International Journal of Agricultural Technology 2017 Vol. 13(1): 37-46
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Quality of Probiotic Fermented Milk using *Lactobacillus casei* subsp. *casei* R-68 as a Starter with the Variation of Skim Milk and Sucrose

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Usman Pato, Yusuf, Y., Rossi, E., Yunaira, R and Githasari, T. (2017). Quality of probiotik fermented milk using *Lactobacillus casei* subsp. *casei* R-68 as a starter with the variation of skim milk and sucrose. International Journal of Agricultural Technology 13(1): 37-46.

The functional food in the form of probiotic fermented milk is one of the alternative foods that can be consumed to keep the body fit and healthy. This research aimed to determine the amount of optimal skim milk and sucrose to produce probiotic fermented milk using *Lactobacillus casei* subsp. *casei* R-68 as a starter. The research was conducted experimentally using a Completely Random Design. The data were analyzed statistically using ANOVA and continued with DNMRT test at the level of 5%. The results show that variation of skim milk or sucrose significantly affected pH, total lactic acid, number of lactic acid bacteria, total of solid without fat as well as the amount of fat and protein, but did not significantly influence the ash and fat contents. In the case of sensory test, the variation of sucrose significantly influenced all sensory parameters. The best treatment was obtained from the use of 15% skim milk and 5% sucrose.

Keywords: probiotic, *Lactobacillus casei*, quality, skim milk, sucrose

Introduction

The tendency of the increase of the kinds of disease has led to the public awareness to keep themselves healthy. A tendency of life style change requires not only the awareness for delicious and nutritious food, but also the food that can keep the healthy body. One of the ways is to be in the habit of consuming functional and healthy foods such as fermented milk produced through a fermentation process by the lactic acid bacteria (LAB).

The skim milk and carbohydrates are used by the bacteria as the source of nitrogen and carbon for its growth. Supplementation of the skim milk medium with either carbohydrates like sucrose, glucose, lactose, galactose and fructose

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or whey protein concentrate (WPC) increased both growth of *Streptococcus thermophilus* ST1 and its polymer formation by different extents, with sucrose being most effective among the carbon sources tested (Zhang *et al*., 2011). In addition, the use of sucrose could become a good source for microorganism that could influence the quality of the skim milk produced. The addition of 5% sucrose could produce the optimum amount of lactic acid in corn yogurt (Etiyati, 2010) and Sintasari (2014) reported that the 7% sucrose concentration and the 9% skim milk was the best treatment according the physical, chemical and microbiological parameters in probiotic drinks of red rice extract. Both sucrose (up to 3%, w/v) and skim milk (up to 12%, w/v) increased the number of lactic acid bacteria. However, the effect of sucrose plus skim milk was not superior to that of skim milk alone (Kim *et al*., 2009). Bills *et al*. (1972) found that growth and acid production by *Lactobacillus bulgaricus* and *Streptococcus thermophiles* were inhibited in media containing 4% or more sucrose.

Dadih is a milk product that is fermented in a bamboo tube that can generally be found in West Sumatra province and in the district of Kampar, Riau province. The result of bacterial isolation and identification has proven that there were 4 genus of LAB contained in *dadih*, i.e. *Lactobacillus* sp, *Leuconostoc* sp, *Streptococcus* sp *and Lactococcus* sp. Some strains of dadih isolates were very useful to health because LAB can reduce cholesterol through a direct binding of dietary cholesterol and taurocolate deconjugation (Pato, *et al*. 2005). The previous studies also found that *Lactobacilus casei* subsp. *casei* R-68, a strain of dadih's LAB had the ability to bind mutagenic substances such as *N*-Nitrosodiethylamine or NDEA (Hosono *et al*., 1990) and mutagenic compound that obtained in '*tauco'*, a kind of fermented bean paste used as condiment, as a result of the heating in high temperature, and it was resistant to gastric acid or bile (Pato, 2003); therefore, *Lactobacillus casei* subsp. *casei* R-68 is potential to be used as probiotic.

This research aimed to find out the variation of the addition of skim milk and sucrose on the quality of fermented milk made by using *Lactobacilus casei* subsp. *casei* R-68.

Materials and methods

Culture and Materials

The main materials used to make this fermented milk drinks were skim milk, sucrose and CMC. The starter used was *Lactobacilus casei* subsp. *casei* R-68 (Cultural Stock in the Laboratory of Agricultural Product Technology, Faculty of Agriculture, Universitas Riau, Indonesia) which was isolated from *dadih*, traditional fermented milk from West Sumatra (Hosono *et al*., 1989).

Research Method

This research was carried out experimentally using Complete Random Design devided into two steps. The first step was to determine the use of optimal milk that consisted of five treatments namely skim milk 7.5%, skim milk 10%, skim milk 12.5%, skim milk 15% and skim milk 17.5%. The second step was to determine the use of optimal sucrose that consisted of 4 treatments were sucrose 0%, sucrose 5%, sucrose 10% and sucrose 15%.

Preparation of Starter

First, the 10% of skim milk containing 1% sucrose were sterilized at 115^{0}C for 10 minutes. Then after the medium became cold (37^{0}C), the medium of the skim milk was inoculated with a culture of 2% and then incubated at 37^{0}C for 12 h signalled by coagulation of skim milk.

Preparation of fermented milk

Skim milk was prepared on the basis of the 7.5%, 10%, 12.5%, 15% and 17% treatments and then added with sucrose according to the 0%, 5%, 10% and 15% treatments as well as 0,05% CMC. These materials were homogenized using a mixer with the speed of 1000 rpm for 5 min and then pasteurized at 85^{0}C for 15 min. After the medium became cold (37^{0}C), then medium were inoculated with the starter of 5% and incubated at 37^{0}C for 12 h. Afterwards, the fermented milks were analysed according to the parameters of yoghurt by SNI 102981: 2009 (10).

Parameters of fermented milk

The observed parameters in this research were the number of LAB, pH value, total lactic acid, fat, total solid without fat, protein and ash contents in accordance with Indonesia National Standard of Yoghurt (SNI 2981: 2009). The sensory test was performed according to Setyaningsih *et al.* (2010) including colour, texture and taste of the probiotic fermented milk by 25 semi-skilled panellists.

Data Analysis

The collected data were analyzed using Analysis of Variance (ANOVA). When *F calculated* was greater than *F table*, then analysis was continued with DNMRT test at the level of 5%.

Results and Discussion

The variation of skim significantly affected to the pH value and total lactic acid of probiotic fermented milk as shown in Table 1.

Table 1. The average value of pH and total lactic acid and the number of lactic acid bacteria of probiotic fermented milk using strain R-68 with the variation of skim milk

Treatments	pH	Total lactic acid (%)	Number of LAB (log cfu/mL)
Skim milk 7.5%	5.02[ab]	1.06[a]	9.43[b]
Skim milk 10.0%	4.85[a]	1.15[a]	9.05[b]
Skim milk 12.5%	5.12[bc]	0.88[b]	8.78[b]
Skim milk 15.0%	5.30[cd]	0.74[c]	7.70[a]
Skim milk 17.5%	5.45[d]	0.56[d]	6.92[a]

The different small letters in the same column are significantly different according to DNMRT test at the 5% level

The data in Table 1 shows that the greater the amount of skim milk added, the pH value increased from 5.02 to 5.45 while the total lactic acid decreased from 1.06 to 1.56% and the number of LAB also decreased from 9.43 to 6.92 log cfu/mL. This was because a great amount of skim milk was not metabolized by the *Lactobacillus casei* subsp. *casei* R-68 so it was accumulated in the medium so that the pH value of product became higher. Rahman *et al*. (1992) stated that lactose had the natural pH of 6.6. Therefore, the greater the remaining lactose that was not metabolized by LAB, the value of pH product increased and the total lactic acid declined. The pH value of the fermented milk obtained through this study was almost the same as the pH value (4.55-5.05) of cocoghurt made by using the *Entrococccus faecalis* UP-11 (Pitrayadi *et al*., 2015) and a bit different from the pH value (4.35-4.68) of the fermented milk by using isolate from Egypt traditional food (Francois *et al*., 2007) and the pH value (4.21-4.30) of probiotic fermented milk (Tonguc *et al*., 2013). The total lactic acid of the probiotic fermented milk obtained from a study that only used one kind of LAB was about 0.56-1.15%, whereas the fermented milk that used 2 to 3 LAB species produced the total lactic acid of around 0.74-0.82% (Tonguc *et al*., 2013). The probiotic fermented milk produced through all treatments had the total lactic acid of about 0.56-1.15% that already fulfilled the quality standard of yoghurt which is between 0.5-2% (SNI 2981 :2009) and Codex standard of 0.3% (Codex Stan 243-2003).

The data in Table 1 also indicates that the more the skim milk was used, the number of LAB significantly declined which may be caused by the presence of non-specific antimicrobial compounds such as lactoferrin in the skim milk protein as antimicrobe that might inhibited the growth of LAB (Pakkanen and Alto, 1997 *in* Zsuzsanna Bosze, 2007). It may also be caused by the peptide activities in the milk (Lahov and Regelson, 2006). Hayes *et al*. (2006) discovered that antimicrobe of peptide originated from casein namely isracidin had antibacterial activity towards *Lactobacilli* and some Gram positive and negative bacteria. The total number of LAB in the probiotic fermented milk in all treatments except treatment Skim Milk 17.5% had met the standard quality of yogurt of minimally 10^7 koloni/g (SNI2981: 2009 and Codex Stan 243-2003).

The chemical composition of probiotic fermented milk analyzed in accordance with the Yogurt Quality Standard (SNI 2981:2009) consisted of fat, total solid without milk fat, protein and ash contents. The variation of skim milk used significantly affected ($P<0.05$) the amount of fat and protein, the total solid without milk fat, but did not influence ($P<0.05$) the ash content of the probiotic fermented milk product (Table 2).

Table 2. Chemical composition of probiotic fermented milk using strain R-68 with the variation of skim milk

Treatments	Fat (%)	Total solid without milk fat (%)	Protein (%)	Ash (%)
Skim milk 7.5%	0.38[a]	10.17[a]	2.14[a]	0.05[a]
Skim milk 10.0%	0.54[ab]	10.29[a]	3.30[b]	0.08[a]
Skim milk 12.5%	0.66[b]	12.01[b]	4.37[c]	0.09[a]
Skim milk 15.0%	0.86[c]	12.94[b]	5.91[d]	0.14[a]
Skim milk 17.5%	0.93[c]	14.73[c]	7.45[e]	0.16[a]

The different small letters in the same column are significantly different according to DNMRT test at the 5% level

Table 2 shows that the more the skim milk used, the higher the amount of fat, total solid without milk fat and protein of the probiotic fermented milk. The increase in protein content of probiotic fermented milk was caused by the high amount of skim milk used that contained great amount of protein. This is supported by USDEC (2014), Codex Stan 207-1999 and Reference Manual for U.S. Milk Powders (2005) who stated that the amount of protein in the skim milk reaches 34-37%. The more the skim milk was used, the greater the total solid without milk fat was obtained because the skim milk contains high solid material such as protein of 34-37%, lactose of 49.5-52% and ash of 8.2-8.6%. It was obvious that the addition of skim milk caused high amount of solid material in fermented milk and that the total solid without milk fat increased.

The research findings also indicated that there was a significant increase of fat in the probiotic fermented milk since more skim milk was used. This happened because the skim milk contained fat between 0.6-1.25% (USDEC, 2014) and 1.5% in maximum (Codex Stan 207-1999). The finding of this study was similar to the previous research findings (Pitrayadi *et al*., 2015). In addition, Table 2 also shows that the increase in skim milk used significantly affected the ash content of the probiotic fermented milk. This finding was contradictory to the previous research findings (Syahputra *et al*., 2015) which indicated that there was an increase of ash content in fermented milk as more skim milk was used in the production process. The amount of fat, the total solid without milk fat, protein and ash contents of the probiotic fermented milk produced by using starter *Lactobacillus casei* subsp. *casei* R-68 already met the yogurt standard quality of low fat (SNI 2981:2009) and Standard Codex for fermented milk (Codex Stan 243-2003), except the amount of protein at the treatment of skim milk 7.5% as much as 2.14% which was lower than the amount of protein according to SNI and Codex Standard, that is 2.7%.

The data in Tables 1 and 2 demonstrate that the use of skim milk of 15% produced good quality of probiotic fermented milk that fulfills the SNI and Codex standards. Based on this fact, it was necessary to continue conducting the study to find out the effect of the variation of sucrose addition on the quality of probiotic fermented milk. The variation of the sucrose used significantly affected (P<0.05) the pH value and total lactic acid of the probiotic fermented milk as shown in Table 3.

Table 3. The average value of pH and total lactic acid and the number of lactic acid bacteria of probiotic fermented milk using strain R-68 with the variation of sucrose

Treatments	**pH**	**Total lactic acid (%)**	**Number of LAB (log cfu/mL)**
Sucrose 0%	5.64^{a}	0.99^{d}	8.11^{a}
Sucrose 5.0%	5.65^{b}	0.93^{c}	8.34^{a}
Sucrose 10.0%	6.72^{c}	0.77^{b}	6.53^{b}
Sucrose 15.0%	6.76^{d}	0.63^{a}	6.96^{b}

The different small letters in the same column are significantly different according to DNMRT test at the 5% level

The data in Table 3 show that the more sucrose was added, the pH value increased from 5.64 to 6.76 and the total lactic acid decreased from 0.99 to 0.70% because the natural pH of sucrose was relatively high, that is 9.0. Other reason might be because the more sucrose was added the more sucrose accumulated in the medium that might inhibit the bacterial activity so that the formation of lactic acid would also declined. This corresponds to the statement of Fardiaz *et* *al*. (1980) who said that if bacteria, yeast or mould are placed in sugar liquid with higher concentration, the water in the cell will go out through the cell membrane known as osmosis. This process would further influence the growth of bacteria and other microbes. The probiotic fermented milk for all treatments had the total lactic acid of 0.63-0.99% and this already met the yogurt standard which is between 0.5-2.0% (SNI 2081:2009).

The amount of bacteria in a fermented milk product is one of the important quality parameters. The data show that the addition of sucrose significantly affected the number of LAB of probiotic fermented milk (Table 3). The data in Table 3 indicate that the use of more than 5% of sucrose significantly influenced the number of LAB. Based on this table, it is clearly seen that the use of greater sucrose between 10 to 15% reduced the amount of LAB in probiotic fermented product. This is because the *Lactobaccilus casei* subsp. *casei* R-68 did not produce sucrose, a type of anzyme that could break sucrose to glucose and fructose. As a result, sucrose accumulation occured in the fermentation medium which could increase the pH and reduce total lactic acid. Consequently, the LAB was unable to grow optimally. A similar phenomenon was also found in a study on the making of yogurt that using *Enterococcus faecalis* UP-11 as starter (Syaputra *et al*., 2015). The lactic acid bacteria especially *Lactobacillus casei* subsp. *casei* R-68 was isolated by Hosono *et al*. (1989) had the ability to grow in the medium without addition of sucrose. *Dadih* whose main ingredient is only buffalo’s milk is produced by a natural fermentation process in a bamboo closed with a banana leaf. Based on the finding of this research, it is clear that LAB could grow well without the addition of sucrose. This indicates that *Lactobacillus casei* subsp. *casei* R-68 prefered to choose lactose in the skim milk than the sucrose as the source of energy. Among four treatments, only Sucrose 0% and Sucrose 5% treatments had met the quality standard the number of LAB of minimum 10^7 colony/g (SNI 2981: 2009 and Codex Stan 243-2003).

The chemical composition of probiotic fermented milk analyzed according to Yoghurt Quality Standard (SN 2981:2009) including fat, total solid without milk fat, protein and ash contents. The variation of sucrose significantly affected ($P<0.05$) the amount of fat and protein but did not significantly influence ($P<0.05$) the total solid without milk fat and ash contents of probiotic fermented milk (Table 4). Table 4 shows that the more the skim milk was used, the greater the total solid without fat and ash contents of probiotic fermented milk. The greater addition of sucrose would cause the bacteria to be unable to degrade sucrose maximally so that a great amount of sucrose could not be degraded resulted in the increase in total solid.

Table 4. Chemical composition of probiotic fermented milk using strain R-68 with the variation of sucrose

Treatments	Fat (%)	Total solid without milk fat (%)	Protein (%)	Ash (%)
Sucrose 0%	0.58[a]	8.36[a]	4.36[c]	0.04[a]
Sucrose 5%	0.59[a]	11.19[b]	4.20[b]	0.10[a]
Sucrose 10%	0.64[a]	12.01[bc]	4.09[a]	0.28[b]
Sucrose 15%	0.66[a]	12.68[c]	4.04[a]	0.37[c]

The different small letters in the same column are significantly different according to DNMRT test at the 5% level

The finding of the study also proved that the Sucrose 0% treatment (without sucrose addition) produced lower protein content compared to the sucrose addition treatments of 5-15%. This might be partly caused by the growth of *Lactobacillus casei* subsp. *casei* R-68 using protein in the fermentation medium originated from skim milk. Therefore, the amount of protein in the fermented milk product decreased. It might also be caused by the increase of sucrose in the medium that slowed down the growth of *Lactobacillus casei* subsp. *casei* R-68 that could be seen from the decline of the amount of BAL in the probiotic fermented milk (Table 6). The data in Table 6 shows that the increase of the sucrose did not significantly affect to the amount of fat of probiotic fermented milk because the sucrose does not contain fat. The amount fat, the total solid without milk fat, protein and ash of the probiotic fermented milk made using *Lactobacillus casei* subsp. *casei* R-68 had met the yoghurt standard quality of low fat (SNI 2981-2009) and Standard Codex for fermented milk (Codex Stan 243-2003).

The sensory analysis consisted of hedonic and descriptive tests towards probiotic fermented milk covering taste, texture and colour analysis. The results of the analysis provided the evidence that the variation of the skim milk use significantly affected all sensory parameters (Table 5). Data in Table 5 show that the addition of sucrose significantly affected (P<0.05) all sensory parameters. In general, panellists chose '*rather like'* to 'like' towards the taste of probiotic fermented milk. This might be caused by its taste which was '*rather sour'* at the Sucrose 0% treatment and '*sweet'* at the treatments with the addition of sucrose. In terms of texture, the panellists preferred choosing '*rather like*' because the texture of the fermented milk was not so solid. On the other hand, in terms of the analysis of colour, they tended to score '*like*' for the fermented milk with the use of sucrose.

Tabel 5. Sensory analysis of probiotic fermented milk using strain R-68 with the variation of sucrose

Treatments	Descriptive test			Hedonic test		
	Taste	Texture	Colour	Taste	Texture	Colour
Sucrose 0%	3.43[b]	2.37[a]	3.50[a]	2.50[a]	2.67[a]	3.33[a]
Sucrose 5%	1.47[a]	2.63[ab]	3.53[a]	3.50[c]	2.63[ab]	3.27[a]
Sucrose 10%	1.37[a]	2.93[bc]	3.57[a]	3.67[c]	2.93[bc]	3.37[a]
Sucrose 15%	1.30[a]	3.13[c]	3.57[a]	2.97[b]	3.13[c]	3.27[a]

The different small letters in the same column are significantly different according to DNMRT test at the 5% level

This was because the colour of probiotic fermented milk produced was almost the same at all treatments. In general, the panellists tended to choose *'like'* towards the probiotic fermented milk made using much sucrose especially at Sucrose 15% treatment. This might be because the taste of the probiotic fermented milk at the Sucrose 15% treatment was sweeter than other treatments.

Based on the findings of the research, it can be concluded that the variation of skim milk or sucrose significantly affected the value of pH, total lactic acid, total lactic acid bacteria, total solid without milk fat, fat, and protein contents, but did not significantly influence ash and fat (especially sucrose variation treatment). In terms of sensory test, the use of 15% skim milk and the variation of sucrose significantly affected all sensory parameters including taste, texture and colour. The best quality of fermented milk was obtained through the use of 15% skim milk and 5% sucrose that had met the standard in accordance with SNI No. 2981-2009 and Codex Stan 243-2003.

Acknowledgement

This study was financed by the Institute of Research and Public Extension, Universitas Riau, the Ministry of State for Research, Technology and Higher Education of the Republic of Indonesia.

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(Received 17 December 2016, accepted 8 January 2017)