Characteristics of Pumpkin (Cucurbita moschata) Fermented Beverage Products With the Addition of a Powder Milk Mixture

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Abstract. One of the efforts in the use of pumpkin is making lactate fermented beverage. In this product there are two components at once, namely inulin from pumpkin that acts as a prebiotic and culture starter of Lactobacillus sp which acts as a probiotic. This study aims to determine the effect of the addition of milk powder on the characteristics of pumpkin juice fermented beverage. Variations in addition to powdered milk (skim and full cream). The results showed the addition of a mixture of 13% skim milk and 2% full cream is a lactate fermentation beverage that panelists prefer. This product has an average pH of 4.22, a total titrated acid of 0.43%, a protein content of 3.78%, a total solids of 17 ° Brix, a viscosity of 8.44 dPa, a fat content of 1.16%, an ash content of 0.83%, antioxidant activity 3.13%, beta-carotene levels 2.41 μg / g, and total lactic acid bacteria 2.1x10⁹.

Keywords: pumpkin, fermented beverage, Lactobacillus sp, probiotic, prebiotic.

1. Introduction
Fermented beverage that are known to originate from cow’s milk or buffalo milk are processed by fermentation with the addition of a starter of lactic acid bacteria (Lactobacillus and Bifidobacterium), such as yogurt or without the addition of a starter such as curd, whereas fermented drinks can be made from ingredients containing carbohydrates or beans. Pumpkin can be used in making fermented drinks, because pumpkin contains carbohydrates in the form of food fiber and antioxidants which is quite high. The fiber content in the pumpkin is 1.16% [1]. Based on the results of research [2] carbohydrates contained in pumpkin is raffinosa from the family oligosaccharides (RFO), namely rafinosa, stakiosa and verbaskosa. Fresh pumpkin contains rafinosa (612.0 mg / 100 g), stakiosa (36.3 mg / 100 g), and verbaskosa (6.0 mg / 100 g). Antioxidants in the pumpkin are beta-carotene. Beta-carotene is one type of carotenoids, besides having biological activity as provitamin A, it can also act as an effective antioxidant at low oxygen concentrations [3]. Betacarotene content in pumpkin is 1.18 mg / 100g [4].

Making the pumpkin juice fermented beverage needs to be added with as much as 15% milk powder which is a mixture of skimmed milk powder and full cream milk powder with varying concentrations. Addition of milk powder is done because lactose is not available in pumpkin. The important components that play a role in milk during the fermentation process are lactose and casein. During the fermentation process, lactose will be converted to lactic acid by lactic acid bacteria. The
formation of lactic acid will cause a decrease in pH so that the casein is coagulated to form a gel. The formation of the gel causes the beverage texture to become semi-solid.

The difference between skimmed milk powder and full cream milk lies in fat content. Kim milk is low fat milk while full cream milk is high fat milk. The addition of both milk powder to fermented beverages is expected to be a source of nutrition for the growth of lactic acid bacteria. While the high fat content in full cream milk can improve the taste and flavor of fermented drinks produced. According to [5] the content of milk fat can also affect the resulting flavor. Flavor produced in milk fat comes from fatty acids. Fatty acids such as butyrate, palmitate, oleate, and myristate.

The purpose of this study was to determine the effect of adding powdered milk mixture to the characteristics of the pumpkin juice fermentation drink and to find out the best treatment in making a functional synbiotic drink from pumpkin juice produced based on chemical analysis, microbiological tests, and organoleptic tests.

2. Methods

2.1 Materials and tools

Yellow pumpkin used is ripe fruit, yellowish green skin. Lactic acid bacteria starter used is Lactobacillus bulgaricus and Streptococcus thermophilus. As additional ingredients used are skim milk, full cream milk, arabic gum, and sugar. The chemicals used are aquades, potassium hydroxide, hydrochloric acid, phenophaline indicators, sodium hydroxide, oxalic acid, potassium sulfate, sulfuric acid, n-hexane, DPPH, methanol, biological salts, MRSA media, PCA media, and petroleum ether.

The tools used consist of juicers, analytical scales, test tubes, erlenmeyers, soxhlets, beaker cups, dropper pipettes, measuring cups, measuring flasks, burettes, porcelain cups, aluminum plates, soxhllets, viscosimeters, petri dishes, oce needles, vortex, incubator, colony counter, water bath, spectrophotometer, pH meter, and autoclave.

2.2 Research design

This research was conducted using a completely randomized design (CRD) with 5 treatments and 3 replications. If the results show differences due to the treatment give then proceed with the Duncant's New Multiple Range Test (DNMRT) at 5% level.

The treatment for this functional pumpkin beverage is the addition of a mixture of powdered milk (skim and full cream) as follows: A (13% skim milk, 2% full cream milk); B (12% skim milk, 3% full cream milk); C (11% skim milk, 4% full cream milk); D (10% skim milk, 5% full cream milk); and E (9% skim milk, 6% full cream milk)

2.3 Making Pumpkin Juice [6]

Yellow pumpkin (ripe, yellowish green skin), peeled off, separated from the head and washed clean. Then the pumpkin is cut into small pieces. Then the pumpkin pieces are crushed using a juicer and the pumpkin juice is obtained. After that chemical analysis is carried out such as pH, viscosity, beta carotene, antioxidant activity and total titrated acid.

2.4 Starter making [7]

The starter used in this study used fresh cow's milk. 100 ml of cow's milk is pasteurized at 80 °C for 10 minutes while stirring. Then cooled to 37 °C. After that, pure cultures of Lactobacillus bulgaricus and Streptococcus thermopillus were inoculated in the milk mixture as much as 5 ml. Inoculated milk was incubated at 37 °C for 12 hours. After that, analysis of pH and total LAB.

2.5 Manufacture of pumpkin juice fermented drinks Modification of [8]

The process of making this functional drink is done by preparing pumpkin extract, starter, milk powder (skim and full cream) according to the formulation, arabic gum, and sugar. Subsequently, mixing and homogenization of all ingredients were carried out, then pasteurized at 80 °C for 10
minutes. Then cool to 37 °C and add a 5 ml starter and the product is packed in a 250 ml jam jar. The product was incubated at 37 °C for 15 hours.

2.6 Observation
Observations made in this study in the form of analysis of raw materials and product analysis. Analysis of raw materials for total acid titration, viscosity, determination of antioxidant activity, beta carotene analysis, and pH. Analysis of fermented beverage products in the form of chemical analysis of pH, viscosity, total titration acid, total dissolved solids, ash content, protein content, fat content, determination of antioxidant activity, beta carotene analysis, and calcium content analysis. Then the microbiological test of the total lactic acid bacteria surface method. Next is sensory assessment,

3. Results and Discussion

3.1 Pumpkin
Raw material analysis of fermented pumpkin juice fermentation is done to determine the pH content, total titrated acid, viscosity, antioxidant activity, and beta-carotene levels found in pumpkin juice. The following are the results of the analysis presented in Table 1.

| Observation                     | Results |
|---------------------------------|---------|
| pH                              | 6.7     |
| Total titrated acid (%)         | 0.19    |
| Viscosity (dPa)                 | 1.78    |
| Antioxidant Activity (%)        | 2.05    |
| Betacarotene (μg / g)           | 2.61    |

The degree of acidity (pH) of the pumpkin is near neutral pH, due to the low acid content of the pumpkin juice before fermentation. Total titrated acid is 0.19%, this shows that fermentation has not yet taken place, the only available acid is from pumpkin juice. The content of beta-carotene in pumpkin juice is 2.61 μg / g while according [9] pumpkin beta-carotene is 1569 μg. The antioxidant activity of pumpkin juice was 2.05% and the viscosity of pumpkin juice was 1.78 dPa.

3.2 Lactic acid bacteria starter
Observations on Lactobacillus bulgaricus and Streptococcus thermophilus starters include pH and total lactic acid bacteria which can be seen in Table 2.

| Observation                          | Results             |
|--------------------------------------|---------------------|
| pH of cow's milk                     | 6.7                 |
| pH of Lactobacillus bulgaricus       | 4.32                |
| pH of Streptococcus thermophilus     | 4.56                |
| Total lactic acid bacteria starter Lactobacillus bulgaricus | 8.3 x 10^{10} |
| Total lactic acid bacteria starter Streptococcus thermophilus | 6.1 x 10^{10} |

During the growth phase of BAL in milk media, there is an overhaul of carbohydrates and proteins present in cow's milk by BAL which produce acid so that it can reduce pH.

3.3 Characteristics of Yellow Pumpkin Fermented Drinks
The results the analysis of the acidity (pH), total titrated acid and total lactic acid bacteria which showed the characteristics of the pumpkin fermented beverage with the resulting milk powder mixture can be seen in Table 3.

3.3.1 Acidity (pH)
Based on the results of analysis of variance in the addition of a mixture of different milk powder (skim milk and full cream milk) significantly affected the level of 5% on the pH of the pumpkin juice fermented beverage. The drink pH test results in Table 3 show a decrease in pH during the fermentation process. This happens because during fermentation, bacteria produce lactic acid. Lactic acid is the result of BAL metabolism by overhauling lactose which is a carbohydrate from milk [10][11][12].

| Table 3. Characteristics of Pumpkin Fermented Beverage and milk powder mixture |
|---------------------------------------------------------------|
| Milk powder mixture | pH ± SD | TTA (%) | LAB (colony/g) |
| A. (skim 13% + full cream 2%) | 4.22 ± 0.02 a | 0.43 ± 0.03 a | 2.1 x 10^9 ± 0.20 |
| B. (skim 12% + full cream 3%) | 4.24 ± 0.03 ab | 0.41 ± 0.03 ab | 2.0 x 10^9 ± 0.20 |
| C. (skim 11% + full cream 4%) | 4.27 ± 0.01 bc | 0.38 ± 0.04 bc | 1.9 x 10^9 ± 0.15 |
| D. (skim 10% + full cream 5%) | 4.27 ± 0.00 c | 0.36 ± 0.01 bc | 1.7 x 10^9 ± 0.28 |
| E. (skim 9% + full cream 6%) | 4.29 ± 0.01 c | 0.32 ± 0.02 c | 1.6 x 10^9 ± 0.20 |

Note: Figures in the same column are followed by unequal lowercase letters which are significantly different at the 5% level according to DNMRT

3.3.2 Total Titrated Acid

Based on the results of analysis of variance in the addition of a mixture of different milk powder (skim milk and full cream milk) significantly affected the level of 5% on the total acid of the yellow pumpkin juice fermented drink. Table 3 shows the total titrated acid in pumpkin fermentation drinks has fulfilled SNI 7552-2009, where the total standard of titrated acid fermented beverage is 0.2-0.9%.

In the pumpkin juice fermented beverage, the source of lactose comes from skim milk and full cream milk. The higher addition of skim milk causes the higher lactose content in fermented drinks. Lactose will be overhauled by lactic acid bacteria to lactic acid.

The higher the lactose in the milk mixture in fermented pumpkin juice, the higher the lactic acid formed. This is because more and more lactose is also converted into lactic acid. Similar to the research of [13], the more bacteria produce lactic acid, the higher the acid formed.

3.3.3 Total Lactic Acid Bacteria

Table 3 shows the total lactic acid bacteria in the pumpkin juice fermented drink ranged from 1.6x10^9-2.1x10^9 colonies / g. The addition of skimmed milk powder and full cream in this study serves as a source of lactose and protein for the activity and growth of lactic acid bacteria, because pumpkin juice does not contain lactose.

Skim milk and full cream milk also provide protein as a source of nitrogen for the growth of lactic acid bacteria and make up bacterial cells. The total lactic acid bacteria of the pumpkin juice fermented drink when compared to the fermented beverage from the red sweet potato juice drink is higher. According to [14], fermented drinks from red sweet potato juice drinks contain a total of acid bacteria with a range of 6.1x10^8-8.3x10^8 CFU / ml. The addition of full cream milk powder to fermented red sweet potato juice serves as a source of lactose and protein for the activity and growth of starter bacteria, because red sweet potato juice does not contain lactose. According to research by [15], the average number of lactic acid bacteria in red guava probiotic drinks ranged from 1.5x10^10-2.0x10^11 colonies / ml.

The total lactic acid bacteria of the pumpkin juice fermented drink for all treatments had fulfilled the SNI 7552-2009 standard regarding the requirements of fermented drinks that contained a total of lactic acid bacteria of at least 106 CFU / ml. The same was conveyed by Tamime (2006) cit. [15], that the minimum total LAB in probiotic food is 106 CFU / ml.

3.4 Functional Characteristics of Fermented Pumpkin Beverage with Milk Powder Mixture

One of the characteristics expected from fermented drinks is that it can be a functional beverage that is beneficial to health. Functional characteristics observed were antioxidant activity, beta-carotene levels and calcium levels, as shown in Table 4.
Table 4. Antioxidant activity, beta-carotene levels and calcium levels

| Milk powder mixture | Antioxidant (%) | Beta-carotene (µg/g) | Calcium levels (mg / 100ml) |
|---------------------|----------------|----------------------|----------------------------|
| A                   | 3.13 ± 0.16 a  | 2.41 ± 0.01          | 86.64 ± 0.02 a             |
| B                   | 2.87 ± 0.07 b  | 2.36 ± 0.01          | 100.61 ± 0.01 b            |
| C                   | 2.64 ± 0.07 c  | 2.32 ± 0.06          | 116.49 ± 0.01 c            |
| D                   | 2.33 ± 0.07 d  | 2.31 ± 0.07          | 128.82 ± 0.01 d            |
| E                   | 2.12 ± 0.04 e  | 2.30 ± 0.01          | 144.50 ± 0.01 e            |

3.4.1 Antioxidant Activity
Based on the analysis of variance, the addition of different milk powder (skim milk and full cream milk) significantly affected the level of 5% on the antioxidant activity of the pumpkin juice fermented beverage. The results of antioxidant testing on fermented drinks can be seen from Table 4. The highest antioxidant activity was found in treatment A which was 3.13%. The antioxidant activity of pumpkin juice extract increases after fermentation. According to [16], an increase in antioxidant activity occurs because of the activity of lactic acid bacteria in the medium. During fermentation compounds are produced which can increase and stabilize the antioxidant activity such as lactic acid, acetic acid, citric acid, succinic acid, malic acid, acetaldehyde, diacetyl and acetoin.

Increased antioxidant activity in fermented drinks may occur due to the presence of Vitamin C and Vitamin A. Both of these vitamins are a source of antioxidants. Antioxidant activity also comes from the content of vitamins found in skim milk and full cream milk. According to [10], skim milk contains Vitamin A around 0.04 SI and Vitamin C around 1.0 µg. Whereas full cream milk contains Vitamin A around 0.02 SI and Vitamin C around 0.8 µg [11].

3.4.2 Beta-carotene Content
Based on the analysis of raw materials, the beta-carotene content in pumpkin juice is 2.61 µg / g, whereas after fermented the beta-carotene concentration in the pumpkin juice is between 2.30-2.41 µg / g. Decrease in beta-carotene levels after the fermentation process, due to carotenoids sensitive to oxygen and acidic environment [17]. Also according to [18] the main reason for losing carotenoids is due to oxidation of the carotenoid unsaturated structure. Oxidation that appears is auto oxidation, this reaction arises spontaneously because of the presence of oxygen. In addition, carotenoid loss can also be caused by photo oxidation that occurs due to the presence of light.

At the best treatment, the average yield of beta-carotene was 2.41 µg / g. These results have met the nutritional adequacy rate according [19] nutritional adequacy rates for the consumption of beta-carotene recommended by the RDA (Recommended Dietary Allowance) in children of 400-700 µg RE / day which is equivalent to 2,400-4,200 µg beta-carotene and in infants at 375 RE / day or the equivalent of 2,250 µg beta-carotene. Consuming fermented pumpkin juice will meet the daily needs of vitamin A.

3.4.3 Calcium Levels
Based on the analysis of variance, the addition of different milk powder (skim milk and full cream milk) significantly affected the level of 5% on ash levels of the yellow pumpkin juice fermented drink. The highest calcium level was in treatment E which was 144.50 mg / 100ml and the lowest calcium level was in treatment A which was 86.64 mg / 100ml. The amount of calcium obtained in pumpkin juice fermented drinks is strongly influenced by the calcium content found in pumpkin, skim milk and full cream milk.

Pumpkin, skim milk, and full cream milk contains calcium. Calcium level is closely related to ash content. If the ash content is high then the calcium level is also high. This is also in line with the opinion of [20], calcium levels are closely related to ash content, the higher the ash content, the higher the calcium content. Calcium levels in bean sprouts fermented drinks ranged from 0.12% -1.08%. The higher the addition of winged bean sprouts extract, the higher calcium levels.

Calcium is a type of mineral that is very important for the growth and maintenance of teeth and bones. Calcium is generally found in milk. Increased calcium levels from treatments A to E in
fermented drinks may occur because calcium has dissolved by low pH. The lower the pH the more dissolved calcium. This is in line with the opinion of Deman (1997) cit [20], at pH 5.2, all calcium and phosphate in milk are dissolved.

3.5 Sensory Assessment

Sensory assessment was carried out to find out the pumpkin juice fermented beverage which was the result of the addition of a different milk mixture which was most accepted by the panelists. The average assessment results for each treatment can be seen in Table 5.

| Treatment | Color      | Aroma      | Taste     | Consistency | Texture |
|-----------|------------|------------|-----------|-------------|---------|
| A         | 3.80 ± 0.52 | 3.40 ± 0.59 | 2.70 ± 0.73 | 3.50 ± 0.68 | 3.75 ± 0.44 a |
| B         | 3.75 ± 0.63 | 3.35 ± 0.48 | 2.75 ± 0.55 | 3.35 ± 0.58 | 3.55 ± 0.60 ab |
| C         | 3.75 ± 0.55 | 3.35 ± 0.67 | 2.90 ± 0.71 | 3.35 ± 0.74 | 3.45 ± 0.68 ab |
| D         | 3.70 ± 0.47 | 3.35 ± 0.80 | 2.90 ± 0.68 | 3.25 ± 0.71 | 3.45 ± 0.68 b  |
| E         | 3.45 ± 0.51 | 3.00 ± 0.79 | 2.95 ± 0.51 | 3.20 ± 0.69 | 3.00 ± 0.91 b  |

Note: 1 = very dislike, 2 = dislike, 3 = somewhat like, 4 = like, 5 = really like

3.5.1 Color

The average value of the colors given by the panelists ranged from 3.45 to 3.80 with criteria like. The color of the pumpkin juice fermented beverage is orange. The color did not have a significant effect on the organoleptic test because the colors of each treatment were the same and the raw material used was the same, namely pumpkin juice extract. The color of this fermented drink is influenced by the main raw material, pumpkin. According to [21], pumpkin having a bright yellow color indicates that it has one of the carotenoid pigments, including beta-carotene.

According to Meyer cit [22], carotenoids are organic pigments found naturally in the chromoplast of plants. Carotenoids are generally yellow to red pigments that are found in plants. The longer the beta-carotene bond, the more orange the color.

3.5.2 Aroma

The average scent value given by the panelists ranged from 3.00-3.40 with somewhat like criteria. The aroma of the pumpkin juice fermented beverage is the sour taste and the characteristic odor of the pumpkin. The typical smell of pumpkin comes from the raw material itself, that is pumpkin. According to [23], the typical flavor of fermented drinks is caused by lactic acid and residual acetaldehyde, acetyl acid, acetic acid and other volatile substances produced by lactic acid bacteria.

3.5.3 Taste

The taste of this fermented beverage is sweet and sour. In treatment A had a sour and slightly sweet taste than treatment B, C, D, and E. So it was less liked by panelists. Whereas in treatment E the taste of the pumpkin juice fermented beverage was not too acidic. The taste of acid in fermented pumpkin juice is caused by the presence of lactic acid as metabolites due to the activity of lactic acid bacteria. According to [23], milk fermentation is sugar components in milk, especially lactose into lactic acid and other acids. At the beginning of the fermentation Sterptococcus thermophilus lowers the pH to 5.0. Below pH 5.0 causes Sterptococcus thermophilus to experience slow growth and Lactobaciluus bulgaricus dominates thereby reducing pH from 4.0 to 4.5.

3.5.4 Consistency

The average consistency value given by panelists ranged from 3.20 to 3.0. This pumpkin juice fermented beverage is homogeneous. If you see homogeneity, all treatments are almost the same. The homogeneity of the pumpkin juice fermented beverage is due to the presence of a stabilizing agent which is Arabic gum. According to [23] the addition of Arabic gum can increase stability by increasing viscosity. Viscosity will increase proportional to an increase in arabic gum concentration.
The homogeneity of the pumpkin juice fermented beverage all treatments looks the same because the amount of stabilizer added for all treatments is equal to as much as 1%. According to SNI 7552-2009 the consistency of fermented drinks is homogeneous.

3.5.5 Texture
The average texture value given by panelists ranged from 3.00 to 3.75 with the criteria of somewhat like to like. The texture or appearance of the pumpkin juice fermented beverage is strongly influenced by the physical state of the product. Texture is a sensory characteristic that is valued by a product through the sense of touch, sight, and feel in the oral cavity Soewarno, 1985 cit [5]. The appearance of this pumpkin juice fermented beverage is in the form of thick orange liquid.

The texture of the thick pumpkin juice fermented beverage is affected by pH, addition of powdered milk (skim and full cream) and Arabic gum. According to [24] the pH value in yogurt products can influence the process of protein denaturation which can cause viscosity in yogurt. The lower the pH value, the higher the viscosity of the yogurt product. Likewise, with the addition of milk powder (skim and full cream) which is the main component of total dissolved solids which causes increased thickness.

4. Conclusion
Addition of a mixture of powdered milk (skim and full cream) that is different from the fermented pumpkin juice fermentation has a significant effect on pH, total titrated acid, total lactic acid bacteria, antioxidant activity, calcium levels, viscosity, and organoleptic appearance, but not different markedly against beta-carotene, organoleptic color, aroma, taste, and consistency.

The best treatment based on chemical analysis, microbiological test, and sensory assessment, namely treatment A with a panelist acceptance level of color 3.80 (likes), aroma 3.40 (somewhat like), consistency 3.50 (likes), and texture, 75 (likes). This product has an average pH of 4.22, a total of 0.43% acid, a total of 2.1x10⁹ lactic acid bacteria, an antioxidant activity of 3.13%, a beta-carotene content of 2.41 μg / g, and a calcium content of 86.64 mg / 100ml.

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