

FOOD SCIENCE AND NUTRITION

Effects of sugar type and concentration on the characteristics of fermented turi (*Sesbania grandiflora* (L.) Poir) milk

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Abstract

Fermented milk products are widely consumed for their positive health image, which can be further enhanced by the addition of probiotic bacteria with therapeutic properties. One of the fermented milk products is fermented turi-milk; however, the turi milk has unpleasant off flavor and sour taste. The objective of this study was to determine the best treatment in producing fermented turi-milks with acceptable flavor and taste. Two treatments were arranged in Randomized Block Design with 3 replications. The first treatment was sugar types which consisted of sucrose, glucose, and a sucrose-glucose mixture at a ratio of 1:1. The second treatment was concentrations of sugar consisting of 29.25%, 35.75%, 42.25%, and 48.75% (w/v). Clean turi seeds were submerged in 0.5% NaHCO₃ solution at 80°C for 15 minutes, crushed using a blender, and filtered with filter cloth to obtain turi milk. The turi milk was added with 10% skim milk powder and 4% glucose, inoculated with *Lactobacillus casei* subsp. rhamnosus, and then incubated at 37°C for 96 hours to produce fermented turi-milks. The fermented turi-milks were added with sugar at different concentrations and then stored at refrigerate temperature for 12 days. After storing for 12 days, samples of the turi-milks were taken, and analyzed to determine their pH, total acid bacteria, and sensory characteristics. Result of this study showed that adding sucrose at a concentration of 35.75% (w/v) was the best treatment to produce fermented turi-milk with acceptable flavor and taste. The best fermented turi-milk had a pH of 3.71, a total lactic acid bacteria of 3.62 x 10¹¹ cfu/g, a taste scores of 3.75, a flavor score of 3.3, and an overall acceptance score of 3.75 (out of 5 scales).

Key words: Fermentation, Fermented milk, Turi, *Sesbania grandiflora*, Sugar

Introduction

Fermented milk products are widely consumed for their benefits and refreshing effects. Their popularity is said to be attributed to the effective use of consumer-driven flavors and milder cultures (Jensen and Kroger, 2000). Valli and Traill (2005) stated that these products already have a positive health image, which can be further enhanced by the addition of probiotic bacteria with therapeutic properties (Lourens-Hattingh and Viljoen, 2001).

Probiotics are living microorganisms that when consumed in sufficient amounts provide health benefits beyond basic nutrition (Food and Agricultural Organization of the United

Nation/World Health Organization, 2002). Potential benefits of these probiotic microorganisms in health conditions are primarily in prevention of common infectious diseases, such as diarrhea, necrotizing enterocolitis, and allergies (Vanderhoof et al., 1999; Rosenfeldt et al., 2002; Mastrandrea et al., 2004; Weizman et al., 2005; Lin et al., 2005, 2009).

Because of health benefits, the probiotic bacteria are emerging as important dietary ingredients in functional foods. Majorities of the probiotics are lactic acid bacteria, especially lactobacilli, and bifidobacteria (Schrezeomeir, 2008). Factors related to technological and sensory aspects of the probiotic food products are of utmost importance since only by satisfying the demands of consumers can the food industry succeed in promoting the consumption of functional products in the future (Mattila-Sandholm et al., 2002).

One of the fermented milk products is fermented turi-milk. Turi (*Sesbania grandiflora* L. Poir) - a plant belong to Leguminosae - grows on tropical and subtropical areas and yields turi beans

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consisting of 38.4% protein, 44.7% carbohydrate, 4.3% fat, and 12.6% water (Anonymous, 2010a). Because of its high protein and carbohydrate contents, turi-beans are utilized as raw material of turi-milk, which is not technically milk, but a beverage made from turi beans (Nurhayati et al., 1996). However, the turi-milk has unpleasant off flavor because of lipoxidase-catalyzed oxidation of unsaturated turi-bean oil. To eliminate the problem, Indriyani (2002) fermented the turi milk using lactic acid bacteria as a starter to produce fermented turi-milk. Flavor of the fermented turi-milk was acceptable, but its taste was too sour because of lactic acid content (Irawan, 2003).

In order to reduce sour taste of fermented turi-milks, sugar was added to the fermented turi-milks. If sugar addition is too much, the fermented turi-milk will be too sweet and lactic acid bacteria cannot survive; if too small, the sour taste of the turi-milk still exists. Thus, objective of this study were to find out the treatment (adding sugar) in producing fermented turi-milks with acceptable flavor and taste.

Materials and Methods

Materials

Materials used in this study consisted of turi seed obtained from South Lampung, Indonesia, *Lactobacillus casei* subsp. *rhamnosus* obtained from Biotechnology Laboratory, Institut Pertanian Bogor, Indonesia, and glucose, sucrose, skim milk, NaHCO₃, aquadest, MRSB media, MRSB media, as well as NaCl which obtained from the Department of Agricultural Product Technology, the University of Lampung, Indonesia.

Experimental design

Two treatments in this study were arranged in Randomized Block Designed with 3 replications. The first treatment was the types of sugar which consisted of sucrose, glucose, and sucrose-glucose mixture at a ratio of 1:1. The second treatment was the concentration of sugar added into fermented turi-milk and consisted of 29.25%, 35.75%, 42.25%, and 48.75% (w/v). The fermented turi-milks were analyzed to determine their total lactic acid bacteria, pH, and sensory characteristics (taste, flavor, and overall acceptance). Data of total lactic acid bacteria and pH were analyzed using analysis of variances and performed Barlett and Turkey tests to differentiate among treatments (Steel and Torrie, 1995). Polynomial orthogonal test was also carried out to determine effect tendencies of the treatments. Data of sensory characteristics were presented in bar diagram and discussed descriptively.

Experimental Procedure

Production of turi milk

Turi milk was produced according to a method developed by Nurhayati et al. (1996). After separating from dirt and damaged seed, turi seed was washed upto clean, and then submerged in 0.5% (w/v) NaHCO₃ solution at 80°C for 12 hours. A ratio of the NaHCO₃ solution and turi seed weight was 3 to 1 (v/w). Furthermore, the turi seed was drained, and then blanched in a solution of 0.003% (w/v) NaHCO₃ for 15 minutes. After peeling, the turi seed was crushed using a blender and added hot (80°C) water with a ratio of 1 to 6. The result of the destruction in the form of gruel was filtered with filter cloth to obtain filtrate, namely turi milk.

Starter preparation

Pure culture of *Lactobacillus casei* subsp. *rhamnosus* in ampoules was entirely transferred to the erlenmeyer containing 50 mL of MRS Broth medium and then incubated for 48 hours at a temperature of 37°C. Two drops of the incubated MRS Broth medium were inoculated into 50 mL of turi milk which has been added with 10% (w/v) skim milk and sterilized at a temperature of 121°C for 15 minutes. After incubation at 37°C for 48 hours, the culture was used as parent culture. The parent culture was then inoculated into the same medium as much as 0.5% (v/v) and then incubated for 48 hours at a temperature of 37°C. After incubation, the result culture was used as between culture. As much as 0.5% (v/v) of the between culture was inoculated into the same medium with the addition of 3.0% glucose. The culture was incubated at a temperature of 37°C for 24 hours in order to obtain working culture which was ready to be used.

Production of fermented turi milk

Fermented turi-milk was produced according to the method developed by Indriyani (2002). Turi milk was added with 10% (w/v) skim milk powder and 4% (w/v) glucose. The mixture was mixed homogeniously, heated at 80°C for 30 minutes, and then cooled upto 37°C. After cooling, the mixture was inoculated with working culture and then incubated at a temperature of 37 °C for 96 hours to produce fermented turi-milk. The fermented turi-milk was added with different types of sugar (sucrose, glucose, or sucrose and glucose mixture at a ratio of 1:1) at concentrations of 29.25%, 35.75%, 42.25%, and 48.75% (w/v) and then stored at refrigerate (5 – 10°C) temperature for 12 days.

Production of sugar solution

Sugar solution was produced by pouring 325 g sugar into 500 mL measuring flask, adding with

water up to tera sign, and shaking until all sugar was dissolved. The sugar solution containing 65% sugar (w/v) was then pasteurized at a temperature of 65°C for 30 minutes. This solution was then added into fermented turi-milks up to sugar concentration treatments were achieved.

Experiment observation

Experiment observations were performed to determine pH value (AOAC, 1990), total lactic acid bacteria (Fardiaz, 1987), and sensory characteristics (taste, flavor, and overall acceptance) (Soekarto, 1985). Observations of total lactic acid bacteria were conducted every four days during the 12 days storage of fermented turi-milk in order to determine the effect of sugar on the growth of lactic acid bacteria. Sensory test and pH analysis were performed after 12 days storage of the fermented turi-milks at refrigerate temperature.

Results and Discussion

pH value

After collecting and tabulating pH data, analysis of variance was carried out. Results of the analysis showed that the type of sugar had a very significant effect on the pH of the fermented turi-milks but the the concentration of sugar and interaction between the two treatments had no significant effects. pH values and their linear lines

were presented at Figure 1. pH values of fermented turi-milks added with glucose were lower (3.64 – 3.67) than that added with sucrose (3.71 – 3.75). It indicates that the lactic acid microba (*L. casei subsp rhamnosusin*) still alive and apparently preferred to utilize glucose than sucrose for producing lactic acid. This finding is similar to the finding of Wang et al. (1974), who also found that fermented soymilk with *L. acidophilus B-1911* had a lower pH when added with glucose than that added with sucrose.

Total of lactic acid bacteria

Total lactic acid bacteria were determined after storing the fermented turi-milks for 0, 4, 8, and 12 days. After collecting and tabulating data, analysis of variance was carried out. Results of the analysis of variance showed that the type of sugar, the concentration of sugar, and interaction between the two treatments had no significant effect on the total lactic acid bacteria on day 0 (without storing). However, after storing for 4, 8, and 12 days, the concentration of sugar had a very significant effect on the total lactic acid bacteria. Orthogonal comparison indicated that there were no significantly interactions between the two treatments. Total acid bacteria and their linear regression lines were presented at Figure 2, 3, and 4.

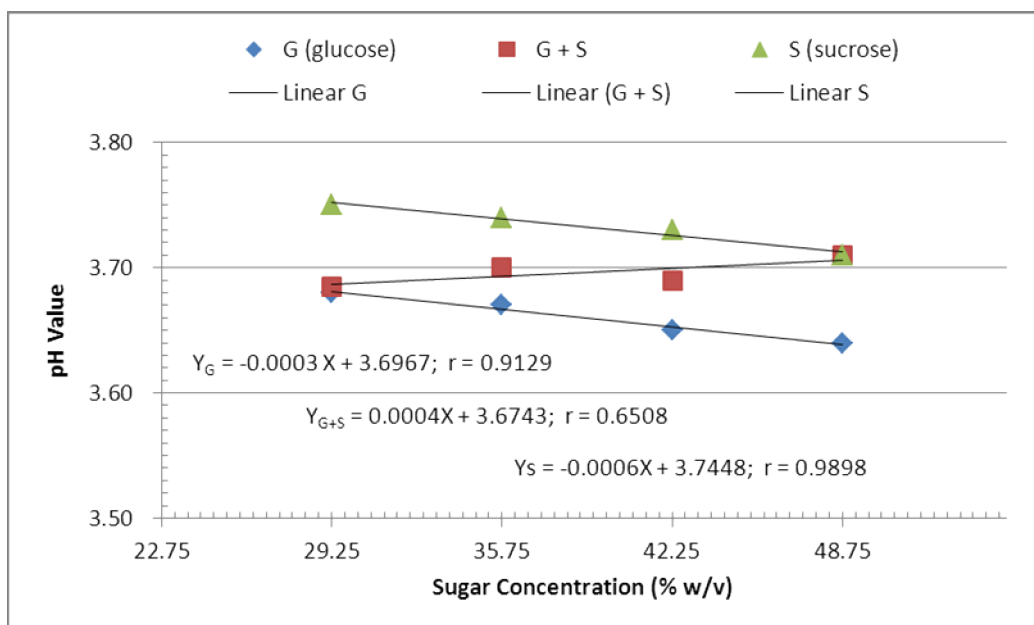


Figure 1. pH values of fermented turi-milks added with different types of sugar at concentrations of 29.25–48.75% and then stored at refrigerate temperature for 12 days.

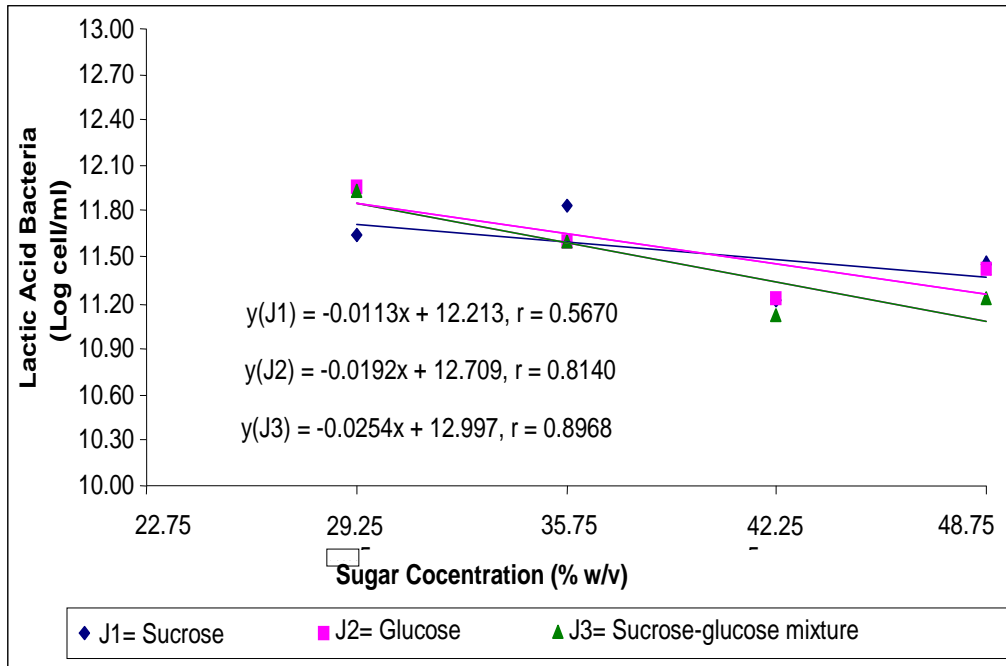


Figure 2. Total lactic acid bacteria of fermented turi-milks added with sugar at concentrations of 29.25 – 48.75% and then stored at refrigerate temperature for 4 days.

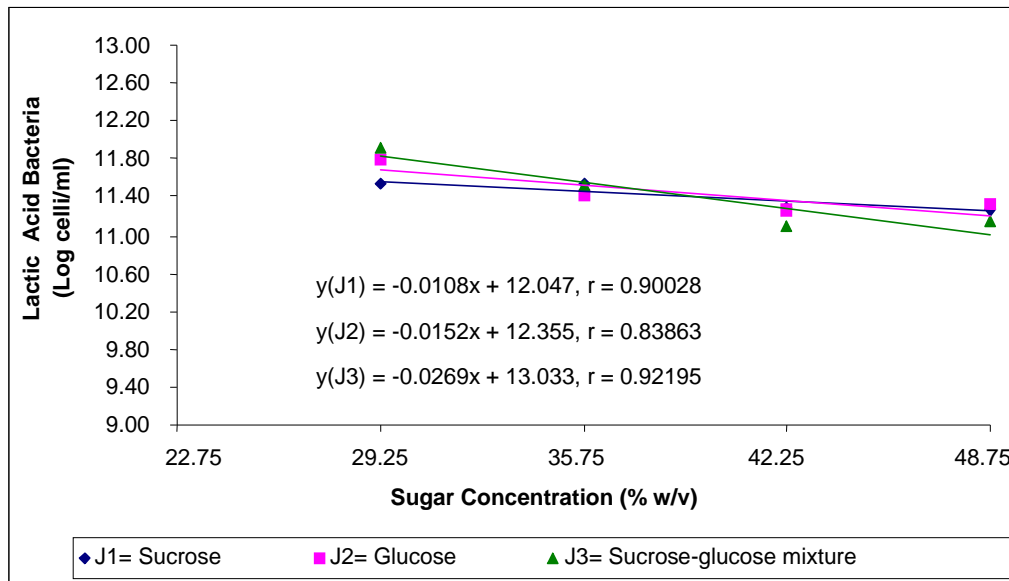


Figure 3. Total lactic acid bacteria of fermented turi-milks added with sugar at concentrations of 29.25 – 48.75% and then stored at refrigerate temperature for 8 days.

On day 0, total lactic acid bacteria was more than 10^{12} cfu/g (data was not presented). On the day 0, sugar was not able to inhibit the growth of lactic acid bacteria because of short exposure. The analysis of total lactic acid bacteria carried out immediately after the sugar was added to the fermented turi-milks.

The concentrations of sugar significantly decreased total lactic acid bacteria of the fermented turi milks after storing at refrigerate temperature for 4, 8, and 12 days (Figure 2, 3, 4). This phenomenon indicated that the bacterial growth rate decreased linearly with increasing sugar concentration. The higher concentrations of sugar,

the higher water removing from bacterial cells, so that the cells lacked of water and then died because of plasmolysis (separation of protoplasm from the cell membrane) (Membre et al., 1999).

As shown at Figure 2, 3, and 4, all fermented turi-milk that added with sugar and stored for 4, 8, and 12 days contained more than 10^{11} cfu/g total lactic bacteria. It means that the fermented turi-milks have fulfilled Codex standar for fermented Milks (Annonymous, 2010b). The standar stated that fermented milks should contain minimum 10^7 cfu/g total microorganisms constituting the starter culture. Thus, all treatments (adding sugar at concentration of 29.25 – 48.75%, w/v) can be applied for producing fermented turi-milks.

Sensory evaluation of fermented turi-milk

After storing at refrigerate temperature for 12 days, fermented turi-milks were subjected to

sensory evaluation to test their tase, flavor, and overall acceptability using a five point hedonic scale, where 1= dislike extremely, 2= dislike moderately, 3= neither like nor dislike, 4= like moderately, and 5= like extremely. Result of the sensory evaluation was presented in bar diagrams and discussed descriptively.

Taste

Taste is an important parameter when evaluating sensory attribute of fermented milks. The milks might be appealing and having health benefit but without good taste, such milks are likely to be unacceptable. Results of taste evaluation were presented at Figure 5. Taste scores for fermented turi-milks - which were added with sugar - were 2.61 - 3.75 (out of 5 scale). Meanwhile, fermented turi-milks - which were not added with sugar – had a score of 1.36 - 1.64 (Figure 5).

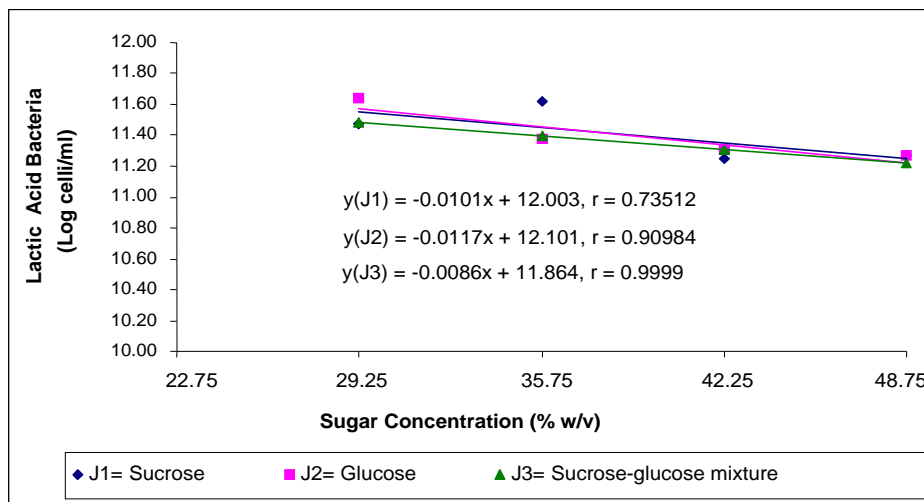


Figure 4. Total lactic acid bacteria of fermented turi-milks added with sugar at concentrations of 29.25 – 48.75% and then stored at refrigerate temperature for 12 days.

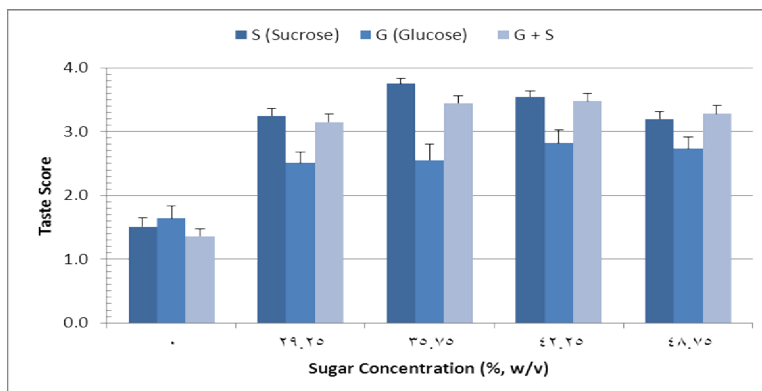


Figure 5. Taste scores of fermented turi-milks with or without sugar addition at concentrations of 29.25 – 48.75% and stored at refrigerate temperature for 12 days.

Adding sugar into fermented turi-milks was able to increase taste scores of the turi-milk (Figure 5). Without adding sugar, the fermented turi-milk went sour and panelists did not like it. By adding sugar, the fermented turi-milk went sweeter and the panelists were more like it. Adding sucrose into fermented turi-milks yielded the turi-milk having higher taste scores than that of adding glucose (Figure 5). Sucrose is sweeter than glucose because sucrose contains fructose, which is a sweet-tasting sugar ring that binds more tightly than glucose does to the sweetness receptor in the human mouth (Hendrickson, 2010). Joesten et al. (2007) stated that relative sweetness of sucrose, glucose, and fructose is 1.00, 0.74, and 1.17, respectively. The fermented turi-milk, which was added with 35.75% sucrose, had the highest taste score i.e. 3.75 out of 5.00 scales. It means that the fermented turi-milk is the most favorable.

Flavor

Flavor is the sensory impression of a food or other substance taken into the mouth which stimulates one or both of the senses of taste and smell. Results of flavor evaluation were presented at Figure 6. Flavor scores for fermented turi-milks - which were added with sugar - were 3.01 – 3.40. Whereas, flavor scores for fermented turi-milks - which were not added with sugar - were 2.64 - 2.88 (Figure 6). It indicates that adding sugar into fermented turi-milks result in better flavor. This phenomenon agrees to the opinion of Tranggono et al. (1990). They said that the purpose of adding sweetness materials into foods or drinks were to improve their flavor so that sweetens taste could improve person's preference.

Lactic acid bacteria produce lactic acid and a small portion of citric, succinic, malic, and acetic acid, as well as carbonyl compounds such as acetaldehyde, diasetil, acetone and asetoin which acts as a flavor component (Ross et al., 2002; Salminen et al., 2004). The carbonyl compounds in high amounts especially diasetil are not desirable because it may cause *off flavor* in fermented beverages. Tamime and Robinson (1989) stated that natural yoghurt which has a high acidity level and a sharp flavor is less favored by consumers. The addition of sucrose would suppress the smell of carbonyl compounds (Tranggono et al., 1990); therefore, adding sucrose into fermented turi-milk results in favorable flavor.

Overall acceptance

Overall acceptance is affected by all sensory characteristics, such as taste, flavor, smell, texture, and appearance of food or drinks. Results of overall acceptance evaluation were presented at Figure 7. Overall acceptance score for fermented turi-milks which were added with sugar were 2.79 – 3.75. The scores for fermented turi-milks which were not added with sugar were 1.64 – 1.73 (Figure 7). Adding sugar into fermented turi-milks, results in higher overall acceptance. This is caused by the facts that sugar can improve taste and flavor of the fermented turi-milks. In addition, fermented turi milks which were added with sucrose have higher acceptance scores than those which were added with glucose or sucrose-glucose mixture (Figure 7) because sucrose is sweeter than glucose (Hendrickson, 2010).

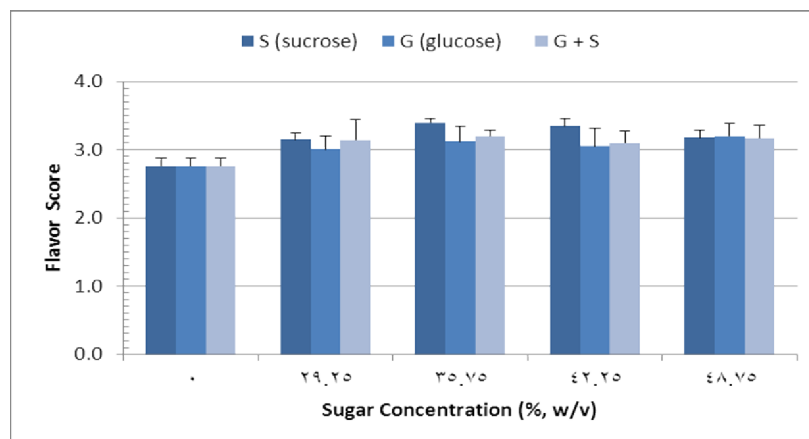


Figure 6. Flavor scores of fermented turi-milks with or without sugar addition at concentrations of 29.25 – 48.75% and stored at refrigerate temperature for 12 days.

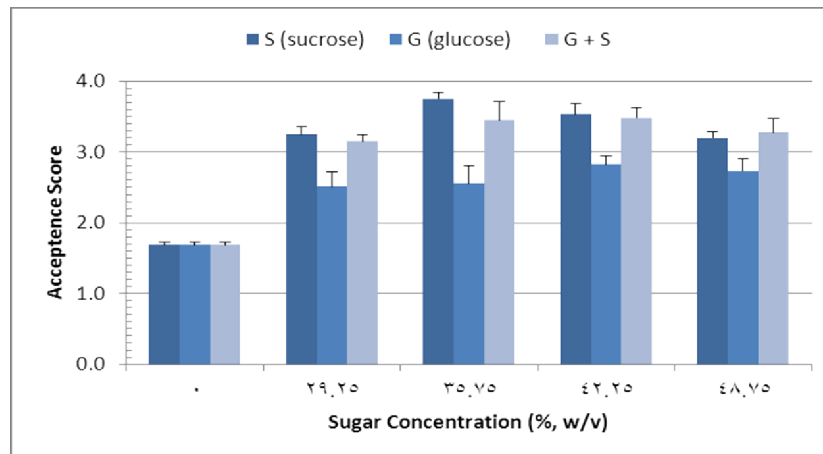


Figure 7. Overall acceptance scores of fermented turi-milks with or without sugar addition at concentrations of 29.25 – 48.75% and stored at refrigerate temperature for 12 days.

Selection of the best treatment

In this study, selection of the best treatment was based on the standard of fermented milks. Total lactic acid bacteria, pH, and sensory characteristics (taste, flavor, and overall acceptance) of the fermented turi-milks are compared to the available fermented milk standards. A treatment that fulfills the standards is considered as the best treatment.

The pH value of the fermented turi-milks which added with sugar has complied with the standar of fermented milks. All fermented turi-milks have a pH of 3.67 to 3.75 (Figure 1). Meanwhile, Australia New Zealand Food Standards Code - Standard 2.5.3 – stated that pH of fermented milk products is maximum 4.5 (Anonymous, 2011). Thus, all the fermented turi milks comply with the standar of fermented milks.

All the fermented turi-milks contain more than 10^{11} cfu/g total lactic acid bacteria (Figure 2, 3, and 4). Australia New Zealand Food Standards Code - Standard 2.5.3– stated that fermented milk products have to have minimum 10^6 cfu/g microorganisms used in the fermentation (Anonymous, 2011). Meanwhile, Codex Standar for Fermented Milks – CODEX STAN 243-2003- stated that total microorganisms constituting the starter culture is minimum 10^7 cfu/g and labeled microorganisms is minimum 10^6 cfu/g. Thus all the fermented turi-milks produced in this research fulfill the standards of fermented milk products.

Since there are no standard for sensory characteristics of fermented milks, fermented turi-milks produced in this research are evaluated based on their overall acceptance scores to determine the

best turi milks. Overall acceptance scores of all fermented turi-milks are presented at Figure 7. As shown at Figure 7, the fermented turi milk which was added with 35.75% sucrose has the highest overall acceptance score (3.75 out of 5.00). It means that the treatment (adding with 35.75% sucrose) is the best treatment for producing fermented turi-milk.

Based on the above discussion, all treatments (sucrose, glucose, and sucrose- glucose mixture at concentrations of 29.5, 35.75, 42.25, and 48.75% w/v) yielded fermented turi-milks which fulfill the available standards of pH and total lactic acid bacteria. Fermented turi-milks added with 35.75% sucrose had the best acceptance. Thus, the best treatment in this study was adding 35.75% sucrose into fermented turi-milk. The treatment yielded fermented turi-milk that had a pH of 3.71, total lactic acid bacteria of 3.62×10^{11} cfu/g, a taste scores of 3.75, a flavor score of 3.3, and an overall acceptance score of 3.75.

Conclusion

Adding sucrose at a concentration of 35.75% (w/v) was the best treatment for producing fermented turi milk. The fermented turi-milk had a pH of 3.71, a total lactic acid bacteria of 3.62×10^{11} cfu/g, a taste scores of 3.75, a flavor score of 3.3, and an overall acceptance score of 3.75 (like moderately).

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