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THE CHARACTERISTICS OF PROBIOTIC DRINK BASED ON MORINGA LEAVES JUICE

[Karakteristik Minuman Probiotik Berbasis Jus Daun Kelor]

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ABSTRACT

Moringa (*Moringa oleifera*) is rich in minerals, vitamins, and other essential phytochemicals. This research aimed to evaluate the effect of addition skim milk powder to the characteristics of probiotic drinks based on moringa leaves juices. The probiotic drink was fermented by *Lactobacillus casei* FNCC 00090 with different levels of skim milk powder (0, 3, 5, and 7%). The results showed that supplementation skims milk powders significantly affected the color of the probiotic product. The higher level of skim milk powders, the lighter, greener, and more yellow in the color ($P<0.05$). The higher concentration of skim milk, the higher of pH and lactic acid content resulted. The number of probiotics among the treatment was not significantly different. The addition of 7% skims milk powder produced the highest protein content, antibacterial activity, phenolic content, and antioxidant activity. This product has a protein content of 2.65%, antibacterial activity: 14.50±1.80 mm (zone inhibition of *E. coli*) and 35.33±4.16 mm (zone inhibition of *S. aureus*), phenolic content of 0.2410±0.0054 mg equivalent gallic acid/mL sample, and antioxidant activity of 75.18±1.45%. The probiotics drink based on moringa leaves juices has the potential to be developed as a functional drink.

Keywords: antibacterial, antioxidant, moringa, probiotic

ABSTRAK

Daun kelor (Moringa oleifera) kaya akan mineral, vitamin dan senyawa fitokimia penting. Penelitian ini bertujuan mengevaluasi pengaruh konsentrasi bubuk susu skim terhadap karakterisitik minuman probiotik berbasis jus daun kelor. Minuman probiotik difermentasi oleh Lactobacillus casei FNCC 00090 pada konsentrasi bubuk susu skim (0, 3, 5, dan 7%). Konsentrasi bubuk susu skim pada penelitian ini berpengaruh nyata terhadap warna produk. Semakin bertambahnya bubuk susu skim, semakin cerah, hijau dan kekuningan warna minuman probiotik (P<0,05). Minuman probiotik dengan penambahan 7% bubuk susu skim memiliki kadar protein yang paling tinggi (2,65%). Semakin bertambahnya susu skim, semakin tinggi pH dan kandungan asam laktatnya. Jumlah probiotik tidak berbeda signifikan pada berbagai perlakuan. Penambahan 7% bubuk susu skim menghasilkan kandungan protein, aktivitas antibakteri, kandungan fenolik dan aktivitas antioksidan tertinggi. Produk yang terbaik memiliki kandungan protein sebesar 2,65%, aktivitas antibakteri: 14,50±1,80 mm (zona penghambatan E. coli) 35,33±4,16 mm (zona penghambatan S. aureus), kandungan fenolik 0,2410±0,0054 mg equivalent asam galat/mL sampel dan aktivitas antioksidan 75,18±1,45%. Minuman probiotik dari daun kelor potensial untuk dikembangkan sebagai minuman fungsional.

***Kata kunci:** antibakteri, antioksidan, kelor, probiotik*

INTRODUCTION

Moringa (*Moringa oleifera*) is a nutritious plant that is a source of essential phytochemicals in its all part of their plant, and it belongs to the family of *Moringaceae* (Gopalakrishnan *et al.,* 2016). Moringa tree is known as miracle trees because of its health benefits, and most parts of the tree are widely consumed as traditional food (Vanajakshi *et al.,* 2015). Leaves, flowers, pods, and fruits of moringa are rich in nutritional content such as proteins, lipids, minerals, and fiber (Melo *et al.,* 2013; Mawouma *et al.,* 2017). Besides it, their pods, flowers, and leaves contain abundant bioactive compounds such as β-

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carotene, tocopherol, vitamin C, phenolic compounds, and some essential amino acids (Ferreira *et al.,* 2008; Martinez *et al.,* 2017). According to Moyo *et al.* (2011), the chemical content of moringa dried leaves is protein 30.29%, fat 6.5%, moisture 9.53%, ash 7.64%, fiber 11.40%, tannin 3.12 mg/g, Vitamine E 77 mg/kg, beta carotene 18.50 mg/100 g and minerals.

The nutrition and bioactive compound of moringa could be utilized as a healthy food source for human beings. In Indonesia and Philippine, moringa traditionally has been processed as traditional cuisine (Aminah *et al.,* 2015). In East Indonesia that is in Sumba Barat Daya, Nusa Tenggara Timur, moringa leaves have been processed to become various products such as tea, snack, *Moringa olifera* meatballs, and pudding.

Other research has been done utilized moringa as a beverage based on its leaves, such as herbal tea moringa (Lalas *et al.,* 2017), cereal grain beverage with moringa leaves (Olusanya *et al.,* 2019) and also become a probiotic drink based on its leaves (Vanajakshi *et al.,* 2015). Nowadays, the probiotic drink has become popular cause it has excellent health benefits and high nutritional value (Zhang *et al.,* 2019). The living microbe in the probiotic drink confer a health benefit on the host (Amara and Shibl, 2015; He and Shi, 2017). A probiotic drink based on *moringa* leaves and beetroot juice showed good potential as antibacterial for *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli*. Moreover, it has antioxidant activity with phenolic content of 5 mg/mL and high mineral content such as calcium and iron (Vanajakshi *et al.,* 2015). There is limited information about probiotic drinks based on moringa leaves juice its self in the highest concentration. The research was conducted to evaluate probiotic drink characteristics based on moringa leaves added with various skim milk powder concentrations.

MATERIALS AND METHODS

Materials

Moringa leaves were collected from Subang, West Java, Honey (MMN Randu Honey), sucrose, carboxyl methylcellulose (CMC), mineral water, and skim milk powder local market. *Lactobacillus casei* FNCC 00090, *Escherichia coli*, and *Staphylococcus aureus* were purchased from Pusat Studi Pangan dan Gizi (PSPG) Universitas Gadjah Mada. The reagents for analysis were buffer peptone water (BPW) (Merck, Germany), de man rogosa sharpe broth and agar/MRSB/MRSA (Merck, Germany), Nutrient Agar (Merck, Germany), NaOH (Merck, Germany), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma, Germany).

Research design and data analysis

This research was conducted used a completely randomized design with one factor. The factor was four levels of supplementation skim milk. The concentrations added as tested were 0, 3, 5, and 7%, then repeated three times. The data was analyzed using SPPS 16.0 by one-way ANOVA. A probability value of $P<0.05$ was considered statistically significant.

Preparation of juice (Agustina *et al.,* 2019)

Fresh moringa leaves were washed, put in a blender cup, added mineral water, and mixed homogeneously. The ratio of moringa and mineral water was 1 (kg):5 (L). The puree of moringa leaves was filtered using a cloth filter and stainless wire. The mixing and filtering steps were repeated until they were fully extracted. moringa juice was prepared by adding moringa extract with 7.5% sucrose, 2.5% honey, and 200 ppm CMC. The next step was to cook moringa juice until boiling.

Preparation of probiotic starter (Desnilasari and Kumalasari, 2017)

L. casei were inoculated in 50 mL of MRSB and incubated at 37°C for 24 hours. Furthermore, the media that consist of 5% skimmed milk was inoculated with *L. casei* and incubated for 24 hours at 37°C. The lactic acid bacteria starter is ready to inoculate in the juice.

Preparation of the probiotic drink (Agustina *et al.,* 2019)

Moringa leaves juice was divided into four treatments (addition with 0, 3, 5, and 7% skim milk powder) with triplicated. All of the samples were pasteurized in a water bath for 15 minutes at 75°C. The mixed juices were tempering until temperature ±37°C before inoculating it with 5% *L. casei* starter, and then, it was incubated at 37°C for 24 hours.

Physicochemical analysis (AOAC, 2004)

The probiotic product's color was determined using a 3NH colorimeter to obtain the L, a, and b scores (Desnilasari and Kumalasari, 2017). The pH of the probiotic product was measured using a pH meter (SI Analytics). The total free acid value was determined with the volumetric method using NaOH 0.1 M. The protein content was analyzed using the Dumas combustion method using DuMaster (Buchi D-480, Switzerland).

Microbiological analysis (Desnilasari and Kumalasari, 2017)

The probiotic drink was evaluated for total lactic acid bacteria (*L. casei*) by serial dilutions. Briefly, 10 mL of the product was mixed in 90 mL of BPW and homogenized using a vortex. The serial dilutions were prepared, and the pour plate method was used to carry out the viable bacteria count.

Antibacterial activity measurement (Balouiri *et al.,* 2016)

The antibacterial analysis was determined using the diffusion agar plate method, according to Balouiri *et al.* (2016), with modification in the media by using nutrient agar. Nutrient agar was inoculated with *E. coli* or *S. aureus*. After that, the paper disc (6 mm) contains the probiotic product was placed on the agar surface. Then, it was incubated at 37°C for 24 hours. Lastly, the inhibition zone has measured the diameter of the clear zone around the paper disk.

Antioxidant properties analysis (Sompong *et al.,* 2011)

Antioxidant activity was determined using DPPH methods as described by Sompong *et al.* (2011). The probiotic drink was pipetted 0.3 mL, and then added 1.5 mL DPPH 0.24 mM and 2 mL ethanol. The solution was mix used vortex and incubated in the dark chamber for 40 minutes at room temperature. The absorbance of the solution was read at 515 nm using Spectrophotometer UV-Vis (Shimadzu 1900, Japan). The solution contained DPPH and ethanol was used as a control solution included in the calculation formula. Antioxidant activity was measured with this equation:

Scavenging Ability=

$$\left(\frac{\text{absorbance control-absorbance sample}}{\text{absorbance control}}\right) \times 100\% \quad (1)$$

Total phenolic content was determined by spectrophotometry method. The sample with 200 µL volume was reacted with 1.5 mL of 10% Folin-Ciocalteau reagent and 1.5 mL of Na_2CO_3 made from 75 g/L. Furthermore, this solution was incubated for 2 hours in a dark room. The absorbance of the solution was measured at λ 725 nm. Total phenolic concentrations are based on linear regression of gallic acid standard curves (Shao *et al.,* 2014).

RESULTS AND DISCUSSION

Physicochemical characteristics

The L value represents the lightness parameter (black= 0, white= 100). A of value represents reflected light which produces a chromatic red-green mixture (redness= +a (positive) from 0-100, greenness= -a (negative) from 0–(–80)). B of value represents a blue-yellow mixture (yellowness= +b (positive) from 0-70, blue= -b (negative) value from 0-(-70)). The color of probiotic drink based moringa leaves was showed in Table 1. According to Table 1, the higher concentration of skim milk powder, the more increased L and b value of the product, and the more decreased the value. Product without skim milk had the lowest lightness, but additional skim milk affected the fermented drink's lightness.

Moreover, skim milk added to the fermented product would stabilize the dominant color of the product. The value of a represents redness to greenness. Additional skim milk caused to decreased the a value so that the probiotic drink was green. Furthermore, the higher concentration of skim milk would affect the product's color to become more yellow. Pasteurized skim milk has a dominant color, yellow (Bruzantin *et al.,* 2016).

Table 1. The color value of probiotic drink based *Moringa oleifera*

Variation of Skim Milk (%)	L Value	a* Value	b* Value
0	28.81[a]	1.82[d]	7.40[a]
3	31.46[b]	1.24[c]	10.72[b]
5	34.20[c]	0.73[b]	13.33[c]
7	35.03[d]	0.26[a]	14.04[d]

Note: Different letter after value showed significantly in $P<0.05$

Lactic acid content was determined based on the titration method. The result showed in Table 2 indicated that skim milk powder could increase the total lactic acid in the probiotic drink. Product without skim milk also has a high total of titrated acid. It indicated that without skim milk, the fermentation process has also occurred. Based on BSN (2009), lactic acid for yogurt is between 0.5-2.0%, but according to the SNI for fermented milk, the lactic acid for fermented milk is 0.2-0.9% (BSN, 2009). According to Gezginc *et al.* (2015), the expected level of lactic acid from yogurt is around 0.95%, and the product has a sharp and acidic taste. Thus, the value of acid as lactic acid from this research is higher than expectations and existing standards. In other research, the value of lactic acid from probiotic drinks made from *Centella asiatica* leaves ranges from 0.40-1.20 with skim milk powder concentration as much as 0-9% (Agustina *et al.,* 2019). The high total of titrated acid is possible because of the high content of ascorbic acid (vitamin C) from moringa leaves. The content of vitamin C in fresh moringa leaves is estimated to range between 2-4 times greater than lemon and mosambi (Sankhyan *et al.,* 2013).

The increase of lactic acid is not in line with the pH. The pH of probiotic drink tends to increase with a higher concentration of skim milk. A good pH value for probiotic drinks (yogurt) according to Food Standards Australia New Zealand Food Standard (2007) has a maximum value of 4.5. According to Gezginc *et al.* (2015), several types of mild yogurts are produced at a pH of around 4.6. Thus, the pH value created is included in a suitable category (Gezginc *et al.,* 2015). The pH value of probiotic drink based moringa did not differ from the pH value of some other probiotic beverages such as those produced from lay durian juice (*Durio kutejensis*), which is around 4.45 (Yuliana *et al.,* 2017), yogurt with pineapple juice ranging from 4.15-4.18 (Insyiroh *et al.,* 2014).

Table 2. Total acid, pH, and protein contents of *Moringa oleifera* probiotic drink

Variation of Skim Milk (%)	Acid Titrated (%)	pH	Protein (%)
0	3.05±0.14[a]	3.83±0.01[a]	0.72±0.07[a]
3	4.80±0.53[b]	4.01±0.08[b]	1.64±0.01[b]
5	5.85±0.21[c]	4.09±0.01[b]	2.06±0.02[c]
7	5.29±0.10b[c]	4.49±0.08[c]	2.65±0.07[d]

Note: Different letter after value showed significantly in *P*<0.05

The protein content of probiotic drink based *moringa* leaves increased significantly with the increase of skim milk concentration. This result in line with the research by Pato *et al.* (2017). After adding the skim milk, this value already fulfilled the SNI (BSN, 2009) for fermentation milk beverage, which is a minimum protein content is 1%. In other probiotic drinks, probiotic drinks from *antanan* (*Centella asiatica*) with the addition of 0-9% skimmed milk powder have a protein content ranging from 3.71-8.59% (Agustina *et al.,* 2019). Probiotic drinks from cow's milk with 5% moringa leaves extract produced the highest protein content, which was 0.87% (Rahmawati, 2015).

Microbiological characteristics

Lactic acid bacteria of the probiotic drink showed in Table 3. Lactic acid bacteria did not significantly differ among the treatments. This data showed that the lactic acid bacteria all treatment has 10^8 CFU/mL amount. Based on the data above, it is known that the viability of lactic acid bacteria in the whole sample of moringa leaves probiotic drinks is following SNI fermented milk beverage, the viability of lactic acid bacteria must be above 10^6 colonies/g or greater than 6 logs CFU/mL. The highest viability sample was a sample with 5% skims milk powder around 8.82 CFU/mL. In other probiotic-based drinks such as lay durian, the best selection is a mixed culture with *L. plantarum* and *L. acidophilus* having live LAB cells as much as 9.33 log CFU/mL (Yuliana *et al.,* 2017). Research by Pato *et al.* (2017), addition 7,5% skims milk powder result in 9.43 log CFU/mL.

Table 3 also showed that probiotic drink based *Moringa oleifera* has antibacterial activity to *S. aureus* and *E. coli*. Increasing the concentration of skim milk in the product did not significantly increase the inhibition zone for *E. coli*. The inhibition zone *S.aureus* higher than *E. coli*. *E. coli* was gram-negative bacteria, and *S. aureus* was gram-positive bacteria. This antibacterial activity's ability increases in both types of bacteria with the increasing amount of skim milk added. Inhibition of *S. aureus* gram-positive bacteria was better than gram-negative *E. coli* bacteria. It is in line with the antimicrobial activity of probiotic drinks from Antanan and probiotic drinks from date palm juice, which can inhibit the growth of *S. aureus* and *E. coli,* the inhibition of *S. aureus* is better when compared to inhibition of *E. coli* (Agustina *et al.,* 2019; Khotimah and Kusnadi, 2014). Gram (+) bacteria were more sensitive to chemical compounds than a gram (-). It is probably caused by differences in the composition and structure of their cell walls. The cell walls of gram (+) bacteria is relatively more straightforward than gram (-) bacteria. Cell walls of gram (+) bacteria were single layer with low lipid (1-4%), while the gram (-) bacterial consists of three layers with a lipid composition of about 11-12%. Three layers, namely the outer lipoprotein, the middle lipopolysaccharide, and the inner was peptidoglycan. Therefore gram (+) bacteria cell wall is more easily penetrated by active compounds derived from plant extracts (Agustina *et al.,* 2019).

Table 3. Viability of probiotic and antibacterial activity of *Moringa oleifera* probiotic drink

Variation of Skim Milk (%)	Lactic Acid Bacteria Content (log CFU/mL)	Zone of Inhibition (mm)	
		E.coli	*S. aureus*
0	8.60±0.03[a]	11.67±2.08[a]	28.33±2.89[a]
3	8.73±0.19[a]	12.33±1.53[a]	29.33±3.79[ab]
5	8.82±0.09[a]	14.33±2.08[a]	32.00±2.00[ab]
7	8.71±0.11[a]	14.50±1.80[a]	35.33±4.16[c]

Note: Different letter after value showed significantly in *P*<0.05

These probiotic drinks ability to inhibit bacterial growth is possible because of phenolic compounds in the product. The number of hydroxyls (OH) functional groups in the phenol group is toxic to microorganisms. The higher the phenol compound was oxidized, the stronger the toxicity and inhibition of microorganism growth. The phenols'content at high concentrations can penetrate and disrupt bacterial cell walls and precipitate proteins in bacterial cells. Also, phenols can cause inactivation of cellular enzymes, coagulation of proteins, change the permeability of bacterial membranes, and finally, membrane cells undergo lysis (die). Whereas at lower concentrations, phenols can form complex bonds with proteins followed by phenol penetration into cells and cause precipitation and denaturation of proteins, thereby activating essential enzyme systems in bacterial cells (Hidayah *et al.,* 2017).

Antioxidant properties

Antioxidant activity is showed in Table 4. It has been seen that probiotic drink based on *Moringa oleifera* has an antioxidant activity of approximately 70 GAE/mL. Besides, it has total phenol between 0.22-0.24 equivalent gallic acid/mL. The higher antioxidant activity and phenolic content was the product with an addition 7% skim milk powder. The antioxidant activity of functional drinks is influenced by the high content of vitamin C in moringa leaves. According to Werdhasari (2014), antioxidants from natural ingredients are obtained from active ingredients such as vitamin C, vitamin E, provitamin A, organosulfur, and flavonoids. The most nutritious moringa plant content is the antioxidants, especially in the leaves that contain high antioxidants such as vitamin E (α-tocopherol) 104 mg/100 g (Mubarak *et al.,* 2017) vitamin C 203.1 mg.100 g-1 DM (Lalas *et al.,* 2017), and vitamin A around 3.31 mg/g (Tahir *et al.,* 2016).

Table 4. Antioxidant properties of probiotic drink based *Moringa oleifera*

Variation of Skim Milk (%)	Antioxidant Activity (%)	Total Phenol mg Equivalent Galic Acid/mL Sample
0	72.53±1.21[b]	0.2355±0.0013[b]
3	71.02±1.64[b]	0.2374±0.0021[bc]
5	64.95±1.42[a]	0.2266±0.0029[a]
7	75.18±1.45[c]	0.2410±0.0054[c]

The difference in antioxidant activity and total phenol content is due to antioxidant compounds, including polyphenols found in moringa leaves. The total phenol in this functional drink ranges from 0.22 to 0.24 mg/mL. The results of the study of Ramdhan and Aminah (2014) stated that the content of phenol fresh moringa leaves was 5.4 mg/mL. Processing methods affect antioxidant content such as polyphenols in vegetables, which can increase or decrease. It is mainly due to the softening or denaturation of plant tissue or cellular disruption and separation of some phenolic compounds from cellular structures when processing vegetables. The decrease in total phenol levels is affected by the process's stages from fresh ingredients, extraction, dilution, and fermentation (Ramdhan and Aminah, 2014).

CONCLUSION

The research found that skim milk powders' addition significantly affects the product's color; it becomes lighter, greener, and more yellow (*P*<0.05). The probiotic drink juices with supplementation of 7% skim milk powder had the highest protein content (2.65%) and already fulfilled BSN (2009) for fermentation milk beverage. The higher the concentration of skim milk, the higher the pH and lactic acid content. Total viable probiotic among the treatment was not significantly different. The addition of 7% skims milk powder resulted in the highest protein content, antibacterial activity, phenolic content, and antioxidant activity. The probiotics drink based on moringa leaves juices has the potential to be developed as a functional drink.

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