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1st International Symposium on Integrated Biorefinery (ISIBio) 2014

"Utilization of Biomass and Biodiversity of Microbes for The Production of Biofuels and Bioproducts"

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The Characteristics of Fermented Beverages of Green Bean Sprouts (*Phaseolus radiatus*)

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Abstract - *The milk from green beans is less desirable by the community because it smells dreadful. Germination is one alternative to improve the digestibility of green beans that can cope with the use of nuts, stripping the skin, shortening the cooking time, reducing the unpleasant odors and reducing the activity of non disgatable oligosacharides lipoxigenase which caused flatulence and eliminate anti-nutritional compounds. Optimizing the use of green bean sprouts milk can be done through process of fermentation, since the fermentation process can improve the taste. The fermented milk products can be produced by the fermentation of lactic acid bacteria such as Lactobacillus bulgaricus, Streptococcus thermophilus, and others. The purpose of this study was to determine the concentration of Lactobacillus bulgaricus and Streptococcus thermopillus in producing a fermented beverage from green bean sprouts (Phaseolus radiatus). The observation was done on the protein content, the water content, the fat content, the total solids, pH, the total acid, the viscosity, the total plate count and the number of lactic acid bacteria as well as the organoleptic assessment of the color, aroma, flavor, and appearance. The optimum result for fermentation can be obtained with the Lactobacillus bulgaricus and Streptococcus thermophilus in the ratio of 1:1accordance with ISO 7552: 2009.The quality characteristics of the resultbeverage product contained 2.28% of protein, 80.04% of water content, 0,58% of fat content, a pH of 4.22, 0.56% of acid titration number, 0.25% of ash content, 5.67 dpa of viscosity, a total of 2.2 x1010 (colonies / g) lactic acid bacteria, 2.4 x1010 (colonies / g) of the total plate count. For sensory evaluation, the panelists' acceptance level was 3.55, the consistency was 3.80, the aroma was 3.75, the color was 3.85 and the flavor was 3.25.*

Keywords - Green beans, fermentation, lactic acid bacteria, Lactobacillus bulgaricus, Streptococcus thermophillus

INTRODUCTION

Generally, fermented milk was produced through a fermentation by lactic acid bacteria, namely *Lactobacillus bulgaricus* and *Streptococcus thermophilus,* and the addition of other allowed materials [1].the lactic acid bacteria are added to the food and drink in order to improve human's health by reducing pathogenic bacteria in the intestines and stimulating the immune system to keep the body fit and resistant to diseases [2]. Nowadays, people tend to reduce the consumption of livestock products including dairy, because of high content of fat and cholesterol. Nowadays, people tend to reduce the consumption of livestock products including dairy, because of high content of fat and cholesterol as well as the lactose intolerance that causes allergies. Vegetable plant can be an alternative to the adequacy of the protein. Soy milk is vegetable milk that is dominantly consumed. However, green bean milk is rarely consumed because it has an aroma that is less preferred.

Germination is one alternative to improve the digestibility of green beans. Green bean sprouts can be prepared easily and cheaply. Germination has been known to have the advantage of being able to cope with the use of nuts. Germination can facilitate the process of stripping the skin and shorten the cooking time, lower lipoxygenase activity which causes unpleasant odors and non disgatable oligosaccharides which cause flatulence, as well as reduce and eliminate the anti-nutritional compounds (Bayu, 2006). [3]. This study was conducted to determine the right concentration of *Lactobacillus bulgaricus* and *Streptococcus thermopilus* to produce fermented beverage of green bean sprouts (*Phaseolus radiatus*) in good characteristics. By doing this research, the authors hope to increase the value of green beans as food and green beans product's diversification as a fermented beverage.

METHODOLOGY

The Making Process of Green Bean Sprouts (Hariyuni 2006 modified) [4].

It is started with sorting and washing the green beans. The green beans are cleaned from the impurities such as sand and stone, and then washed.

The next step is soaking the green beans in the water for 8 hours. The process is followed by draining the water from the beans and spreading the beans to the hollow basin that has been coated with a damp cloth. During the germination, the beans should be avoided from direct sunlight by covering the damp cloth. According to Hariyuni 2006 [4], the length of the seed's germination process that can result in optimum quality and flavor is 8 hours.

Making the Milk of Green Bean Sprouts (Hariyuni 2006 modified) [4]

The making process of the milk is started with sorting, cleaning of the dirt and wash the sprouts. Blanch the sprouts in hot water with a temperature of 80-90 ˚C for 3 minutes and dry them. Then puree them in a blender until it becomes mush, add at a ratio of 1: 6 weight of dried green beans. The mush is filtered and squeezed with filter cloth. The result of the filter is called raw milk.

Making the Starter (Hidayat, 2006 modified) [2]

The initial phase of making a starter is to mix 100 ml of mung bean sprouts raw milk with a 12% (w/v) skim milk, 5%(w/v) sucrose, 0.1% (w/v) xanthan gum. The process is followed by pasteurization at a temperature of 80 ˚ C for 15 minutes, and then it is poured into a glass jar that has been sterilized at 180 ˚ C for 60 minutes. Then cool the starter to a temperature of 37˚ C. After that the starter is inoculated with pure cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as much as 2%. Cover the container with aluminum foil that has been sprayed with alcohol and then cover the container with a lid. Incubate the starter on 37˚ C for 15 hours.

Making the Fermented Milk of Green beans (Karleni 2010 modified) [5]

The substrate is made by adding 12% (w/v) skim milk, 5% (w/v) sucrose, 0.1 g (w/v) in 100 ml of green bean sprouts milk. The mixture is heated at 80° C for 5 minutes and then cooled to a temperature of 37° C, then put in a glass jar that has been sterilized at 180° C for 60 minutes and inoculated by adding a starter (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) based on the treatment. The bottle is closed and fermented at 37°C for 15 hours. After 15 hours, the fermentation is stored by placing the product at a temperature of 5°C in the refrigerator.

Observation

The observation was made on the fermented beverage of mung bean sprouts including protein content [6], fat content [6], pH, total acid, viscosity, total plate count and the number of lactic acid bacteria [7], as well as the organoleptic assessment of the color, aroma, flavor, and appearance.

The Research Method

The method used in this study was a Complete Random Design (CRD) with 4 treatments and 3 replications. The data was analyzed with statistical F test and if it was significantly different, it was followed by Duncan's New Multiple Range Test (DNMRT) at the 5% significance level. The treatments in this study were A, B, C and D i.e. the percentage concentration of *Lactobacillus bulgaricus* and *Streptococcus thermopillus* given (v / v) consists of 4 standards: A = 100%: 0%; B = 50%: 50%; C = 75%: 25%; D = 0%: 100%.

RESULTS AND DISCUSSION

Chemical Characteristics

In the manufacture of fermented beverages, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, that had been added, took the advantage of nitrogen's sources in the green bean sprouts milk to multiply and reproduce them. The data of the lowest protein content was on treatment D, namely *Streptococcus thermophilus* 100%. It happened because the pH of the product reached the point isoelectric 4.61, therefore the protein's solubility was low. According to Deman (1997) [8], the point of isolektris on milk protein is at pH 4.6 to 4.7. When it was compared with the use of a single cell and double cell, it indicated that the double cell of the product had higher protein content. This is in accordance with Weitter and Web's opinion (1970) in Prasetyo (2010) [9] that the symbiosis of *L. bulgaricus* and *S. thermopillus* can produce glycine and histidine.

TABLE 1
PROTEIN AND FAT CONTENT (%) OF GREEN BEAN SPROUTS FERMENTED BEVERAGES

Treatment (stater ratio)	**Protein (%)**	**Fat (%)**
A (100% *Lb*)	2,13	0,66
B (50% *Lb*;50% *St*)	2,28	0,58
C(75% *Lb*;25% *St*)	2,34	0,59
D (100% *St*)	2,03	0.64

Fat content has a relationship with the total plate count. The higher the total plate count, the fat content will decrease. The increasing number of microorganisms will also increase the absorption of fat as an energy source, thus the fat content decrease after fermentation. According to SNI 7552: 2009, the minimum fat content in a drink is 0.5%, thus the fermented beverage of green bean sprouts (*Phaseolus radiatus*) has been in accordance with SNI standards.

The total of acid titration is associated with pH values that can be seen in Table 2. The lactic acid levels has inversely proportional to the pH value. The higher the value of lactic acid, the lower the pH value to be. According to Winarno (2007), [1] the resulting lactic acid from the metabolism of carbohydrates will be able to lower the pH value of the environment. This is in accordance with the opinion of Chancan and Shahani (1993) cit Yusmarini and Efendi (2004) which states that the lactic acid produced in the process of making yogurt can reduce the pH of milk.

TABLE 2
PH AND TOTAL ACID OF GREEN BEAN SPROUTS FERMENTED DRINK

Treatment (stater ratio)	pH	Total acid (%)	Viskositas (dPa)
A (100% *Lb*)	4,36	0,56	4,53
B (50% *Lb*;50% *St*)	4,22	0,49	5,67
C(75% *Lb*;25% *St*)	4,16	0,57	5,43
D (100% *St*)	4,61	0,48	3,63

The mixture culture between *Lactobacillus bulgaricus* and *Streptococcus thermophilus* lactic acid levels were almost close. In mung bean sprouts fermented beverage that used multiple cultures produced the higher lactic acid levels when compared with single culture. Based on opinion of Winarno and Fernandez (2007) [1], the growth of *Streptococcus thermophilus* at first is fast and give a little sour taste, then *Lactobacillus bulgaricus* provides a stronger sour taste in the milk. Both of these bacteria will ferment lactose to lactic acid. Double cultures can produce a high amount of lactic acid compared to using a single culture. In line with the opinion Tamime and Robinson (1999) cit Taufik (2004) [11], the mixture starter will produce higher acid than the non-mixture starter.

Viscosity

The viscosity value seemed to have a relationship with the pH level. The higher the pH level, the lower the viscosity of fermented beverages mung bean sprouts (*Phaseolus radiatus*) to be. Yusmarni and Efendi (2004) [10] argues that the fermentation of carbohydrates will produce organic acids, especially lactic acid which lowers the pH of the milk. At the low pH, the protein coagulates and will increase the viscosity. Viscosity has an important role in the manufacture of yoghurt.

Microbiological Analysis

The data in Table 4 shows that the growth of lactic acid bacteria is higher by using a mixture of cells when compared with single cells. Kasmiati (2002) cit Adiyati [12] states that the number of cells will increase higher in a mixed cell than in a single cell. It is agreed by Winarno and Fernandez (2007) [1] which states that yogurt cells generally involve two or more bacteria for fermentation.

Table 3 shows the presence of other microbes beside LAB (Lactic Acid Bacteria) which might come from other microbes or other ingredient that contaminated during processing, such as during the cooling of milk after heating. According to Yasa (2006) cit Mahendra (2009) [13], the food fermentation by lactic acid bacteria can suppress the growth of pathogenic bacteria that cause diarrhea, so the possibility of the growth of harmful bacteria can be reduced. Furthermore, Arisman (2009) [14] states that pathogenic bacteria cannot grow or grows very slowly at low pH.

TABLE 3
TOTAL LACTIC ACID BACTERIA AND MICROBES IN MUNG BEAN SPROUTS FERMENTED BEVERAGE

Treatment (stater ratio)	Lactic Acid Bacteria (coloni/g)	Total Microbial
A (100% *Lb*)	1,2 x10^{10}	1,5 x10^{10}
B (50% *Lb*;50% *St*)	2,2 x10^{10}	2,4 x10^{10}
C(75% *Lb*;25% *St*)	2,1 x10^{10}	2,3 x10^{10}
D (100% *St*)	1,3 x10^{10}	1,7 x10^{10}

Organoleptic Test

The appearance of a product is influenced by the physical state of the product. The viscosity (appearance) is an element of texture that assessed the organoleptic properties of the product via the sense of touch, sight and feel in the mouth (Soewarno, 1985 cit Chairunnisa, 2009) [15]. The average score of the panelists on the appearance of the product was in the range of 3.50 - 3.70. The appearance of this fermented beverage is a green viscous liquid. The structure of green bean sprouts fermented beverages which was thick occurred because denaturation of proteins from skim milk which was one of the major components of the total solids. According Yusmarini and Efendi (2004) [10], carbohydrate fermentation will produce organic acids, especially lactic acid that causes the milk to a low pH of about 3.96 to 5.01 and at that pH, the protein clotting will occur thereby the viscosity of the products increase

TABLE 4
THE RESULTS OF ORGANOLEPTIC TEST GREEN BEAN SPROUTS FERMENTED BEVERAGE

Treatment	Appearance	Consistency	Flavor	Color	Taste
A (100% *Lb*)	3,60	3,55	3,65	3,70	2,80
B (50% *Lb*;50% *St*)	3,55	3,80	3,75	3,85	3,25
C (75% *Lb*;25% *St*)	3,65	3,70	3,70	3,85	3,20
D (100% *St*)	3,70	3,55	3,60	3,75	3,15

The green bean sprouts fermented beverage looked homogeneous due to the addition of stabilizer xanthan gum as much as 1%. According to Mahendra (2009) [13] the addition of xanthan gum 0.08% can increase the stability of lactic acid of resulting fermented beverage. Homogeneity milk green bean sprouts all looked the same treatment as the amount of stabilizer was added to all the same treatment. In addition, the consistency can be caused by the clotting proteins by the acid.

The opinion of Mahendra (2009) [13], who stated that yogurt has a thick texture to moderately viscous or semi-solid with a homogeneous consistency as a result of the clotting protein from organic acids produced by the starter culture. According to SNI 7552: 2009, the consistency of fermented milk is homogenized.

The flavour in this green bean sprouts fermented beverage was ranging from 3.60 to 3.75.The flavour of this green bean sprouts fermented beverage was the sour smell and odor typical of green beans. Distinctive odor of green beans derived from the raw material itself was still there, but the unpleasant smell of green beans did not appear. According Yusmarini and Efendi (2004) [10], the fermentation process can reduce the unpleasant odor, because the fermentation process of nuts milk produced organic acids that can improve the flavor. According to Prassetyo (2010) [9], the volatile components that have been produced by lactic acid bacteria in yogurt give a distinctive aroma.

The average color value given by the panelists ranged between 3.70 - 3.85 with the criterion rather like colours have no significant effect on the organoleptic test for each color of the equal treatment and the raw materials used are also the same.

Table 4 shows that the interaction between *Lactobacillus bulgaricus* starter additional concentration and *Streptococcus thermophilus* is not significant. The taste of this green bean sprouts fermented beverage was sweet and sour. The most preferred flavor of the product by the panelists was the treatment with the addition of 50% *Lactobacillus bulgaricus* and 50 % *Streptococcus thermophilus* with a slightly sour taste and the lowest score was on the treatment with the addition of 100% Streptococcus. In the treatment B (50% *Lactobacillus bulgaricus* and *Streptococcus thermophilus* 50%). Treatment D (*Streptococcus thermophillus* 100%) tasted sweet and produced less acid that made less preferred by the panelists. Treatment D (*Streptococcus thermophillus* 100%) failed to give a sour taste in the green bean sprouts fermented beverage. It can be seen from the pH of treatment A (*Streptococcus thermophillus* 100%) that was 4.61.Sour taste is the characteristic of fermented beverage which is caused by the chemical reorganizing of the sugars by lactic acid bacteria that affect the acidity of the product.

CONCLUSION

The starter concentration of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* significantly affected the pH and viscosity (DPA), protein content (%), number of lactic acid bacteria (colonies / g), number plate (colonies / g) and did not have a significant influence on the level of water (%), fat content (%), total solids (%), number of titration acid (%), and sensory (appearance, consistency, aroma, color and flavor). The best products were the treatment B (*Lactobacillus bulgaricus* and *Streptococcus thermophilus* 50% : 50%) met for ISO 7552: 2009 requirements with a protein content of 2.28%, 80.04% moisture, 0.58% fat content, pH 4, 22, acid total 0:56%, ash 0.25% rate, viscosity of 5.67 dpa, the amount of lactic acid bacteria 2.2 x1010 (colonies / g), total plate 2.4 x1010 (colonies / g) and the level of acceptance panelist appearance 3:55 , the consistency of 3.80, 3.75 aroma, color of 3.85, and a sense of 3.25.

From the research it can be concluded that the concentration of *Lactobacillus bulgaricus* starter and *Streptococcus thermophilus* affect the pH value and viscosity (DPA), protein content (%), total lactic acid bacteria (colonies / g), total plate (colonies / g) and no effect real fat content (%), total solids (%), total acid (%), and sensory (appearance, consistency, aroma, color and flavor). The best treatment was the product B (*Lactobacillus bulgaricus* and *Streptococcus thermophilus* 50% 50%) already met the standards in accordance with SNI 7552: 2009 with a protein content of 2.28%, 0.58% fat, 4.22 pH, total acid tertitrasi 0, 56%, ash content 0.25%, viscosity of 5.67 dpa, a total of 2.2 x1010 lactic acid bacteria (colonies / g), total plate 2,4x1010 (colonies / g) and 3.55 appearance acceptance level by panelists, consistency 3.80, 3.75 aroma, color of 3.85, and a sense of 3.25.

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