10^7

The values in the table are the mean of two replicates and two batches

Anthocyanin levels and antioxidant activity.

Table 2 presents the average anthocyanin levels and antioxidant activity of probiotic tape from glutinous rice varieties with the addition of inoculum types. In general, the average anthocyanin content in black glutinous rice tape (2.35 mg/100 g) was relatively higher than that of white glutinous rice tape (0.63 mg/100 g). The addition of *Saccharomyces boulardii* simultaneously with *Lactobacillus plantarum* Dad-13 or separately resulted in higher anthocyanin levels in black glutinous rice tape than in white glutinous rice tape. The anthocyanin levels of black glutinous rice are not much different from the results of the research by Fauziyah et al (2018). The results showed the average anthocyanin content in black glutinous rice tape on the 3rd to 5th day of fermentation was 3.02 mg/100 g. Furthermore, this study's average anthocyanin content was 3.73 times that of white glutinous rice tape. This is owing to the higher anthocyanin concentration of black glutinous rice raw material than white glutinous rice. According to Indrasari et al. (2010), the anthocyanin content in Ciherang rice (white rice) with a milling degree of 100% was 0.26 mg/100 g, while the brown rice variety BH390-MR-11-1-1-6 with a milling degree of 80% and 100% were 2.02 mg/100 g and 2.01 mg/100 g, respectively. The average reduction in anthocyanin content during the cooking process from milled rice with 80% and 100% milling degrees to cooked rice was about 81% and 83%, respectively. Sompong et al. (2011)reported anthocyanin levels in black rice (glutinous) ranged from 109.52 - 256.61 mg/100 g, while in brown rice, they ranged from 0.33 to 1.39 mg/100 g. Furthermore, cyanidin 3-glucoside and peonoidin 3-glucoside were confirmed as dominant anthocyanins in black rice varieties, with contents ranging from 19.4 to 140.8 mg/100 g dry weight and 11.1–12.8 mg/100 g dry weight, respectively. Anthocyanins present in plants are glycosides linked to sugar components (Ávila et al., 2009). The first step in the breakdown and absorption of anthocyanins in the body is the hydrolysis of anthocyanin glycosides (Keppler and Humpf, 2005). Several species of lactic acid bacteria have glucosidase enzyme activity and contribute to the hydrolysis of dietary glycosides (Ávila et al., 2009). The β-glucosidase enzyme is an enzyme that is trapped in the cell wall and has extracellular activity. It is produced by the bacteria *L. plantarum* Mut 7 (Suhartatik et al., 2013), *Pediococcus pentosaceus* N11.16 (Suhartatik et al., 2014), a subspecies of *Lactobacillus plantarum*, *Lactobacillus casei* LC-01 and *L. casei* IFPL7190 (Ávila et al., 2009).

Table 2. Anthocyanin Content and Antioxidant Activity of Glutinous Rice Tape Type with the Addition of Probiotic Cells

Type of probiotic addition	Anthocyanin content (mg/100 g)		Antioxidant activity (%RSA)	
	Type of glutinous rice			
	White	Black	White	Black
NKL	$0,85 \pm 0,00^{a}$	$1,90 \pm 0,11^{abc}$	$15,58 \pm 0,36^{a}$	$87,57 \pm 3.20^{e}$
NKL + *Saccharomyces boulardii*	$0,41 \pm 0,08^{a}$	$3,13 \pm 0,13^{c}$	$20,95 \pm 0,24^{b}$	$87,99 \pm 4.17^{e}$
NKL + *Lactobacillus plantarum* Dad-13	$0,62 \pm 0,08^{a}$	$1,46 \pm 0,19^{ab}$	$32,93 \pm 0,33^{c}$	$88,02 \pm 8.12^{e}$
NKL + *Saccharomyces boulardii* + *Lactobacillus plantarum* Dad-13	$0,62 \pm 0,08^{a}$	$2,92 \pm 0,48^{bc}$	$51,22 \pm 1,09^{d}$	$88,61 \pm 3.24^{e}$

Numbers followed by different letter notations in the same column are significantly different ($p<0.005$)

Table 2 shows that the addition of the inoculum did not significantly affect the anthocyanin levels in white glutinous rice tape. Meanwhile, the content of black glutinous rice was significantly affected ($p<0.05$) and adding *S. boulardii* increased the anthocyanin levels compared to adding *L. plantarum* Dad-13. The increase in anthocyanin levels in the black glutinous rice tape could be due to the higher β-glucosidase activity produced by *S. boulardii* than that of *L. plantarum Dad-13*. This enzyme helps the process of hydrolysis of glycosides into sugars and their aglycones.

The antioxidant activity of white glutinous rice tape is not affected by anthocyanin levels. Although, it can be induced by enzyme activity, the number of probiotic cells, and microorganisms from NKL. Table 2 also shows that adding inoculum did not significantly affect anthocyanin levels (0.41-0.85 mg/100 g) in white glutinous rice tape but greatly increased its antioxidant activity. Adding *L. plantarum* Dad-13 with *S. boulardii* on white glutinous rice tape either simultaneously or separately increased antioxidant activity compared to control glutinous rice tape. The increase in antioxidant activity can be due to the action of *L. plantarum* Dad-13 and *S. boulardii,* which can potentially help the degrading of anthocyanin content and total phenol to increase antioxidant activity. According to Fauziyah et al. (2018), the highest activity of black glutinous rice tape at 70.2% with a total phenol of 73.38 mg/100 g and anthocyanins (flavonoids) of 2.57 mg/100 g was achieved on the 3rd day of fermentation. The combination of *L. plantarum* Dad-13 *and S. boulardii* provided the maximum antioxidant activity in white glutinous rice tape (51.22%), followed by *L. plantarum* Dad-13 (32.93%), *L. plantarum* Dad-13 (20.95). %), and NKL (15.58%). Table 1 shows that the magnitude of this antioxidant activity is in line with the size of the total cells of lactic acid bacteria and yeast in white glutinous rice tape.

Meanwhile, the antioxidant activity of black glutinous rice tape ranged from 87.57-88.61 (%RSA). This was higher than that of white glutinous rice tape (15.58-51.22%RSA) and was not affected by the addition of inoculum type. The high antioxidant activity of black glutinous rice tape may be due to a combination of anthocyanin levels and compounds fermented by black glutinous rice tape. According to Datta et al. (2017), the extracellular fraction of the *S. cerevisiae var. boulardii* cultures was rich in polyphenolic metabolites such as vanillic acid, cinnamic acid, phenyl ethyl alcohol (rose oil), erythromycin, amphetamine and vitamin B6. This resulted in the strain having a 6-10-fold more significant antioxidant potential judged by the DPPH assay. Moreover, Avila et al. (2009) reported that with malvidin-3-glucoside as a substrate, aglycones would be fermented into gallic acid, homogentisic, syringic, p-coumaric acid, sinapic, and DMB propionic acid. This also included other compounds not identified by cell-free extracts of *Bifidobacterium lactis* BB-12, *Lactobacillus plantarum* IFPL722, *L. casei* LC-01, *L. acidophilus* LA-5. These bacteria also showed β-glucosidase enzyme activity. According to Parrella

et al. (2012), the probiotic yeast *S. boulardii* could use fermented goods to develop, preserve the stability of lactic acid bacterial strains while stored and boost the antioxidant characteristics of the final fermented product. The development of probiotic yeast in combination with LAB seems to increase the stability of these microorganisms. Furthermore, *L. plantarum* was previously found capable of degrading hydroxycinnamic and hydroxybenzoic acids (gallic and protocatechuic acid) through phenolic acid decarboxylation and reduction processes (Rodríguez et al., 2008). Gallic acid, for example, is decarboxylated to form pyrogallol, a potent antioxidant.

Preference level.

The panelists' preference for probiotic glutinous rice tape was determined using an organoleptic test. Aroma, color, taste, texture, and the overall probiotic glutinous rice tape produced were the quality attributes evaluated. Table 3 shows the preference test results on white and black glutinous rice tape with the addition of the inoculum type.

Table 3. Preference Level of Glutinous Rice Tape Type with the Addition of Probiotic Cells

Type of glutinous rice tape	Type of probiotic addition	Test parameters				
		Aroma	Color	Taste	Texture	Overall
White	NKL	3,43±0,73^{a}	3,67±0,70^{b}	3,90±0,89^{b}	2,87±1,14^{ab}	3,63±0,85^{b}
	NKL + S.b	3,87±0,73^{ab}	3,73±0.74^{b}	3,77±0,77^{b}	3,43±1,04^{c}	3,77±0,82^{b}
	NKL + L.p	3,53±0,68^{b}	3,50±0,68^{b}	3,60±0,93^{b}	3,23±0,77^{bc}	3,50±0,73^{a}
	NKL + S.b + L.p	4,47±0,63^{c}	4,20±0,81^{c}	4,40±0,72^{c}	4,13±0,073^{d}	4,60±0,56^{c}
Black	NKL	3,60±0,81^{ab}	3,03±0,85^{a}	2,90±0,76^{a}	2,70±1,02^{a}	3,17±0,59^{a}
	NKL + S.b	3,80±0,61^{ab}	3,53±0,63^{b}	2,80±1,13^{a}	3,10±0,85^{abc}	3,17±0,91^{a}
	NKL + L.p	3,90±07^{b}	3,57±0,63^{b}	2,50±1,04^{a}	2,90±1,03^{ab}	3,17±0,87^{a}
	NKL + S.b + L.p	4,47±0,63^{c}	4,43±0,73^{c}	3,77±0,50^{b}	4,30±0,75^{d}	4,27±0,69^{c}

Numbers followed by different letter notations in the same column are significantly different (p<0.005); S.b= *Saccharomyces boulardii*, L.p= *Lactobacillus plantarum* Dad-13; 1=dislike very much, 2=dislike, 3=a little like, 4=like, 5= like very much

Aroma. Glutinous rice tape with the addition of *Lactobacillus plantarum* Dad-13 and *Saccharomycess boulardii* simultaneously provided the highest aroma value (like to very like) with a rating of 4.47 for white glutinous rice tape and 4.47 for black glutinous rice tape. The addition of *Lactobacillus plantarum* Dad-13 can also significantly increase the preference level for the aroma of both white (3.53) and black glutinous rice tape (3.90) compared to the addition of NKL yeast separately (3.43-3.60). This addition produced the right amount of lactic acid and provided the conditions for growing bacteria, yeast, and mold from NKL. Therefore, it yields volatile compounds (aroma) favored by the panelists, such as, ethanol, acetic acid, and esters. The ethanol content of white glutinous rice and black glutinous rice in this study was 1.30% and 1.79%, respectively (Yulianto and Pujimulyani, 2021). Utaminingdyah et al. (2022) reported that tape from various types of rice (non-glutinous) inoculated with *Lactobacillus plantarum* Dad-13 produced lactic acid levels, acetic acid, and ethanol, respectively, at 0.21-0.53%, 0.14-0.35%, and 0.81-0.91%. Cronk et al. (1979) showed that *Amylomyces rouxii*, separately or in combination with different yeasts, yielded a higher range of alcohols, including isobutanol (2-methyl-1-propanol), 2-methyl-1-butanol and isoamyl alcohol (3-methyl - 1-butanol).

Color. The most preferred colors of white and black glutinous rice tape are the results of adding *Lactobacillus plantarum* Dad-13 and *Saccharomyces boulardii* simultaneously. For the

black glutinous rice tape, the most preferred color is black because the anthocyanin content and yellowness value (b*) are higher than the control tape (Yulianto and Pujimulyani, 2021). Meanwhile, the preferred white glutinous rice tape color tends to be yellowish-white and slightly lighter. In addition to the anthocyanin content, especially in black glutinous rice tape, the growth of yeasts such as *Saccharomyces cerevisiae, Candida,* and *Hansenulla* can affect the color intensity of glutinous rice tape.

Taste. The tape has a sweet, alcoholic, and slightly sour taste. Sweet taste is produced from sugar formed primarily from the hydrolysis of starch by amylase produced by fungi. Furthermore, alcohol is formed from the fermentation of glucose by yeast, and lactic acid is formed from the fermentation of sugar to lactate by lactic acid bacteria. Meanwhile, acetic acid is produced by the oxidation of alcohol by acetic acid bacteria. The fungi, yeast, and bacteria were on the tape from the NKL inoculum and the addition of the probiotic cells (*Lactobacillus plantarum* Dad-13 and *Saccharomyces boulardii*). Table 3 shows that the white and black glutinous rice tapes with the probiotic cells were favorites of the panelists with scores of 4.40 (like to very like). The sugar, alcohol, and acid ratio may have influenced the decision of this taste preference level. Furthermore, the total sugar content, ethanol, and pH of white and black glutinous rice tape with the inclusion of the two probiotic cells were 7.73%, 3.20%, 1.30%, 1.79%, 5.04, and 4.57, respectively (Yulianto and Pujimulyani, 2021).

Texture. The simultaneous addition of LAB (*Lactobacillus plantarum* Dad-13) with yeast (*S. boulardii)* increased the panelists' preference for texture features on white and black glutinous rice tape. Moreover, NKL inoculum containing fungi, especially those with high activity of amylase, plays a vital role in the hydrolysis of starch into sugar, which also impacts the softness of the glutinous rice tape texture produced. The combination of the LAB and yeast also gave a certain softness level, which the panelists may prefer. Meanwhile, only LAB or yeast alone did not provide the texture the panelists liked. This could be because the inclusion of the two probiotic cells was sufficient to enable conditions for the growth of amylolytic fungus, resulting in a texture of glutinous rice tape that was neither too soft nor too hard. Panelists prefer probiotic glutinous rice tape with a relatively soft and watery texture.

Overall. The results of the level of preference test of the overall quality attributes of white and black glutinous rice tapes generally have a rhythmic relationship with the results of the preference level for each feature on aroma, color, taste, and texture quality. Furthermore, white glutinous rice tape and white glutinous rice were added with both probiotics simultaneously, and the panelists preferred this. The tape with the two types of probiotic cells had the highest overall preference rating (4.6 = like to very like), followed by *S. boulardii*, the control (NKL), and finally *L. plantarum* Dad-13. Moreover, adding only *L. plantarum* Dad-13 to the white glutinous rice tape production decreased the preference level of the panelists compared to the control. This was due to sufficient lactic acid formed, which causes the sour taste and is less liked by the panelists. Meanwhile, the panelists' preference for black glutinous rice tape that was only inoculated with *L. plantarum* Dad-13 or *S. boulardii* was not significantly different from the tape with NKL separately (control).

CONCLUSION

The addition of *Lactobacillus plantarum* Dad-13 simultaneously with *Saccharomyces boulardii*, or separately in the production of white and black glutinous rice tape increased the number of lactic acid bacteria by 2 log cycles and adding *Saccharomyces boulardii* simultaneously with *Lactobacillus plantarum* Dad-13 increased yeast count by 1 log cycle than control (NKL). The addition of *Saccharomyces boulardii* simultaneously with *Lactobacillus plantarum* Dad-13 or separately resulted in higher anthocyanin levels in black glutinous rice tape than in white glutinous rice tape. The antioxidant activity of black glutinous rice tape (87.57-88.61 %RSA) was higher than that of white glutinous rice tape 15.58-51.22 (%RSA). The addition of *Lactobacillus plantarum* Dad-13 simultaneously with *Saccharomyces boulardii* increased the preference

level for aroma, color, taste, texture, and overall, as well as resulted in white glutinous rice tape and glutinous rice, which the panelists most favored. Glutinous rice tape added with *Lactobacillus plantarum* Dad-13 and *Saccharomyces boulardii* has the potential as a probiotic food.

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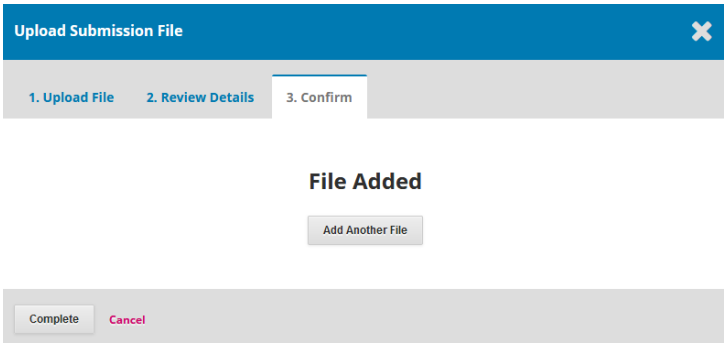

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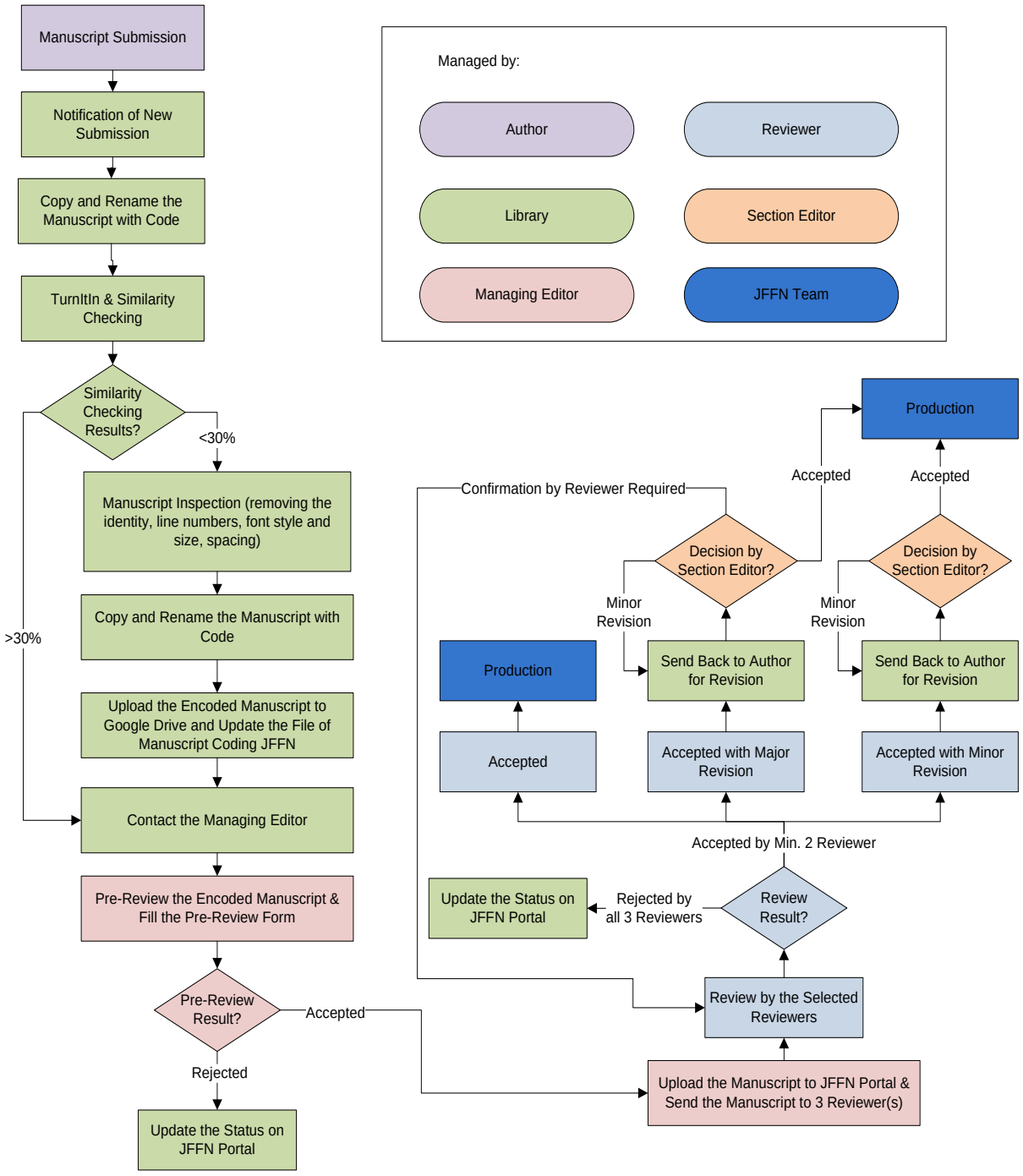

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