

Total Lactic Acid Bacteria (LAB), Antioxidant Activity, and Acceptance of

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Total Lactic Acid Bacteria (LAB), Antioxidant Activity, and Acceptance of Synbiotic Yoghurt with Binahong Leaf Extract (*Anredera cordifolia* (Ten.) Steenis)

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Abstract Alternative treatment for metabolic syndrome can be done by providing a diet consist of functional foods or beverages. Synbiotic yoghurt containing binahong leaf extract which high in antioxidant, total LAB and fiber can be selected to reduce the risk of metabolic syndrome. The effect of binahong leaf extract in synbiotic yoghurt against total LAB, antioxidant activity, and acceptance were analyzed. The experiment was done with complete randomized design with addition of binahong leaf extract 0% (control); 0.12%; 0.25%; 0.5% in synbiotic yoghurt. Analysis of total LAB using Total Plate Count test, antioxidant activity using DPPH, and acceptance were analyzed by hedonic test. The addition of binahong leaf extract in various doses in synbiotic yoghurt decreased total LAB without significant effect ($p=0,145$). There was no effect of addition binahong leaf extract on antioxidant activity ($p=0,297$). The addition of binahong leaf extract had an effect on color, but not on aroma, texture and taste. The best result was yoghurt synbiotic with addition of 0,12% binahong leaf extract. Conclusion of the research was the addition of binahong leaf extract to synbiotic yogurt did not significantly affect total LAB, antioxidant activity, aroma, texture and taste; but had a significant effect on color.

Keywords: total lactic acid bacteria, antioxidant activity, binahong leaf, yoghurt, synbiotic.

1. Introduction

The prevalence of metabolic syndrome in some developed and developing countries has increased, by 7.9 - 43.6% in men and 7 - 56.7% in women [1]. National Health and Nutrition Examination Survey (NHANES) 2003-2012 The prevalence of metabolic syndrome in the United States averaged 33%, 35.6% for women, men 30.3% [2]. The prevalence of metabolic syndrome in Jakarta was 24.8%, respectively in women by 30.4%, male by 25.4% [3]. The metabolic syndrome defined by the National Cholesterol Education Program Adult Treatment Panel III (NCEP / ATP III), if a person has 3 or more of the following data; Waist circumference greater than 102 cm in men or greater than 88 cm in women, serum triglyceride levels of 150 mg / dL or greater, high-density lipoprotein (HDL) cholesterol levels less than 40 mg / dL in men or less than 50 mg / dL in women, systolic / diastolic blood pressure 130 / 85mmHg, or fasting plasma glucose levels of 100 mg / dL or more [4].



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Risk factors which play a role in the development of metabolic syndrome included overweight, lack of physical activity and diet. Diet contributed to the reduction of some metabolic syndrome markers, one of the alternatives of dietary feeding or functional beverage [5,6]. Research development of food for people with metabolic syndrome is very necessary, especially in terms of the use of foodstuffs one of which can lose weight, anti hypertension, anti hyperlipidemia, and anti-hyperglycemia.

Yoghurt is a functional drink made from cow's milk fermented using probiotic bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus*[7,8]. Probiotics produce organic acids such as glucuronic acid, propionic acid, folic acid, and lactic acid that potentially lower non-HDL cholesterol levels. Cholesterol reduction by *Lactobacillus* strain anaerobically can reach 27-38% [9]. Probiotics also have a hypoglycemic effect that can decrease the inflammatory status in patients with DM type 2 [10,11]. Probiotics provide health effects on the human body that is at a minimum of 10^7 CFU / ml before consumed [12]. The number and activity of BAL can be increased by the addition of prebiotics. The combination of probiotics and prebiotics is called sinbiotik. In yogurt, probiotics and prebiotics work synergist [13]. One of the prebiotics is inulin. The addition of inulin is intended to increase the survival and activity of probiotics during the shelf life. As a water-soluble food fiber, inulin can affect homeostasis and decrease fat stores, triglycerides and cholesterol [10].

The addition of high antioxidant herbs is also necessary in preventing the progression of the metabolic syndrome due to the high oxidative stress due to increased ROS. Natural antioxidants in foods containing flavonoid components can inhibit enzymes that produce ROS and reduce oxidized ROS. The phytochemical process of binahong leaf (*Anredera cordifolia* (Ten.) Steenis) contains flavonoids, saponins, steroids / triterpenoids and coumarin. Saponin (bousingosida A1) leaf binahong inhibits cholesterol formation by inhibiting hydroxy enzyme methylglutaril co-acetate reductase at cell phosphorylation stage. The content of saponin fresh binahong leaf that is equal to 28.14 mg / g. Provision of leaf extract of binahong lowers blood cholesterol levels of male white mice induced foods high in fat and propylthiouracil significantly at doses of 500 mg / kgBB with the duration of administration 21 days [14].

High-fiber ingredients are also needed in the metabolic syndrome diet. Carrageenan are a natural polysaccharide type of Rhodophyceae seaweed. A study of 344 mice showed feeding containing 15% kappa/lambda-carrageenan from *Gravicela radula* produced a significant cholesterol-lowering effect [15]. The addition of carrageenan into the yogurt as stabilizer, emulsifier, gel-forming thickener is intended to improve the texture. In addition to carrageenan, stevia is added as a sweetener to increase acceptance.

The development of functional beverage products form of synbiotic yoghurt with binahong leaf extract which high antioxidant, fiber, total lactic acid bacteria can be an alternative treatment for metabolic syndrome. The purpose of this study to determine the effect of addition of binahong leaf extract to total lactic acid bacteria, antioxidants activity and acceptance.

2. Materials dan Methods

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That was Food Production research with true experimental research with one factor's complete randomized design, that was addition of binahong leaf extract in yoghurt of synbiotic herb by 0%, 0,12%, 0,25% , and 0.5%. Each treatment performed 3 replications for total analysis of lactic acid bacteria and antioxidant activity, while for the acceptance was not any repetition. Determination of the formulation of synbiotic yoghurt yoghurt for inulin addition based on previous research that was functional drink product of srikaya yoghurt. The addition of stevia and carrageenan was determined by preliminary test. The process of making binahong leaf extract was done based on previous research using maseration method with 95% ethanol solvent for 5 days.

The process of making synbiotic yoghurt with the addition of binahong leaf extract starts from pasteurized fresh cow's milk at 85 ° C for 15 minutes then cooled to 40 ° C. Pasteurized milk added 4% inulin as prebiotics, 0.2% stevia, and 10% starter *Streptococcus thermophilus* and *Lactobacillus*

bulgaricus. Incubated at incubator at 37 ° C for 24 hours. Incubation process added 6% carrageenan and extract of binahong leaf, then be homogenization.

Fresh milk obtained from the Faculty of Animal and Agriculture Diponegoro University, inulin obtained from PT DPO Indonesia, karagenan obtained from Suket Segoro SMEs Semarang, and bacteria *Streptococcus thermophilus* and *Lactobacillus bulgaricus* obtained from PAU Gajah Mada University Yogyakarta.

This research was done by analyzing total lactic acid bacteria using Total Plate Count (TPC) method, antioxidant activity was analyzed using DPPH method and acceptance was done by hedonic method on 30 panelists of Nutrition Science Study Program Diponegoro University.

The data obtained is processed using statistical software. Before analyzing all the data was tested normality using *Shapiro-Wilk* test because the sample size <30. The effect of binahong leaf extract treatment on total lactic acid bacteria and antioxidant activity was analyzed by One Way Anova. The recording analysis is carried out by Friedman test and continued with Wilcoxon test if there was a significant effect.

3. Results

3.1. Total Lactic Acid Bacteria

That was no significant effect of addition binahong leaf extract to total lactic acid bacteria yoghurt sinbiotik ($p = 0,145$), although there decreased total lactic acid bacteria (Table 1). Total lactic acid bacteria in synbiotic yogurt with addition of binahong leaf extract still fulfilled probiotic criteria according to Indonesian National Standard that is more than 10^7 CFU / ml.

Table 1. Results of Total Lactic Acid Bacteria Analysis

Dose	Total Lactic Acid Bacteria (10^{13} CFU/ml) ¹
0%	90,37±65,02
0,12%	86,96±53,13
0,25%	42,60±22,56
0,5%	8,93±5,22
	$p=0,145$

¹= One Way Anova Test

3.2. Antioxidant Activity

The addition of various dose binahong leaf extracts had no significant effect on antioxidant activity of synbiotic yoghurt ($p = 0,297$). The highest antioxidant activity in synbiotic yoghurt was by adding 0.5% binahong leaf extract with antioxidant content of 8.66% (Table 2).

Table 2. Results of Antioxidant Activity Analysis

Dose	Antioxidant Activity (%) ¹
0%	5,63±4,08
0,12%	4,33±1,52
0,25%	5,33±3,05
0,5%	8,66±0,57
	$p=0,297$

¹= One Way Anova Test

3.3. Acceptance

The acceptance of synbiotic yoghurt with the addition of binahong leaf extract includes the aspect of the assessment of flavor, color, texture and taste (Table 3). The addition of binahong leaf extract significantly influenced the color acceptance ($p=0.001$), but not on aroma, texture and taste.

Table 3. Results of Acceptance Analysis on Yoghurt Sinbiotik with Addition Binahong Leaf Extract

Dose	Aroma		Color		Texture		Taste	
	Mean	Result	Mean	Result	Mean	Result	Mean	Result
0%	2,93±0,82	Like	3,30±0,59 ^a	Like	2,46±0,68	Like	2,40±0,62	Dislike
0,12%	2,90±0,60	Like	3,13±0,51 ^a	Like	2,86±0,73	Like	2,53±0,57	Like
0,25%	2,96±0,66	Like	2,63±0,55 ^a	Like	2,60±0,67	Like	2,50±0,86	Like
0,5%	2,60±0,93	Like	1,76±0,67 ^b	Dislike	2,53±0,89	Like	2,50±1,16	Like
	$p=0,346$		$p=0,001$		$p=0,062$		$p=0,877$	

3.3.1. Aroma

The aroma of synbiotic yoghurt with the addition of binahong leaf extract was a typical fermentation sour aroma. The addition of various dose binahong leaf extracts had no significant effect on the flavor of acceptance ($p = 0.346$). The more addition of the binahong leaf does not decrease level of aroma preference.

3.3.2. Color

More addition of binahong leaf extract on synbiotic yoghurt decreases the level of color preference. Sinbiotik yogurt will be more brown if the amount of extract is added more and more. The addition of binahong leaf extract of 0% and 0.12% has the highest level of favorite, with 0% binahong extracts of 3.30 and 0.12% binahong extract of 3.13. The product color is not preferred in the addition of 0.5% extract.

3.3.3. Texture

Yoghurt sinbiotik's texture with addition of binahong leaf extract that was homogeneous. There was no significant influence between the addition of binahong leaf extract with texture acceptance level ($p = 0.062$). The higher levels of leaf extract of binahong added to the yoghurt sinbiotik did not decrease level of texture preferences.

3.3.4. Taste

The addition of leaf extract of binahong in various doses did not show a significant effect on the acceptance of the taste of synbiotic yoghurt ($p = 0.877$). The taste of synbiotic yoghurt with the addition of binahong leaf extract is a typical yoghurt sour taste. Control of synbiotic yoghurt (0%) is least favored compared with the other three dose.

4. Discussion

4.1. Total Lactic Acid Bacteria

The results showed that the addition of binahong leaf extract did not affect the total lactic acid bacteria although decrease total lactic acid bacteria when added amount of extract of binahong leaf. The decrease in the number of lactic acid bacteria can be caused by the presence of secondary metabolite compounds contained in binahong leaf extracts such as flavonoids, steroid saponins, and terpenoids. These secondary metabolite compounds proved to act as antibacterials, so added more binahong leaf extracts will lead to a decrease in the total number of lactic acid bacteria. The content of saponins in fresh leaf of binahong is 28.14 mg / g. The result of antibacterial activity of binahong leaf extract to gram positive bacteria showed that flavonoid of binahong leaf extract able to inhibit the growth of *Staphylococcus aureus* [16].

4.2. Antioxidant Activity

The addition of various dose binahong leaf extracts did not significantly influence antioxidant activity ($p = 0.297$). Compared with controls, the addition of 0.12% binahong extract decreased the value of antioxidant activity, but slowly increased when added 0.25% and 0.5% binahong leaf extract. In the preliminary study known the average of antioxidant activity of pure binahong leaf extract that is equal to 30%. Yoghurt sinbiotik with addition of leaf extract of binahong have antioxidant content from binahong leaf extract. Binahong leaf contain flavonoids, saponins, steroids / triterpenoids and coumarins. Flavonoids are unstable to changes in the effects of oxidation, light, and chemical changes, so that when the oxidized structure changes and its function as an active ingredient decreases and even disappears and its solubility is low [17]. Decrease in antioxidants due to antagonistic interactions of antioxidant extracts of binahong leaf with components of synbiotic yoghurt.

4.3. Acceptance

4.3.1. Aroma

The addition of binahong leaf extract did not decrease the level of preference to aroma. This is due to the similarity of the distinctive aroma of yoghurt is a typical fermented acid [18]. There was fluctuating aroma acceptance, but did not reduce the scale of the level of aroma acceptance. The preferred level of aroma was the addition of binahong leaf extract with a content of 0.25%. The typical fermentation aroma was derived from the activity of *Lactobacillus bulgaricus* bacteria that play an important role in producing aroma of yoghurt [18]. The aroma of yoghurt sinbiotik with the addition of leaf extract of binahong that was aroma typical of fermentasi. Aroma of leaf extract of binahong when mixed into yoghurt sinbiotik didn't give significant influence ($p = 0,346$).

4.3.2. Color

Statistical analysis on color acceptance had significant effect with giving of binahong leaf extract ($p = 0,001$). The highest color acceptance is on synbiotic yogurt with the addition of 0% binahong leaf extract. The results showed a decrease in color acceptance on the addition of the higher binahong leaf extract. This is due to the color of brown leaf extract binahong fresh maceration. Binahong leaf extract will be green when using the method of extraction by means of sokletasi and dry maceration, while the fresh maceration of leaf extract binahong will be brown. Soaking in 95% ethanol solution causes a change of green leaf color to brown due to browning reaction in maseration process [19,17].

4.3.3. Texture

The statistic analysis showed that the addition of binahong leaf extract did not significantly affect the texture acceptance ($p = 0.062$). This is because the addition of carrageenan in each treatment equal to 6%. The addition of carrageenan 0.6% based on preliminary test results that have been done previously.

4.3.4. Taste

The addition of binahong leaf extract did not significantly influence the taste acceptance ($p = 0,877$). Synbiotic yoghurt is given stevia sugar of the same amount at each treatment, which is 0.2%, but it does not affect the taste of synbiotic yoghurt. The taste of synbiotic yoghurt with the addition of binahong leaf extract is a typical yoghurt sour taste. Fermented milk using *Lactobacillus bulgaricus* bacteria and *Streptococcus thermophilus* produce lactic acid. Both types of bacteria will decompose lactose (milk sugar) to lactic acid and various components of aroma and flavor. *Lactobacillus bulgaricus* is more concerned with the formation of aroma, while *Streptococcus thermophilus* is more concerned with the formation of yoghurt flavors [18].

5. Best Products

Based on total lactic acid bacteria, antioxidant activity and acceptance the best product that is synbiotic yoghurt with addition of binahong leaf extract equal to 0,12%, that have total lactic acid bacteria equal to $86,966 \times 10^{13}$ CFU / ml fulfill criteria minimum probiotic amount based on Indonesian National Standard more Of 10^7 CFU / ml, 4.33% antioxidant activity and acceptance of taste, color, texture and aroma favored by the panelists rather than the addition of other binahong leaf extracts.

6. Conclusion

The addition of binahong leaf extract to synbiotic yoghurt had an effect on the color acceptance, but it did not significantly affect the aroma, texture and taste; Antioxidant activity and total lactic acid bacteria. The addition of binahong leaf extract in various doses of synbiotic yoghurt decreased total lactic acid bacteria, but still qualified probiotics based on Indonesian National Standard that is more than 10^7 CFU / ml. No effect of addition of binahong leaf extract on antioxidant activity. At reception gives effect to color, but not on aroma, texture and taste. The best product is synbiotic yoghurt with addition of binahong leaf extract with amount of 0.12%.

7. Acknowledgements

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