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Article

Study of Proportion of Milk with Lesser Yam Filtrate and Starter Concentration for Producing Synbiotic Yoghurt

Rosida[1,a*] , S.S.Santi[2,a], F.R.Rohman[1]

1 Food Technology Department, Engineering Faculty, UPN "Veteran" Jawa Timur
2 Chemical Engineering Department, Engineering Faculty, UPN "Veteran" Jawa Timur
Jalan Raya Rungkut Madya, Gunung Anyar, Surabaya, Jawa Timur, Indonesia
E-mail: [a*]rosidaupnjatim@gmail.com, [b]sinthaay@gmail.com, [c]rocky.rahman@gmail.com
*Corresponding author: rosidaupnjatim@gmail.com | Phone number: -

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Abstract

This study aimed to determine the effect of the proportion of cow's milk with lesser yam filtrate and starter concentration on the characteristics of synbiotic yoghurt. The research used a factorial completely randomized design (CRD) with two factors, factor I was the proportion of cow's milk with lesser yam filtrate (100:0; 60:40; 50:50; 40:60; 0:100) and factor II was the starter cocentration (3%, 5%, 7%). The data obtained were analyzed using analysis of variant (ANOVA) and if there was a significant difference between treatments, then continued with Duncan's test at the 5% level. Synbiotic yoghurt from proportion of cow's milk with lesser yam filtrate (50:50) and 5% starter concentration was the best treatment with total Lactic Acid Bacteria of 7.23 log CFU/ml; pH 4.20; total dietary fiber 3.05%, soluble fiber content 1.3%, inulin content 1.2%, fat content 0.41% and soluble protein content 2.66% with an average texture preference score of 6.13 (like much); taste core 6.02 (like much) and smell score 5.20 (like).

Keywords: yoghurt, synbiotic, milk, lesser yam, inulin

1. Introduction

Yoghurt is a pasteurized milk product, fermented until distinctive acidity, odor and taste are obtained, with or without the addition of other permitted ingredients [1]. Yoghurt is made using a mixture of two bacteria *Streptococcus thermophilus* and *Lactobacillus bulgaricus* bacteria where these two bacteria have the ability to convert lactose into lactic acid during the fermentation process [2]. Probiotics are defined as live microorganisms that are consumed by humans or animals in sufficient quantities, are able to live and pass through the digestive tract and are beneficial to host cells by improving the health of the host [3].

The combination of probiotics and prebiotics can be called synbiotics. Prebiotics are components of food that cannot be digested and have a good effect on the host by triggering the activity, selective growth of colonic bacteria [4]. Synbiotic drink is fermented beverage that combines probiotics and prebiotics. Probiotics are live microorganisms which, when administered in sufficient quantities, will benefit the host. While prebiotics are substances that cannot be digested but can increase bacterial growth [5].

In general, the bacteria used in making yoghurt are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, but these two bacteria are not able to survive long in the digestive tract [6]. One of the

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probiotic bacteria added in making yoghurt is *Bifidobacterium bifidum. Bifidobacterium bifidum* is one of the probiotics that can survive in the gastrointestinal tract [7] and has a fairly high prebiotic activity [8] compared to other probiotic bacteria, so that in this study the type of probiotic bacteria Bifidobacterium was used. bifidum in the manufacture of synbiotic yoghurt.

One of the prebiotics recognized by BPOM is inulin. Inulin is a prebiotic because of its ability to stimulate the growth of good bacteria in the gut. Inulin is a polysaccharide built by fructose monosaccharide units through β-2-1 fructo-furanside bonds initiated by a glucose molecule so it is called fructooligosacarisa (FOS) [9]. Inulin is soluble in water, cannot be digested by digestive enzymes.

Lesser yam (*Dioscorea esculenta)* is one of the plants that grow in Indonesia and contain high inulin content [10]. Istianah (2010) stated that inulin levels in lesser yam were 14.77% [11]. The purpose of this study was to determine the effect of the proportion of cow's milk with lesser yam filtrate and starter concentration on the characteristics of synbiotic yoghurt. The addition of lesser yam extract to cow's milk in yoghurt fermentation is expected to increase the content of dietary fiber, especially inulin, in synbiotic yoghurt.

2. Material and Method

The materials used in the rearch were lesser yam tubers purchased at the Mangga Dua market in Surabaya, fresh cow's milk purchased at dairy vendor on Jl. Raya Jemursari Surabaya, and bacterial cultures of *Lactobacillus bulgaricus, Streptococcus thermophilus* and *Bifidobacterium bifidum* obtained at the Biology Laboratory, Faculty of Science and Technology, Airlangga University, Surabaya. Additional ingredients used are sucrose and skim milk.

The tools used for this research were scales, blenders, stoves, autoclaves, incubators, inoculation cases, colony counters, test tubes, and other glassware.

Making Lesser yam Filtrate

Fresh lesser yam tubers were peeled, washed, cut and crushed using blender with the addition of water (1:3) then filtered using filter cloth. The lesser yam tuber filtrate obtained was precipitated for 1 hour then heated at 80°C for 15 minutes and cooled.

Making Lesser yam Synbiotic Yoghurt

Cow's milk was pasteurized at 70-80°C for 15 minutes and added with 5% (w/v) skim milk and 8% (w/v) sucrose. Then cow's milk was mixed with lesser yam tuber filtrate according to treatment (0:100; 60:40; 50:50; 40:60; 100:0) and cooled. After that, it was inoculated with 3%, 5%,or 7% (v/v) starter *Lactobacillus bulgaricus, Streptococcus thermophilus*, and *Bifidobacterium bifidum* and incubated at 37°C for 18 hours.

3. Results and Discussion

At the beginning of the study, total analysis of initial lactic acid bacteria was carried out to determine the initial number of mixed lactic acid bacteria (*Lactobacillus bulgaricus, Streptococcus thermophilus* and *Bifidobacterium bifidum*) and obtained total BAL of 8.05 log CFU/ml or (10^8 CFU/ml) while according to FAO (2002) the minimum amount of yoghurt starter is 10^7 CFU/ml [12].

Total LAB and pH of synbiotic yoghurt

Table 1. Total LAB and pH of synbiotic yoghurt

Treatment		Total LAB (log CFU/ml)	pH
Milk:lesser yam filtrate	Starter Concentration (%)		
100 : 0	3	6,49^{ab}±0,016	4,50^{b}±0,141
100 : 0	5	6,54^{ab} ±0,088	4,50^{b} ±0,141
100 : 0	7	7,62 ^{d}±0,396	4,35^{b}±0,070
60 : 40	3	6,39 ^{a}±0,124	4,23^{ab}±0,240
60 : 40	5	6,54^{ab}±0,088	4,17^{ab}±0,183
60 : 40	7	7,73 ^{d}±0,075	4,12^{ab}±0,113
50 : 50	3	6,79 ^{b}±0,075	4,13^{ab}±0,070
50 : 50	5	7,24 ^{c}±0,120	4,12^{ab}±0,028
50 : 50	7	6,82 ^{b}±0,049	6,74^{ab}±0,056
40 : 60	3	6,74 ^{b}±0,057	4,07^{a}±0,042
40 : 60	5	6,88 ^{b}±0,105	4,03^{a}±0,014
40 : 60	7	7,72 ^{b}±0,058	4,01^{a}±0,014
0 : 100	3	6,39 ^{b}±0,124	4,12^{a}±0,028
0 : 100	5	6,93 ^{b}±0,036	4,12^{a}±0,028
0 : 100	7	7,41 ^{b}±0,426	4,11^{a}±0,014

*score with different notation revealed significantly difference

The results showed that the higher the proportion of gembil filtrate and the concentration of starter, the total lactic acid bacteria increased and the pH of yoghurt decreased. This is because of addition of lactose from cow's milk as carbon source, besides that addition of lesser yam tuber filtrate contains

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various kinds of nutrients such as dietary fiber, inulin and oligosaccharides which are also used as carbon sources for bacterial growth and are hydrolyzed into simple compounds.

According to Roberfroid (2007), several types of Bifidobacteria are capable of producing an extracellular enzyme inulinase that can hydrolyze the -2-1-D-fructose bond into simple sugars, which are then used as energy source for their growth [13]. Inulin will be hydrolyzed into simple sugars which will then be converted into lactic acid. During the fermentation process. During the decomposition into simple compounds, energy will be used for synthesis so that the number of cells increases and increases the total lactic acid bacteria in lesser yam synbiotic yoghurt.

From Table 1, it can be seen that the highest number of bacteria was found in the treatment of the proportion of milk: lesser yam filtrate (50:50) and 5% starter concentration. This treatment probably was the most optimal one in increasing the number of bacteria because of the content of nutrient sources needed by lactic acid bacteria for metabolism. This treatment is thought to provide optimum conditions for microbes to growth due to the presence of lactose in skim milk, sugar and inulin which are used by microbes as a carbon source.

The more concentration of starter used, the lower the pH value of yoghurt. This is in accordance with the opinion of Setianto *et al.* (2014) which states that the addition of starter will cause lactose degradation and lactic acid production which results in the decrease in pH and the formation of yoghurt clots [14]. According to Ihsan *et al.* (2017) which states that the decrease in pH value is caused by the formation of lactic acid as microorganism activity at the beginning of fermentation [15]. The decrease in pH occurs due to the fermentation process of carbohydrates, glucose and lactose which produces lactic acid by lactic acid bacteria [16].

Total Dietary Fiber, Soluble Fiber,and Inulin content

Tabel 2. Total Dietary Fiber, Soluble Fiber,and Inulin content of synbiotic yoghurt

Treatment		Total dietary fiber	Soluble fiber content	Inulin content
Milk:lesser yam filtrate	Starter concentration (%)			
100 : 0	3	0,30^{a}±0,028	0,25^{a} ±0,007	0,25^{b} ±0,007
100 : 0	5	0,27^{a}±0,021	0,23^{a} ±0,035	0,23^{a} ±0,007
100 : 0	7	0,26^{b}±0,007	0,21^{b}±0,014	0,21^{a}±0,007
60 : 40	3	2,77^{b}±0,163	1,33^{b}±0,035	1,10^{cd}±0,011
60 : 40	5	2,81^{b}±0,031	1,34^{b}±0,021	1,09^{c}±0,011
60 : 40	7	2,88^{c}±0,015	1,35^{b}±0,007	1,09^{c}±0,022
50 : 50	3	3,11^{d}±0,046	1,41^{b}±0,035	1,20^{e}±0,005
50 : 50	5	3,05^{d}±0,101	1,43^{b}±0,141	1,20^{e}±0,005
50 : 50	7	2,94^{c}±0,007	1,44^{b}±0,028	1,20^{e}±0,005
40 : 60	3	2,91^{c}±0,023	1,49^{b}±0,523	1,18^{e}±0,011
40 : 60	5	2,89^{c}±0,047	1,50^{b}±0,028	1,16^{d}±0,006
40 : 60	7	2,81^{b}±0,016	1,51^{b}±0,156	1,16^{d}±0,034
0 : 100	3	2,83^{b}±0,039	1,41^{b}±0,014	1,14^{d}±0,034
0 : 100	5	2,78^{b}±0,039	1,44^{b}±0,057	1,13^{d}±0,011
0 : 100	7	2,74^{b}±0,016	1,44^{b}±0,057	1,12^{cd}±0,034

*score with different notation revealed significantly difference

From Table 2, it can be seen that the proportion of 100% milk contains almost no dietary fiber, soluble fiber and inulin. While the treatment with the addition of lesser yam filtrate will produce yoghurt which contain different levels of dietary fiber and inulin. This is because some dietary fiber and inulin are used by lactic acid bacteria for their growth. According to Triyono (2010) skim milk is used by microbes as carbon source because there is lactose, while lesser yam filtrate contains inulin as source of prebiotics that can support microbial growth [17].

The treatment of proportion milk : lesser yam filtrate (50:50) and 5% starter concentration resulted in relatively high levels of dietary fiber, soluble fiber and inulin. This is probably because the proportion is optimal for microbial growth, where skim milk provides source of Carbon in the form of lactose and lesser yam filtrate provides inulin and dietary fiber as source of Carbon. The more carbon sources available, the more simple compounds such as simple sugars can be used as source of carbon. source of nutrition for lactic acid bacteria in breaking down simple sugars into lactic acid.

Sensory score of Synbiotic Yoghurt

Tabel 3. Sensory score of Synbiotic Yoghurt

Treatment		Texture score	Taste score	Smell score
Milk:lesser yam filtrate	Starter concentration (%)			
100 : 0	3	4,40 ^{c}±0,99	5,13 ^{c}±1,06	5,07^{b}±0,80
100 : 0	5	4,93^{cd}±0,96	5,33 ^{c}±0,98	5,13 ^{b}±1,13
100 : 0	7	4,53 ^{c} ±1,25	5,67^{cd}±1,11	5,00^{b}±1,13
60 : 40	3	5,73^{de}±0,88	5,53^{cd}±1,06	4,33^{a} ±1,18
60 : 40	5	5,27 ^{d}±0,96	5,33 ^{c}±0,82	4,93^{b}±1,22
60 : 40	7	4,13 ^{b}±1,41	4,53^{bc}±0,99	4,60^{a}±0,99
50 : 50	3	3,67 ^{b}±1,29	3,73 ^{b}±1,22	4,27^{a}±1,28
50 : 50	5	6,13 ^{e}±0,74	6,20 ^{d}±0,68	5,20^{b}±1,21
50 : 50	7	3,67 ^{b}±1,11	4,53^{bc}±0,99	4,60^{a}±1,24

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40 : 60	3	2,60[a]±0,83	2,80 [a]±1,08	3,87[a]±1,73
40 : 60	5	2,93[ab]±0,88	3,40[ab]±1,55	3,93[ab]±1,49
40 : 60	7	2,87[a]±0,99	3,53[ab] ±1,46	4,53[a]±1,55
0 : 100	3	3,60[b]±1,45	4,13 [b]±1,41	4,47[a]±1,36
0 : 100	5	3,07[a]±1,22	4,07 [b]±1,44	4,40[a]±1,45
0 : 100	7	3,07[a]±1,03	4,07 [b]±1,44	4,73[a]±1,33

*score with different notation revealed significantly difference

Sensory test results showed that the panelists preferred yoghurt from the treatment of proportion of skim : lesser yam filtrate (50:50) and 5% starter concentration, in terms of texture, taste, and smell compared to other treatments. Panelists prefer thick yoghurt texture with score of 6.13 (like much) from this treatment. This is because the formation of yoghurt texture due to coagulation of casein in milk was optimal. The viscosity of yoghurt was formed due to the decrease in pH so that casein coagulation occurs and made the semi-solid texture. This is in accordance with the opinion of Utami *et al.* (2013) which stated that the formation of lactic acid by LAB causes the pH to decrease so that casein undergoes coagulation resulting in an increase in viscosity and semi-solid texture [18]. The use of lesser yam filtrate provides additional nutrition in the form of inulin which can be broken down into simple sugars by LAB. Karlina and Rahayuni (2014) stated that the level of yoghurt viscosity can be influenced by the main ingredients of yoghurt, storage time and the addition of fiber [19]. Yoghurt texture is formed due to protein clumping when the pH reaches the isoelectric point [20].

Based on the taste parameters, panelists tend to like sour taste compared to less sour taste. Sour taste is the dominant taste in yoghurt. The treatment of the proportion of milk : lesser yam filtrate (50:50) and 5% starter concentration produced a sour taste that was preffered by the panelists, with a hedonic score of 6.20 (like much). According to Martin *et al.*, (2003) LAB metabolism during fermentation results in the conversion of carbohydrates into simple sugars, generally the main compound produced is lactic acid [21]. The lactic acid formed is the result of yoghurt starter fermentation which is homofermentative. According to Tamime and Robinson (2002) the main metabolite of homofermentative LAB is lactic acid. Lactic acid builds up, leading to more acidic conditions [22].

Similarly, in terms of smell, panelists prefer the typical sour smell of yoghurt compared to the less sour smell. The additional treatment of lesser yam filtrate causes a distinctive smell so that it has an effect on the smell of yoghurt. According to Antara (2012), the starter culture used is the main reason responsible for the formation of flavor compounds in the smell of yoghurt [23]. According to Surajudin *et al.* (2005) *Lactobacillus bulgaricus* is more involved in the formation of smell, *Streptococcus thermophillus* is more involved in the formation of taste [24]. The distinctive flavor of yoghurt is caused by the presence of lactic acid, acetaldehyde and other volatile substances produced by bacterial fermentation. Usmiati (2005) added that *Bifidobacteria* are LAB which have the ability to produce preferred flavor components [25].

In this study, the proportion of milk : lesser yam filtrate (50:50) and 5% starter concentration was determined as the best treatment because it produced synbiotic yoghurt with the best physico-chemical properties and was favored by panelists, and additional analysis was carried out, namely fat and protein content, and the results obtained fat content of 0.41% and protein content of 2.66%. The results of the analysis are in accordance with the requirements according to SNI (2981: 2009) which states that the yoghurt product has a minimum number of starter bacteria 10^7 and a minimum protein of 2.7% [26].

4. Conclusions

Based on the research conducted, it can be concluded that the best treatment is the proportion of milk : lesser yam filtrate (50:50) and 5% starter concentration which produced yoghurt with the following characteristics: total Lactic Acid Bacteria 7.23 log CFU/ml; pH 4.20; total dietary fiber 3.05%, soluble fiber content 1.3%, inulin content 1.2%, fat content 0.41% and protein content 2.66%. Panelists preferred yoghurt from this treatment, in terms of texture (6,13) taste (6,20) and smell (5,20) so that this synbiotic yoghurt can be developed into a functional drink.

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