Short communication

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# Black Grass Jelly Encapsulated *Lactobacillus plantarum* Mar8 in Honey and D-Allulose Beverage Enriched with Mangosteen Pericarp Extract

**Titin Yulinery[1*], Novik Nurhidayat[1], Nilam Fadmaulidha Wulandari[2], Sri Widawati[1] Suliasih Suliasih[1] Lusianawati Widjaja[1]**

1- Research Center for Applied Microbiology, National Research and Innovation Agency (BRIN), Cibinong, Indonesia
2- Research Center for Biosystematics and Evolution, National Research and Innovation Agency (BRIN), Cibinong, Indonesia

## Abstract

**Background and Objective:** Black grass jelly served in sweet syrup is one of the Chinese and East and Southeast Asian traditional beverages. An innovative enrichment can make it a better functional food. This study innovatively enriched the black-jelly food with formulas of probiotic *Lactobacillus plantarum* Mar8, honey, D-allulose and mangosteen pericarp extract. The probiotic viability, antioxidant and hypoglycemic potential were investigated as well.

**Material and Methods:** Ready-to-drink functional beverages included mangosteen pericarp extract varied in concentrations of 0.1, 0.2 and 0.4 mg $ml^{-1}$, D-allulose in honey and encapsulated probiotic *Lactobacillus plantarum* Mar8 in black grass jelly containing konjac and carrageenan. The probiotic viability, antioxidant activity and hypoglycemic potential were the selective parameters for the functional beverage formulas. The viability of probiotic *Lactobacillus plantarum* Mar8 was assessed using total plate count method. Antioxidant activity was assessed based on the reaction of 2,2-Diphenyl-1-picrylhydrazyl radical scavenging. Hypoglycemic potential was investigated by counting petite yeast cells after treating with black grass jelly formulas. Significant differences were reported using one-way analysis of variance and Duncan's test. Statistically significance included *p*-values≤0.05.

**Results and Conclusion:** The probiotic *Lactobacillus plantarum* Mar8 encapsulated in black grass jelly survived well in the honey, D-allulose and mangosteen pericarp extract formulated beverages. Honey supported the probiotic viability better, producing further antioxidants and high potentials in hypoglycemia than that those of other formulas did. Mangosteen pericarp extract enriched the functionality of the black grass jelly probiotic beverages. However, further studies are needed to assess favorability and stability of this functional food.

**Conflict of interest**: The authors declare no conflict of interest.

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***Corresponding author:**

**Titin Yulinery***
Research Center for Applied Microbiology, National Research and Innovation Agency (BRIN), Cibinong, Indonesia
Tel: +628161980768

E-mail:
titi003@brin.go.id

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## 1. Introduction

One of functional foods is the black grass jelly (BGJ) made from the leaf of Cincau [(*Platostoma palustre* (Blume) A.J. Paton)] as a lipid lowering agent and antioxidant. The BGJ is traditionally served in a sweet syrup, mostly sweetened with common powder and rock sucrose sugars as well as brown sugar [1]. Honey and D-allulose have been used as substitute sweeteners. Honey has generally been addressed for nutritious beneficial, especially for digestive system microbiome [2]. Moreover, D-allulose is a low-calorie sweet that good for food ingredient and dietary supplement. It acts in better insulin resistance, antioxidant enhancement and hypoglycemic control [3]. One of the popular nutritious herbals is mangosteen (*Garcinia mangostana* L.) pericarp extract (MPE). The MPE includes xanthanes of mangostin, mangosterol, mangostinons A and B, trapezifolixanthone, tovophyllin B, flavonoid, epicatech-in, garcinone B and gartanin [4]. Alfa mangostin is the major xanthone that may serve as an antioxidant, anti-inflammatory and antidiabetic agent. Those xanthones can prevent diseases associated to obesity [5,6].

*Lactobacillus* species plays essential roles in preventing diseases and maintaining functional digestive system and the immune system [7]. The probiotic *Lactobacillus* (*L.*) *plantarum* produces extracellular mannose-specific adhesion,

specifically for mannose receptors on the epithelial surface of digestive system [8,9]. It allows probiotic cells to compete with the pathogenic bacteria and to facilitate pathogen agglutination inducing macrophage phagocytosis and immune system cascade activation [8]. The probiotic needs protection ensuring its viability from the food processes and storage and must be consumed to reach its functional task. Encapsulation is a choice method of coating bacterial cells [10]. Alternative to the common polysacch-arides and proteins, BGJ can be used as coating encaps-ulation material with carrageenan and konjac to preserve the jelly's elasticity [11]. However, carrageenan and konjac are relatively expensive and may include high water-binding capacities for a tighter space between the particles, where further water is bound and trapped, decreasing occurrence of syneresis. The aim of this study was to innovatively enrich a ready-to-drink functional beverage of the BGJ carrying encapsulated probiotic *L. plantarum* suspended in presence of D-allulose or honey as natural sweeteners. Functional parameters included the probiotic viability, antioxidant characteristic and petite yeast number as indicator assay for hypoglycemic and anti-obesity situations.

## 2. Materials and Methods

### 2.1. Probiotic culture and encapsulation

The probiotic *L. plantarum* Mar8 was cultured in glucose yeast peptone [10 g of glucose (Merck, Germany), 10 g of yeast extract (Himedia, India), 5 g of tryptone (Himedia, India), 2 g of beef extract (Himedia, India), 1.4 g of sodium acetate (Himedia, India), 5 ml of salt solution (Himedia, India), 0.5 g of Tween 80 (Applichem, Germany) and distilled water up to 1 l]. For the solid media, 20 g agar (Himedia, India) were added. To achieve the probiotic biomass, several steps were carried out. First, the probiotic bacteria were cultivated in 100 ml of glucose yeast peptone broth for 24 h at 37 °C. Second, a volume of 10 ml of the culture was transferred into and culture in 1,000 ml of glucose yeast peptone broth for 24 h at 37 °C (Sanyo, Japan). Third, culture was harvested for its biomass by centrifugation (Kubota, Japan) at 5,000× g for 10 min at 4 °C. Fourth, pellets were washed twice with 0.85% NaCl (Merck, Germany) prior to the encapsulation. Practically, the harvested probiotic biomass was suspended in the warm (40 °C) liquid containing the coating materials of 4% BGJ, 0.5% konjac and 0.2% carrageenan and then set to solidify at room temperature (RT) for 1 h. The solidified BGJ containing probiotic was cut into cubes of 1 × 1 × 1 cm [11].

### 2.2. Preparation of mangosteen pericarp extract

The MPE was extracted from 10 g of dried mangosteen peels with 200 ml boiling sterile water for 15 min, filtered and then centrifuged at 6,980 g for 5 min. Extract was transferred into a sterile tube and stored until use [12].

### 2.3. Ready-to-drink beverage formulations

The three formulated BGJ ready-to-drink beverages in 100 ml were: 1. BGJ beverages contained 10 cubes of BGJ encapsulated probiotics and sweetened with 3.5% (w $w^{-1}$) honey containing 1.3% D-allulose (mangosteen honey and d-allulose), 2. BGJ beverages contained 10 cubes of BGJ encapsulated probiotics sweetened with 3.5% (w $w^{-1}$) natural honey (mangosteen honey) and 3. BGJ beverages contained 10 cubes of BGJ encapsulated probiotics and water not sweetened (mangosteen and water). The MPE was added to each beverage in total concentrations of 0.1, 0.2 and 0.4 mg $ml^{-1}$, respectively.

### 2.4. Enumeration of viable probiotic Lactobacillus plantarum cells

Probiotic cells in BGJ were enumerated immediately after formulation and 14 d later using total plate count method in three replications. Practically, 1 g of the BGJ sample was homogenized, suspended in 9 ml of sterile distilled water and serially diluted up to nine serial dilutions. Then, 0.1 ml of the last three dilutions were spread-plated onto GYP media. Plates were incubated at 37 ºC for 48 h. Bacterial colonies were counted and viable numbers were reported based on the dilutions [8,9].

### 2.5. Antioxidant activity assessment

Antioxidant capacity of the BGJ was assessed based on 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging. Basically, 500 µl of each BGJ formula sample and the standard ascorbic acid antioxidant samples of various concentrations with two-fold serial dilutions were added to 500 µl of 0.2 mM methanolic solution of DPPH. All solutions were homogenized using vortex mixers (Sibata, Japan) and then incubated for 30 min at RT. Absorbances at λ517 nm of 1/20 diluted solutions were measured using UV-vis spectrophotometer (Shimadzu, Japan). Ability of scavenging DPPH radical was expressed as percentage inhibition [13].

### 2.6. The petite yeast-cell frequency

The aim of this assay was to report the best selected BGJ formula potential in prevention of obesity-linked diseases [20]. *Saccharomyces cerevisiae* yeast as a cell model [14] was cultured in yeast malt broth (peptone, 5.0 g yeast extract, 3.0 g; malt extract, 3.0 g; dextrose, 10.0 g; and distilled water, up to 1 l; final pH at 25°C = 6.2 ±0.2). Positive control of petite yeast cell was cultured in the yeast malt petite media; in which, YM was supplemented with additional 0.1% glucose concentration with 1% ethanol. All cultures were incubated at 30 °C for 24 h to reach cell density as measured optical density at λ 600 nm was ~0.1. The yeast cells were then centrifuged at 5,000× g for 3 min at 4 °C to harvest the cells. This was resuspended and washed twice with sterile DW. Assay was carried out in 1 ml total volume

of YM containing 100 µl of yeast cell suspension and with or without 18 µl of the test BGJ formula. All treatments were incubated at 30 °C for 24 h; then, yeast suspension was diluted to $10^{-4}$ and plated onto the YM petite media agar and YM for the controls. All plates were incubated at RT for 48 h. Petite colonies of approximately 50% smaller of the normal ones were counted. The petite frequency (%) was calculated for the total colonies of petite and normal ones.

### 2.7. Statistical analysis

The IBM SPSS statistics software v.24.0 and Microsoft Office Professional Plus 2019 Excel were used for the analysis of variance. Then, Microsoft Excel was used to create tabular and graphical representations of the results. Significant differences were recorded using one-way analysis of variance with Duncan's test. In general, *p*-values $\leq 0.05$ were considered statistically significant.

## 3. Results and Discussion

### 3.1. The viable probiotics

The BGJ encapsulated probiotics survived in BGJ ready to drink formulas (Figure 1). The probiotic viability was significantly slightly reduced, yet it remained in log of 9 after 2 weeks of storage. Probiotics remain viable and active even under high water content during storage [15]. This log 9 number is in line with the general effective probiotic dosage in the range of log 7 to log 9 per day. Pharmaceutical preparation of probiotics usually contains around log 9 per dose, whereas in the food products usually have often between log 6 to log 7 so that it required a relatively high volume to provide enough amount of about log 9 per dose [16]. The MPE affected the probiotic viability. Addition of 0.4 mg $ml^{-1}$ MPE supported the probiotic viability better than that other concentrations did (Figure 1). This water extracted MPE included relatively further polar compounds that supported better probiotic growth, promoting gastrointestinal health. The polarity extract played important roles; for example, polar methanol extract of mangosteen pericarp promoted the growth of *Lactobacillus acidophilus* [17] better than that of less polar chloroform extract [18].

Active probiotics in BGJ ready-to-drink could play important roles in production of important functional molecules such as mannose specific adhesion, gamma-aminobutyric acid, amino acids such as Asp, Glu, Gly and Ala and minerals such as iron and zinc. The probiotic *Lactobacillus plantarum* produces extracellular mannose-specific adhesin specifically for mannose receptors on the epithelial surface of digestive system [8,9,19]. It allows the probiotic cells to colonize epithelial surfaces of the intestines actively and hence compete with the pathogenic bacteria. Moreover, it binds to agglutinate pathogenic bacterial cells and induces macrophage phagocytosis, leading to the immune system cascade activation [8,20,21].

After two weeks of storage, natural honey in the ready-to-drink significantly supported probiotic viability relatively better than that of honey containing D-allulose. Probiotics fermented oligosaccharides in honey to yield beneficial metabolites promoting the probiotic growth [2]. Certain lactic acid bacteria produce further acid without altering their probiotic activity [22].

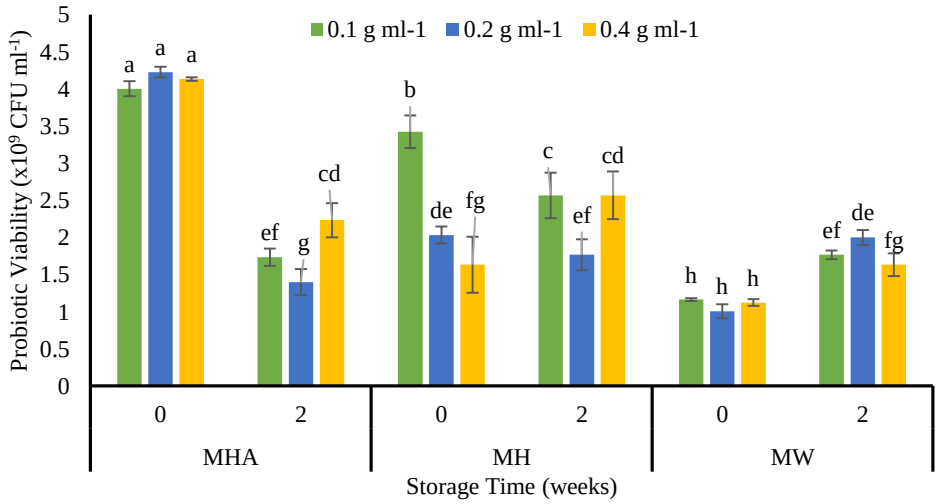


**Figure 1.** The viable number of probiotics *Lactobacillus plantarum* Mar8 (log 9 CFU.$g^{-1}$) in three various ready-to-drink beverage formulations for two weeks of storage. Different letters state significant differences ($p \leq 0.05$). Mangosteen honey and D-allulose was the beverage contained ten cubes of black grass jelly (BGJ) encapsulated probiotics and sweetened with 3.5% (w $w^{-1}$) honey containing 1.3% D-allulose. Mangosteen honey was the beverage contained ten cubes of BGJ encapsulated probiotics sweetened with 3.5% (w $w^{-1}$) natural honey. Mangosteen and water was the beverage contained ten cubes of BGJ encapsulated probiotics and water not sweetened. All formulas were supplemented with mangosteen pericarp extract at concentrations of 0.1, 0.2 and 0.4 mg $ml^{-1}$, respectively.

This might occur in BGJ encapsulated probiotic *L. plantarum* Mar8. However, added D-allulose did not alter growth efficiency in other probiotics such as *L. delbrueckii*, *Streptococcus thermophilus* and 4 strains of lactococci [22]. Interestingly, *L. plantarum* play important roles in D-allulose production from honey and other sugar such as Jujube syrup [23]. However, without added sweeteners, the probiotic cells were able to grow. Organic substances in BGJ and MPE supported the heterotrophic probiotic cell growth [17].

### 3.2. Antioxidant activity

Simple DPPH analysis indicated that all of the BGJ beverage formulas included antioxidant activity and varied according to their enriched components (Figure 2). All of the components alone and in combination played their antioxidant activities in the formulated BGJ ready-to-drink. However, it is not clear which component played the major or minor activity. The BGJ high antioxidant activity might be due to its natural phenolic component [13]. The BGJ ready-to-drink formulas containing honey and D-allulose included relatively higher percentage inhibition values that indicated a higher radical scavenging potential than those of the beverage containing natural honey. In fact, increasing concentration of D-allulose decreased the DPPH radical scavenging activity [19]. Supplementation of MPE at lower concentration relatively increased antioxidant activity in the beverage formulation (Figure 2).

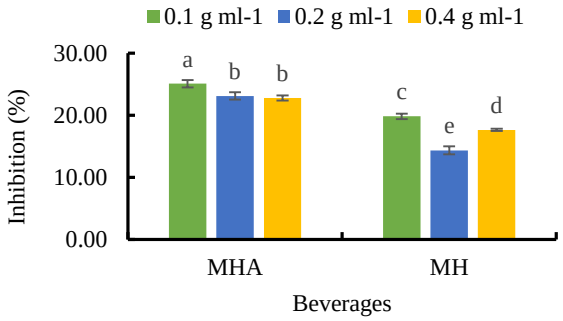


**Figure 2.** Antioxidant activity in three various ready-to-drink beverage formulations. Different letters state significant differences ($p \leq 0.05$). Mangosteen honey and D-allulose (MHA) was the beverage contained ten cubes of BGJ encapsulated probiotics and sweetened with 3.5% (w $w^{-1}$) honey containing 1.3% D-allulose. Mangosteen honey (MH) was the beverage contained ten cubes of BGJ encapsulated probiotics sweetened with 3.5% (w $w^{-1}$) natural honey.

The MPE contains various kinds of xanthones verified to include strong antioxidant activities [5,12]. In addition to vitamin C and folate, the MPE can serve as a source of natural phenolic antioxidant. However, the extract and its antioxidant capacity depended heavily on the extraction methods and solvent. The water extract such this formulation included the strongest capacity, compared to the methanol and hexane extracts [12].

The BGJ beverage potential antioxidant was enriched by the probiotic *L. plantarum* that produced antioxidants and facilitated liberation of flavonoids from their complex form in the plant materials such as BGJ and MPE through microbial transformation and de-polymerization [24]. Naturally, *L. plantarum* produces glucosidase that hydrolyzes flavonoid conjugates; thereby, increasing the antioxidant activity [25]. The probiotic *Lactobacillus* naturally produces antioxidant compounds such as cytochrome c peroxidase, as defense systems preventing free radicals from occurring in cells. The *L. plantarum* strains include high antioxidant activities within the fermented food *Lactobacillus* group. This has been shown by its high resistance to peroxide and high scavenging activity against hydroxyl, superoxide and free radicals [26]. Such a high *in-situ* radical scavenging potential can stimulate natural antioxidants for cell and body defenses. At cellular levels, probiotic *L. plantarum* protected the mammalian cells such as HT-29 cell cultures against $H_2O_2$ injuries. The probiotic microorganism scavenged free radicals and inhibited intracellular reactive oxygen species production. Moreover, detailed analysis indicated that the probiotic microorganism decreased the heat shock protein 70 (HSP-70) expression, the ratio between the Bax and Bcl-2 decreased malondialdehyde level of HT-29 cells that is damaged by $H_2O_2$ and increased superoxide dismutase and glutathione peroxidase activities [27]. In an animal model, dietary *L. plantarum* enhanced antioxidant activities in serum and ruminal fluid. The probiotic supplementation decreased serum lipid peroxidation and increased the hepatic antioxidant enzymes [28].

### 3.3. The yeast petite colony formation

Yeasts are excellent models since they share similar basic processes of fat metabolism with the mammalian cells [20]. This analysis investigated the BGJ formula potential for hypoglycemic and anti-obesity controls, as indicated by the formation of the petite yeast colonies [21]. All BGJ ready-to-drink formulas induced yeast petite colony frequency at various rates (Figure 3). The BGJ containing natural honey induced further petite colonies than that the other formulas did. Honey and its D-allulose derivate are low energy sweets, playing important roles in improvement of insulin resistance, antioxidant enhancement and hypoglycemic control [3]. Addition of MPE decreased petite colonies in BGJ mangosteen honey and BGJ mangosteen and water formulas but not in BGJ mangosteen noney and D-allulose formulas. This might be due to the fact that supplementation of MPE at lower concentrations that increased the antioxidant activity could protect the yeasts. The petite colony was relatively smaller than the normal one due to impaired mitochondrial function and increased oxidative stress, suggested as mechanisms of resistance to insulin [29].

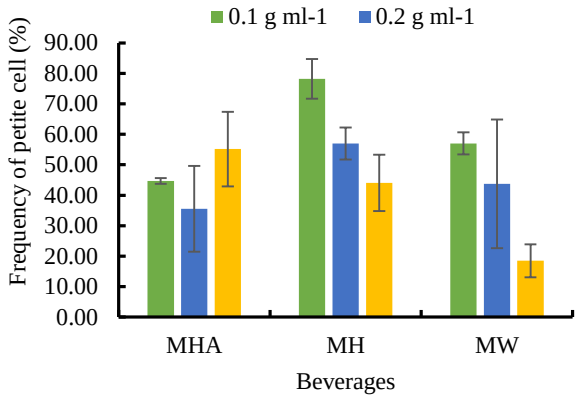


**Figure 3.** Frequencies of petite yeast colony formations in three various ready-to-drink beverage formulations. Mangosteen honey and D-allulose (MHA) was the beverage contained ten cubes of BGJ encapsulated probiotics and sweetened with 3.5% (w $w^{-1}$) honey containing 1.3% D-allulose. Mangosteen honey was the beverage contained ten cubes of BGJ encapsulated probiotics sweetened with 3.5% (w $w^{-1}$) natural honey. Mangosteen and water was the beverage contained ten cubes of BGJ encapsulated probiotics and water not sweetened. All formulas were supplemented with mangosteen pericarp extract at concentrations of 0.1, 0.2 and 0.4 mg $ml^{-1}$, respectively.

One of the key characteristics of petite yeasts included decreased succinate dehydrogenase enzyme activity in the mitochondrial inner membrane. The enzyme catalyzed oxidation of succinate to fumarate in the tricarboxylic acid (TCA) cycle. The yeast mutant lack of the succinate dehydrogenase activity resulted in a high frequency of petite colony formation [14]. This succinate dehydrogenase included an essential part of complex of the electron transport chain and suggested playing important roles in obesity [30]. Figure 4 shows ready-to-drink products in this study containing D-allulose honey and mangosteen pericarp extract beverages with small cubic slices of black grass jelly containing probiotics.

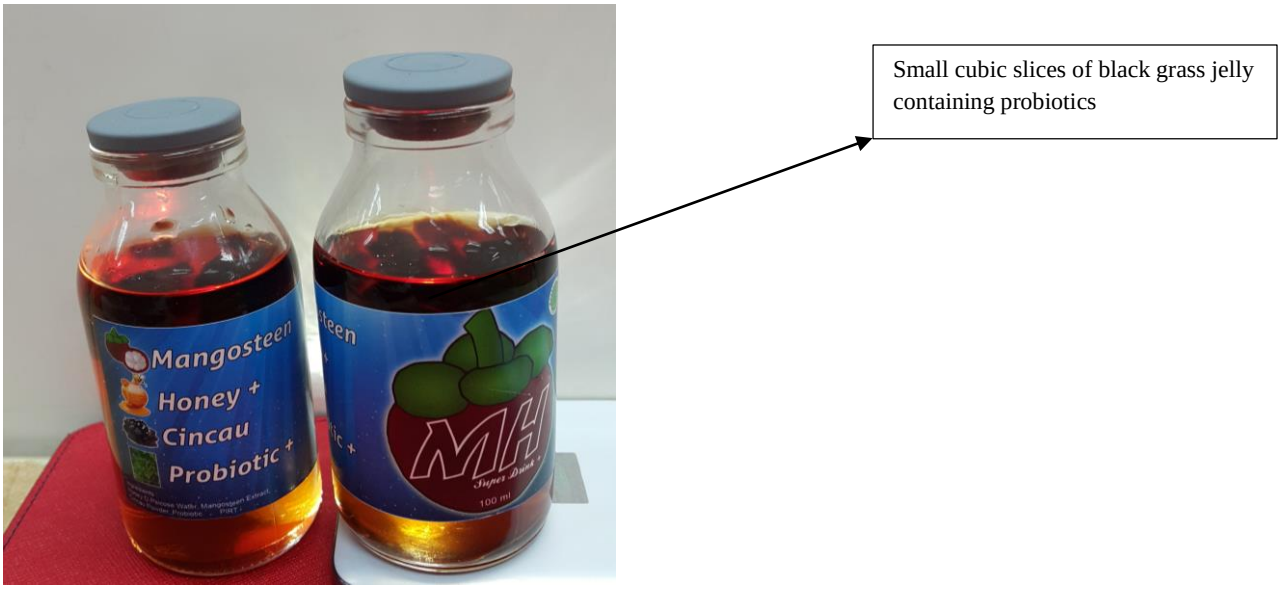


**Figure 4**. Ready-to-drink D-allulose honey and mangosteen pericarp extract beverages with small cubic slices of black grass jelly containing probiotics

## 4. Conclusion

The probiotic *L. plantarum* Mar8 encapsulated in black grass jelly survived well in honey, D-allulose and mangosteen pericarp extract formulated beverages. Honey supported the probiotic viability better, producing further antioxidants and high potentials in hypoglycemic, compared to that other formulas did. The mangosteen pericarp extract partially enriched functionality of the black grass jelly probiotic beverages. However, further studies are necessary to verify favorability and stability of the functional food.

## 5. Acknowledgements

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## 6. Conflict of Interest

The authors report no conflict of interest.

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# ژله علف سیاه حاوی *لاکتوباسیلوس پلانتاروم* Mar8 ریزپوشانی شده در عسل و نوشیدنی دی-آلولوز غنی شده با عصاره پریکارپ ترنجبین

تیتین یولینری[1*]، نوویک نورحیدات[1]، نیلام فدمولدها وولندری[2]، سری ویدواتی[1]، سولیاسیه سولیاسیح[1]، لوسیاناواتی ویجاجا[1]

۱- مرکز تحقیقات میکروبیولوژی کاربردی، آژانس ملی تحقیقات و نوآوری، سیبینونگ، برین، اندونزی
۲- مرکز تحقیقات بیوسیستماتیک و تکامل، آژانس ملی تحقیقات و نوآوری، سیبینونگ، برین، اندونزی

## چکیده

**سابقه و هدف:** ژله علف سیاه که در شربت شیرین سرو می‌شود یکی از نوشیدنی های‌سنتی چین و کلا شرق و جنوب شرقی آسیاست. غنی سازی نوآورانه می‌تواند آن را به یک غذای فراسودمند[1] بهتری تبدیل کند. این مطالعه به‌طور نوآورانه‌ای نوشیدنی ژله را با فرمول‌های زیست‌یار[2] *لاکتوباسیلوس پلانتاروم* Mar8، عسل، دی-آلولوز و عصاره پریکارپ ترنجبین غنی کرد. زنده‌مانی زیست‌یار، آنتی‌اکسیدان و پتانسیل هیپوگلیسمی نیز مورد بررسی قرار گرفت.

**مواد و روش ها:** نوشیدنی‌های کاربردی آماده شامل عصاره پریکارپ ترنجبین در غلظت‌های ۰/۱، ۰/۲و ۰/۴ میلی‌گرم در میلی‌لیتر، دی-آلولوز در عسل و زیست‌یار *لاکتوباسیلوس پلانتاروم* Mar8 در ژله علف سیاه حاوی کنجاک و کاراگینان بود. زنده ماندن زیست‌یار، فعالیت آنتی‌اکسیدانی و پتانسیل هیپوگلیسمی پارامترهای انتخابی برای فرمول های نوشیدنی فراسودمند بودند. زنده‌مانی زیست‌یار *لاکتوباسیلوس پلانتاروم* Mar8 با استفاده از روش شمارش کل پلیت بررسی شد. فعالیت آنتی‌اکسیدانی بر اساس واکنش مهار رادیکال ۲،۲-دی فنیل-۱-پیکریل هیدرازیل ارزیابی شد. پتانسیل هیپوگلیسمی با شمارش سلول‌های مخمر کوچک پس از تیمار با فرمول‌های ژله علف سیاه بررسی شد. تفاوت معنی داری با استفاده از آنالیز واریانس یک طرفه و آزمون دانکن گزارش شد. از نظر آماری معنی داری شامل مقادیر $p \leq ۰/۰۵$ بود.

**یافته‌ها و نتیجه‌گیری:** زیست‌یار *لاکتوباسیلوس پلانتاروم* Mar8 ریزپوشانی شده در ژله علف سیاه به خوبی در نوشیدنی‌های فرموله شده عسل، دی-آلولوز و عصاره پریکارپ ترنجبین زنده ماند. زنده‌مانی زیست‌یارها در عسل بهتر بوده، آنتی اکسیدان‌های بیشتری و پتانسیل بالایی در هیپوگلیسمی نسبت به سایر فرمول‌ها ایجاد می‌کند. عصاره پریکارپ ترنجبین عملکرد نوشیدنی‌های زیست‌یار ژله علف سیاه را بهبود می‌بخشد. با این حال، مطالعات بیشتری برای ارزیابی مطلوبیت و پایداری این غذای فراسودمند مورد نیاز است.

**تعارض منافع:** نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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**واژگان کلیدی**

- فعالیت ضداکسایشی
- زیست‌یار لاکتوباسیلوس پلانتاروم ریزپوشانی شده
- نوشیدنی فراسودمند
- توانایی کاهش قند خون
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***نویسنده مسئول**

**تیتین یولینری**

بزرگراه جاکارتا-بوگور KM 46، سیبینونگ، جاوا غربی، اندونزی ۱۶۹۱۱

تلفن: ۶۲۸۱۶۱۹۸۰۷۶۸+

پست الکترونیک:
titi003@brin.go.id

[1] functional food

[2] probiotic