The addition of Yam Tuber (Dioscorea alata) flour as a source of prebiotic on biomilk synbiotic characteristics

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The addition of Yam Tuber (*Dioscorea alata*) flour as a source of prebiotic on biomilk symbiotic characteristics

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Abstract. Yam tuber is quite abundant in Indonesia and has high carbohydrate and has inulin content. The cyam has not been widely used as an ingredient of biomilk synbiotic or yoghurt products. This study aimed to investigate the effects addition of yam Tuber (Dioscorea alata) flour as a source of prebiotic, on physicochemical and microbial characteristics of biomilk synbiotic. Starter culture on biomilk process using Streptococcus thermophillus and Lactobia 15 us bulgaricus as probiotic bacteria used Lactobacillus rhamnosus. The experiment design in this study using a Completely Randomized Design with six treatments of three replicates. The treatments of three replicates. The treatments of three replicates. The treatments of three replicates will be continued with Least Significant D 33 rence Test (LSD). The results of the research showed that addition of yam tuber flour has a significant effect (P<0.05) on pH, total Lactic Acid Bacteria and viscosity of biomilk synbiotic. It was concluded that yam tuber flour could be used as a source of prebiotic on biomilk synbiotic. The addition of 1.0 % of yam tuber flour produced the best of biomilk synbiotic. The addition of the best of biomilk synbiotic characteristics.

1. Introduction

Biomilk is a fermentation processing product from milk or UHT milk by Lactic Acid Bacteria (LAB) from *Streptococcus thermophillus* and *Lactobacillus bulgaricus*. Biomilk, better known as yogurt, is a fermented milk drink from milk are preferred by consumers because they have health benefits besides containing acid with low or without alcohol, semi-solid and soft texture, as well as fresh taste. To increase the functionality of the fermented drink, biomilk needs to be added by probiotics bacteria. The ability of probiotic bacteria to grow in the intestine and pressure growth of enteric pathogenic bacteria was useful for keeping man health. Some probiotics bacteria commonly used in the fermented drink or biomilk are *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, and *Bifidobacterium*. Probiotics bacteria of *Lactobacillus rhamnosus* species have ability to reduce cholesterol levels in experimental rats (*Rattus novergicus*)[1].

Synbiotic biomilk is a biomilk containing probiotics bacteria and prebiotics substances. Prebiotics are food ingredient that cannot be digested but it is possible to be used by lactic acid bacteria or probiotic bacteria to increase its viability [2,3]. Some prebiotics commonly used is *fructo oligosaccharides* (FOSs) like *inulin*, *galakto saccharida* (GOSs). *Lactitol*, *lactulose*, and *inulin* are special FOSs. *Lactulose*, *oligofructose* and *lactitol* are compounds that can increase the growth in *Bifidobacterium*. Fructo oligosaccharide (FOSs) is an important and natural prebiotic. Inulin has the

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characteristic β (1-2) bond with terminate molecule in a group in glucose unit. Inulin is considered to be able to reduce blood glucose level and cholesterol level, as well as to improve bone health and absorption when being added to food. Additionally, it is rich of calcium[4,5].

One of the local tubers that are rich with inulin and can be used as a source of prebiotics are known as yam tuber (Diosorea alata). Yam tuber (Dioscorea spp) contains inulin that can be functioned as prebiotics[6], and also rich which fiber and inulin contained in tuber yam is a bioactive component that can be functional. Food fiber is a part of plant that cannot be digested by digestive enzymes in healthy human intestine, especially from polysaccharides[7]. Generally, yam tuber flour (Dioscorea spp.) can be consume, and some of it can be used as medicine due to its contents of alkaloid and steroid as anti-inflammatory. In local area, yam is processed as food substitute and chips as snack. The use of tuber yum as a prebiotic source in the process of making biomilk or fermented products is not widely known and used. Inulin and foo be represented to be used as prebiotic source for fermented drink or Biomilk. Hence the purpose of this study was to investigate the physico-chemical and microbial characteristics of biomilk synbiotic from UHT milk added with different amount of tuber yam flour (Dioscorea alata), though analysis of Total Lactic Acid Bacteria, pH, water content and viscosity.

30 Material and method

This research was conducted at the Laboratory of Animal produt technology study program, Faculty of Animal Science, Sam Ratulangi University in Manado and The Laboratory of Microbiology Study program Food and Nutrition of Gadjah Mada University.

2.1 Material and equipment

The main materials used in this research were pasteurized milk (UHT) milk, skim milk, sucrose, distilled water, starter of lactic acid bacteria using *Streptococcus thermophiles, Lactobacillus bulgaricus*, and cultures of probiotic bacteria were *Lactobacillus rhamnosus*, sodium bicarbonate (CaCo₃), MRS agar (MRSA), MRS broth (MRSB), peptone water, alcohol 75%, aquadest, and other materials. The Equipment used in this study were, autoclave, incubator, cooler box, laminar air flow, electric oven, bottle scale, water bath, digital pipette, spatula, refrigerator, erlenmeyer, petridish, desicator, buns 10 lamp, ose needle, and test tube. Instruments used include digital pH meters, digital thermometers, and colony counters.

2.2 Research methods

This research was conducted using Completely Randomized Design (CRD), consisted of six (6) treatments with three replications [8]. Data were analyzed by variance analysis. Significantly different treatment effect on variables were tested using honestly significant difference (HSD)

The Treatments used in this research were as follows:

P1: Without addition of yam tuber flour (control)

 P_2 : Addition of yam tuber flour 0.5 %

P3: Addition of yam tuber flour 1.0 %

P4: Addition of yam tuber flour 1.5%

P5: Addition of yamtuber flour 2.0% P6: Addition of yamtuber flour 2.5%

2.3 Research procedure

The research procedures are explained below:

2.3.1 Starter culture process. Lactic acid bacteria used before being reproduced were firstly refreshed; the bacteria used as starter yoghurt was (L. bulgaricus, S. thermophillus) and probiotic bacteria was (L.



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rhamnosus). All bacteria used were obtained from Food and Nutrition Microbiology Laboratory, Faculty of Gadjah Mada University.

- 2.3.2. Yam tuber flour making. Yam tuber flour was made by peeling yam tuber then washing it thoroughly. After that, it was thinly sliced and soaked in water for 3 hours. After being soaked, it was dried in oven for at temperature of 50°C during 16 hours. After being dried, was blended and sifted using mesh 80. The flour obtained was stored until it was used.
- 2.3.3 Preparation starters from lactic acid bacteria. Starters from Lactic Acid Bacteria that can be used refers to a method [9] that was done by skim milk solution of 8% sterilized a 112 temperature of 1150C during 10 minutes, then cooling to reach 450C. After that, inoculated using lactic acid bacteria, was used S. Thermophillus, L bulgaricus, and L. rhamnosus. The incubation is carried out at the temperature of 360C during 18 hours.
- 2.3.4 Preparation of fermented drink of biomilk synbiotik. The process of fermented drink of biomilk refers to the process of making modified yoghurt that previously has been conducted by[1]. Process of making biomilk synbiotik, was done by adding the main material used (UHT milk) with yam tuber flour (based on the treatments), that was 0%, 0.5%, 1.0%, 1.5%, 2.0% and 2.5 % then added sucrose 6% and skim milk 6%. All the materials was mixed using mixer for 3 minutes then pasteurized at the temperature of 85°C during 15 minutes. After that, it was cooled until the temperature at 40°C. Inoculation with starter that has been prepared of 5%, while the comparison between Streptococcus thermophillus, Lactobacillus bulgaricus, and Lactobacillus rhamnosus, one starter to another was 1:1:1. The stabilizer on the product used gelatin 0.1%. Incubation were conducted in the incubator at the temperature at 42°C (for 6 hours), and was stored in refrigerator until being analyzed.
- 2.3.5 Laboratory analysis. For microbiological analysis used Plate Count Method were sampel (1.0 ml) of yoghurt decimally diluted in sterile peptone water (Merck Germany 0.1%) and 1% aliquot diluted in were take into petridish and added with MRS agar steril and incubated at 376, for 48 hours and colony forming units (cfu/g) were counted by a colony counter. pH measurents were carried out using a 32 tal pH meter (metrohm 691 Swiss). Water content measurement was done using dry method. Samples were estimated by the method described in AOAC [10], Viscosity was done using visco meter (Brookfield viscometer).

3. Results and discussion

Statistical analysis, showed that treatment of different amount of yam tuber flour utilization on biomilk synbiotic, had significantly difference (p < 0.01) on the viscosity and significant effect (p < 0.05) on water content, pH and total lactic acid bacteria (LAB). The result of statistical analysis of viscosity, pH value, water content, and total lactic acid bacteria were presented in table 1.

3.1 The influence of yam tuber flour on total of lactic acid bacteria of biomilk synbiotics

The statistical analysis showed that utilization of yan tuber flour on different level of biomilk synbiotic which was significant different (P≤0.05) on the total of Lactic Acid Bacteria of the biomilk synbiotic. The process of fermented drink shown that highest lactic acid bacteria of biomilk synbiotic was found on utilization 1,0% and 1,5.4. The utilization of yam flour had limitation were used more than 1.5%, it will caused a decrease of the growth of Lactic Acid Bacteria on biomilk synbiotic.

The quality of biomilk influenced by Lactic Acid Bacteria and the amount of yam flour added influenced the growth of Lactic Acid Bacteria. Total Lactic acid bacteria of biomilk synbiotic showed average ranging from 8.84 (Log cfu/ml) was found on P1 or without addition of tuber yam flour to 9.57(Log Cfu/ml) for utilization 1.0% tuber yam flour was found on P3. The highest lactic acid bacteria of biomilk synbiotic was found on P3 or addition 1.0% tuber yam flour. Total lactic acid bacteria of biomilk synbiotic with six (6) treatments were total lactic acid bacteria

still including into normal standard lactic acid bacteria in yoghurt quality of more than 106 cfu/ml or 6.0 (Log Cfu/ml) [11] and all of a fermented drink that as good to be consumed. Based on the Standard Nasional Indonesia(SNI)[12], it was stated that the total of Lactic Acid Bacteria (LAB) on the fermented drink or yoghurt should be 107 cfu/ml, or 7.0 (Log Cfu/ml). The result of the treatment of biomilk product showed that the total of Lactic Acid Bacteria (LAB) in all treatments is more than 107 CFU/ml, meaning that biomilk obtained includes as a good fermented drink. The research result states that good yoghurt is yoghurt containing more than 106 CFU/ml of bacteria. The utilization of yam tuber flour in the process of fermenting biomilk showed quite big influence on the growth of LAB. The increase of total LAB continues until the used of yam tuber flour at the level of 1.5%. Utilization of yam tuber flour above 1.5% showed reduction growth of total LAB. It is probably due to the fact that the higher amount of yam tuber flour, the more viscous the yoghurt that is obtained, so it can disturb the growth of LAB. Physical characteristic of yam tuber flour was slimy, and it was as to binding the cell of LAB, resulting in the decrease of LAB's growth. Other possibility were that the higher the concentration of flour, the higher the content of phytate on biomilk that was obtained, so it will disturb the LAB's growth.

Table 1. The average value of statistical analysis on the total of lactic acid bacteria, pH, water

content and viscosity of synbiotic biomilk				
Treatment	Total of Lactic Acid Bacteria (Log ,CFU/ml)	рН	Water Content (%)	Viscosity (cP)
P1 (control)	8.84±0.02a	5.01 ±0.01 ^a	81.09 ± 0.03b	239.75 ±1.47a
P2 (0.5%)	8.88±0.04a	4.73 ±0.08a	81.55 ± 0.10^{b}	355.38 ±2.67b
P3 (1.0%)	9.57±0.01 ^b	4.87 ± 0.08^{a}	81.95 ± 0.61b	588.93 ±1.18°
P4 (1.5%)	9.36±0.04b	5.18 ±0.07 ^b	80.28 ± 0.06^{a}	980.28 ±1.39d
P5 (2.0%)	9.07±0.05 ^b	5.35 ±0.01 ^b	79.66 ± 0.30^{a}	1843.0 ±2.23°
P6 (2.5%)	8.86±0.02a	5.19 ±0.07 ^b	80.09 ± 0.41^{a}	1256.75±2.27f

^{*}Different superscript are significant different (P<0.05)

The result of LSD test showed that the total of LAB on biomilk fermented drink at control treatment (P1) it wasn't different from the treatment of P2, P5, and P6. However, it was significantly different from biomilk drink with the addition of 1.0% of yam tuber flour or treatment P2 by adding 1.5% of yam tuber flour or treatment P3. On table 1, it was showed that the highest total of LAB of biomilk was in treatment P4 or used of 1.5% of yam tuber flour it was 109 CFU/ml, while the lowest total of LAB in treatment P1 without adding of yam tuber flour was 108 CFU/ml. The decrease of total LAB followed by the increase of concentration of yam tuber flour and also influenced by the viscosity. The higher of the viscosity obtained, the more viscous the yoghurt will be. Thus, the available water content was reduced as well. States that the low availability of water as a growth media and hindered diffusion process, due to the low amount of free water, results in the hindered growth and metabolism of bacteria [13].

3.2 The influence of yam tuber flour on pH value of biomilk synbiotics

Based on the data of observation on pH value of fermented drink of biomilk added by yam flour, pH value was different from one treatment and other treatments. The pH value at the end of fermentation was ranged from pH 5.35 - to pH 4.73. The best or the lowest pH value at the end of the fermentation in treatment P1 by addition of 0.5% yam tuber flour was 4.73, while treatment P2 by addition of 1.0% of yam flour was 4.87. The highest pH value obtained at P5 treatment by addition of 2.0% yam tuber flour was 5.35. Overall, based on pH values obtained from above treatments, it was seen that the pH values are still included in good fermented drink[14].

Statistical analysis showed that treatment of different yam tuber flour utilization had significant difference ($p \le 0.05$) on pH values of biomilk synbiotic. The lowest pH of biomilk synbiotic was found on addition yam tuber flour of 0.5% (P2) and 1.0% (P3) which was not different with pH biomilk

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synbiotic. In addition pH of biomilk synbiotik utilization of 1.5% (P4) yam tuber flour, was not different with pH of biomilk synbiotic utilization of 2.0% (P5) and utilization of 2.5% (P6) yam tuber flour. The pH value of the fermented drink decreases due to the increase of flour concentration used until the level of 1.5%. The utilization of yam tuber flour at the level above 1.5% causes the increase of pH value on biomilk drink.

The utilization of yam tuber flour until the level of 1.5% could be decrease pH value due to carbohydrate renovation inside the milk used by LAB. Lactic Acid Bacteria, as the starter addition, will separate carbohydrate into the simples form of glucose and change it as lactic acid, resulting in the decrease of pH on biomilk synbiotic. Based on the research conducted by [15], it was stated that the activity of LAB is able to change lactose into lactic acid so that the acid content on fermented drink increases. The decrease on pH and acid formed in the fermented drink during the process of fermentation depends on the content of solid material used. Meanwhile, according to [16], it is explained that the activity of LAB forming acid is not the same so that it increase the acidity of milk and causes different decrease on pH values.

3.3 The influence of yam tuber flour on the viscosity of biomilk synbiotic

The analysis statistic of viscosity value of biomilk fermented drink added yam tuber flour in different consentration during the process of making biomilk synbiotic, while the viscosity was different from one treatment and other treatments, and there was highly significant difference (p<0.01). The best viscosity obtained on the used of yam tuber flour added by yam 2.5% (P5) of yam flour was 1843.0 cP. Meanwhile, the lowest viscosity of biomilk without using yam flour 0% (P0) was 239.75 cP. The high viscosity of biomilk using of yam tuber flour was due to the higher carbohydrate content. The increase of yam flour obtains starch content as many as 75.6-84.3% that can influence the scosity of biomilk synbiotic generated. The starch of yam tuber can be used as thickener. Goncalves et al [17] states that the higher the thickener added, the higher the possibility to increase the binding capacity, which can thicken the product obtained.

The result of LSD shows that the viscosity of biomilk synbiotic fermented drink on control treatment (P1) was significantly differ [21] from the viscosity of biomilk fermented drink with the addition of yam tuber flour at treatment P2 (0.5%), P3 (1.0%), P4 (1.5%), P5 (2.0%) and P6 (2.5%). Likewise, other treatments have highly significant difference (p<0.01) between one treatment and other treatments.

The data of observation on viscosity of fermented drink of biomilk added by yam flour, was different from one treatment and other treatments. The Viscosity value at the end of fermentation was ranged from 239,75 cP - to 1843 cP. The higher viscosity was due to biomilk synbiotic containing 2,0% yam tuber flour, and the lowest viscosity value was due to biomilk synbiotic without containing yam tuber flour (P1).

3.4 The effect of yam tuber flour on water content of biomilk synbiotic

Water content of biomilk synbiotic using treatment were presented in table 1. Variance analysis showed that treatments of yam tuber flour had significan difference (P<0.05) on water content of biomilk synbiotic. Utilization of yam tuber flour with difference consentration showed that average water content ranging of 79,66 % to 81.95 %.

The Statistical of analysis on the water content of biomilk synbiotic by addition from the flour on different concentration during the process of making biomilk synbiotic had significant difference (p < 0.05). on the water content. The lowest water content was found on utilisazion yam tuber flour 2.0% (P5) of 79.66% and utilization of yam tuber flour 2.5% (P6) of 80.09%, which was not difference each other, and also was not difference with addition of yam tuber flour 1.5% (P4). In addition water countent utilisazion yam tuber flour 1.0% (P3) of 81,95% and utilization yam tuber 0.5% (P2) of 81.55 was not different and control treatment or without utilisazion of yam tuber flour (P0.) was not difference, but had significan difference with P4, P5 and P5 treatment.

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Difference water content of biomilk synbiotik using different level of concentration of yam tuber flour could be affected solidity of biomilk synbiotic but averages of water content of biomilk synbiotic were still ranging into normal standar of quality biomilk synbiotic. The addition of total solid in yoghurt formation my affect water content production.

4. Conclusion

It could be concluded that the addition of yam tuber flour could increased viability of Lactic acid Bacteria (LAB), decreased pH value, improved viscosity and reduce the water content. Yam tuber flour could be used as source of prebiotic on biomilk synbiotic. The addition 1.0 % of yam tuber flour produced the best of biomilk synbiotic.

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