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# Development of Corn Milk Yoghurt Using Mixed Culture of *Lactobacillus delbruekii*, *Streptococcus salivarus,* and *Lactobacillus casei*

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**The main objective of this research was to diversify the utilization of corn as commercial commodity by a corn-based new product development in the form of yoghurt. The first step was carried out to make corn yoghurt with the addition of fresh milk into heated corn extract using mixture starter of *Lactobacillus delbruekii*, *Streptococcus salivarus,* and *Lactobacillus casei* and the best formulation was determined through hedonic evaluation along with weighing method. The selected formulation from this step was corn extract with the addition of 50% fresh milk. The corn extract was produced from corn kernels that were blended and mixed with water in ratio of 3:1, the mixture was further heated and concentrated until the total volume remained 2/3. Afterwards, this selected formulation was added with sugar and full cream milk powder. The hedonic evaluation results showed that the mixture with 10% sugar and 5% full cream milk powder addition possessed the highest score. Lastly, the selected formulation was observed for physical, microbiological, and chemical assay during 4 weeks period. The ultimate observation concluded that the product could be classified as probiotics with total lactic acid bacteria reached 1.5 x $10^9$ CFU/ml with medium fat content (1.8%).**

Keywords: corn milk, probiotics, yoghurt, lactic acid bacteria

## INTRODUCTION

Yoghurt has become a popular fermented product for Indonesian, and it was initially invented to prolong the shelf life of fresh milk through fermentation (Tamime & Robinson 1989). Nowadays, most of the commercial yoghurts in the market are cow milk based, because of its authentic taste and aroma, and also its nutritional value which is very beneficial for health. As the consumers' awareness on health related issues increases, the current development of yoghurt based products has favored the utilization of probiotic microorganisms that is proven to promote the growth of beneficial microbes in human gastrointestinal organs (Ataie-Jafari *et al.* 2009) and resulted into several therapeutic effects (Lourens-Hattingh & Viljoen 2001) such as lowering the cholesterol level of serum cholesterol (Agerbaek *et al.* 1995; Anderson & Gilliland 1999; Agerholm-Larsen 2000), treating inflammatory bowel disease (Lorea-Baroja *et al.* 2007), preventing gastrointestinal problems, increasing mineral bioavailability and its immunological effects (Wynckel *et al.* 1991; Meydani & Ha 2000; Anukam *et al.* 2008).

Corn milk is considered as a new innovation especially in the making of yoghurt based products. It is going to be an alternative to substitute the animal based cow milk with newly introduced vegetable based corn milk. This effort is paramount to boost the domestic consumption of corn which is still considered low, while the corn production has been increasing these recent years. Based on this reason, developing a new corn based product in the form of healthy yoghurt is an avenue to raise the awareness on corn consumption. Besides, the sweet taste of corn milk along with its aroma and balanced nutritional content compared to other types of vegetable based beverage are seen as the upper hand of this product (Supavititpatana *et al.* 2008). Furthermore, the development of this product will be based on sensory acceptance so that it is expected that the product will be preferred in the market. There are several studies that emphasized a focus on cow milk. However, the number is very inadequate when it comes to the studies on corn milk yoghurt properties especially the sensory properties of corn milk yoghurt with mixed culture. This research aimed to develop yoghurt product with corn extract as functional food using mixed culture of *Lactobacillus delbruekii* subsp. *bulgaricus*, *Streptococcus salivarus* subsp. *thermophiles,* and *Lactobacillus casei* subsp. *rhamnosus*.

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## MATERIALS AND METHODS

**The Preparation of Sweet Corn Kernels.** The sweet corn was purchased at a traditional market in Bogor. Sweet corn was chosen as the main raw materials because of its high sugar content. The whole corn was weighed to obtain the gross weight, husked, removed from the silk and finally washed. After that, the cleaned kernels were boiled with water for 10 minutes. Lastly, the corn kernels were separated from its cob to ease the extraction process.

**The Preparation of Stock Culture.** The mixed cultures, consisted of the strains of *S. thermophiles* FNCC0015, *Lactobacillus delbrueckii* FNCC259, and *Lactobacillus casei* subsp. *rhamnosus* FNCC099, were obtained from Food Nutrition and Culture Collection, Center for Food and Nutrition Studies, Gadjah Mada University. The culture stock was grown on MRS chalk semi solid (oxoid) media and incubated at 43-45 $^{o}$C incubator for 24 h. Afterwards, the stock culture was stored in refrigerator at 5 $^{o}$C. A loop of microorganism was taken from the media and inoculated on MRS broth (oxoid) and further incubated at 43-45 $^{o}$C for 24 h and stored in refrigerator at 5 $^{o}$C. Around 0.5% (v/v) of inoculums was transferred to 50 ml of sterilized mixture containing 10% (w/v) of skim milk powder. This mixture was incubated again at 43-45 $^{o}$C for 24 h. This mixture was referred as stock culture and was kept in refrigerator prior to the usage (Hariyadi 2001).

**The Preparation of Mother Culture.** The mother culture was prepared by transferring 5% of stock culture to 50 ml of sterilized mixture containing 10% (w/v) of skim milk powder. This mixture was incubated again at 43-45 $^{o}$C for 24 h. This mixture was referred as mother culture and was kept in refrigerator prior to the usage (Hariyadi 2001).

**The Mixing of Corn Extracts and Fresh Milk (Step I).** The corn kernels were blended with water using the ratio of 4:1 and 3:1 (v/w), and then filtered to get fine solution. The extract with 4:1 (v/w) ratio was heated at 70-80 $^{o}$C with appropriate stirring until the total volume remained half of initial volume. Another extract with 3:1 (v/w) ratio was processed in two different ways i.e. heated at 70-80 $^{o}$C with appropriate stirring until the total volume reached ⅓ and ⅔ of initial volume. After that, fresh milk with concentration of 50, 30, and 10% (v/v) were added into each extract so that there were nine formulations obtained (Table 1). Out of these formulations, the best formula was determined using sensory evaluation with hedonic assay method.

**The Addition of Sugar and Reconstituted Milk Powder (Step II).** After the basic formulation from Step I had been selected, the formulation was then added 6, 8, and 10% (w/v) sugar and 5% of both skim milk and full cream milk powder. Subsequently, the mixture gained from each formulation was homogenized and pasteurized at 80-90 $^{o}$C for 15-20 min and eventually cooled at 45 $^{o}$C. Thus, there were 6 formulations resulted from Step II. Subsequently, 5% (v/v) of yoghurt starter culture consisted of *S. thermophilus*, *L. bulgaricus*, and *L. caseiatwitha* ratio of 1:1:1 was inoculated to each formulation. Each inoculum was incubated at room temperature for 24 h and stored in refrigerator at 5 $^{o}$C. Each formulation (Table 2) was later analyzed by sensory evaluations to get the best final formulation.

**The Sensory Evaluations.** The sensory evaluation for formulations in step I and step II was conducted by following hedonic scale rating test using 30 untrained panelists. The panelists were asked to score the samples for preference of corn milk yoghurt characteristic on a scale of 1 to

Table 1. Formulation used to make corn yogurt in step 1

| Composition/Treatment | Formulation | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | a1 | b1 | c1 | d1 | e1 | f1 | g1 | h1 | i1 |
| Water to corn extract ratio | 3:1 | 3:1 | 3:1 | 4:1 | 4:1 | 4:1 | 3:1 | 3:1 | 3:1 |
| Remaining volume (from the initial volume) | 1/3 | 1/3 | 1/3 | 1/2 | 1/2 | 1/2 | 2/3 | 2/3 | 2/3 |
| Fresh milk added (v/v) | 50% | 30% | 10% | 50% | 30% | 10% | 50% | 30% | 10% |

Table 2. Formulation used to make corn yogurt in step 2

| Composition | Formulation | | | | | |
|---|---|---|---|---|---|---|
| | a2 | b2 | c2 | d2 | e2 | f2 |
| Sugar (w/v) | 6% | 8% | 10% | 6% | 8% | 10% |
| Full cream milk powder (w/v) | 5% | 5% | 5% | - | - | - |
| Skim milk powder (w/v) | - | - | - | 5% | 5% | 5% |

6, where scale 6 = really like, 5 = like, 4 = rather like, 3 = rather dislike, 2 = dislike, and 1 = really dislike. Thus, the scores were subjected to one-way analysis of variance and significant differences was evaluated by Duncan multiple range test using SPSS Statistic version 15.0 software. The best product was determined by giving weight to every characteristic based on prioritized score of the characteristic, i.e. 4 as the most prioritized characteristic; 3 as prioritized characteristic; 2 as less prioritized characteristic and 1 as the least prioritized characteristic. The priority of every characteristic can be seen at Table 3. The results from every characteristic, based on hedonic evaluation, were presented in the order from the highest to the lowest on the average of hedonic scale. The total score of each formula was calculated by multiplying the weight with the score of ranking.

**The Physicochemical and Microbiological Analysis.** The best formula in Step II was analyzed every week for 4 weeks to observe physical characteristic: viscosity using dial reading viscometer (Brookfield LVT, Middleborough, USA), chemical characteristics. pH using Orion 210 A (Thermo Electron Corp. USA), total soluble solid using handheld optical refractometer (Atago), total titrated acid (AOAC 2000), moisture content (AOAC 2000), ash content (AOAC 2000), protein based on total N content using Kjeldahl method (AOAC 2000), fat content using soxhlet (AOAC 2000) and total carbohydrate content and total lactic acid bacteria (Hariyadi 2001).

## RESULTS

**The Results of Sensory Evaluation Analysis of Step I.** In Step I, there were six out of nine formulations observed with syneresis phenomenon and bad consistency. Therefore, only three remaining formulations that could be selected and analyzed using sensory evaluation to determine the best formula. The three remaining formulations were formulation d1, a1, and g1 (Table 1). The results of hedonic evaluation on taste, color, aroma and viscosity of corn milk yoghurt were presented in Figure 1. The general color of corn milk yoghurt was observed to be light yellow and different from the color of other cow milk-based yoghurts. The a1 had darker color than the other formulas and according to hedonic evaluation on color property, a1 also had the highest mean of hedonic scale. From the aroma property, formula g1 showed the highest mean of hedonic score while formula a1 had the highest mean of hedonic score in viscosity. Based on the overall results of hedonic evaluation, it could be concluded that corn milk yoghurt was still at the level of ‘rather like’ in terms of the consumer’s preference. The total score from weighing method (as shown in Table 4) concluded that formula g1 was the best formula from Step I.

**The Results of Sensory Evaluation Analysis of Step II.** From Step I it was justified that formulation g1 was the best. However, since consumer’s preference was still at the level of ‘rather like’, this formula was further modified by the addition of sugar and milk at different concentration to improve its sensory properties. The results of hedonic evaluation for Step II formulations are shown in Figure 2. Formula e2 had the highest mean of hedonic score based on its taste property. The color of formula c2 had the highest mean of hedonic scale. The aroma of formula e2 had the highest mean of

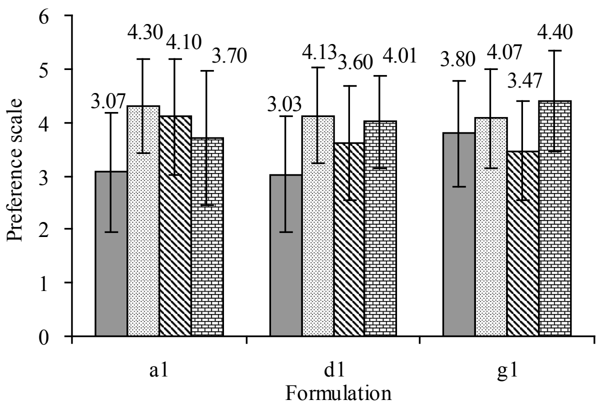


Figure 1. Results of hedonic assay on taste, color, viscosity, and aroma of corn milk yogurt in step I (n = 30). ■ taste, ▨ color, ▧ viscosity, ▦ aroma.

Table 3. Value of priority and weigh of each character

| Characteristic | Score | Weight |
|---|---|---|
| Color | 2 | 0.15 |
| Taste | 4 | 0.31 |
| Aroma | 4 | 0.31 |
| Viscosity | 3 | 0.23 |
| Total | 13 | 1.00 |

Table 4. Total score of tree formulation in step 1 using weighing method

| Formulation | Taste | Color | Aroma | Viscosity | Total score |
|---|---|---|---|---|---|
| a1 | 0.62 | 0.45 | 0.31 | 0.69 | 2.07 |
| d1 | 0.31 | 0.30 | 0.62 | 0.46 | 1.69 |
| g1 | 0.93 | 0.15 | 0.93 | 0.23 | 2.24 |

hedonic scale. The viscosity of formula d2 had the highest mean of hedonic scale. The determination of the best formula in Step II was also based on the weighing method by multiplying the ranking score and the weight, as in Step I. The total score of each formulation could be seen in Table 5. The results showed that formula e2 was the best formulation in step II because formula e2 had the highest total score.

**The Physical and Chemical Properties of Corn Milk Yoghurt.** The complete physical properties of corn milk yoghurt product from the best formulation (e2) is described in Table 6. The corn yoghurt observed-properties were the same as typical yoghurt made from cow milk. The slight difference was on aroma in which the sweet aroma of corn could be detected. In addition, the color of corn yoghurt was light yellow because of the yellowish pigment in sweet corn. The detailed chemical composition of the resultant product could be seen in Table 7.

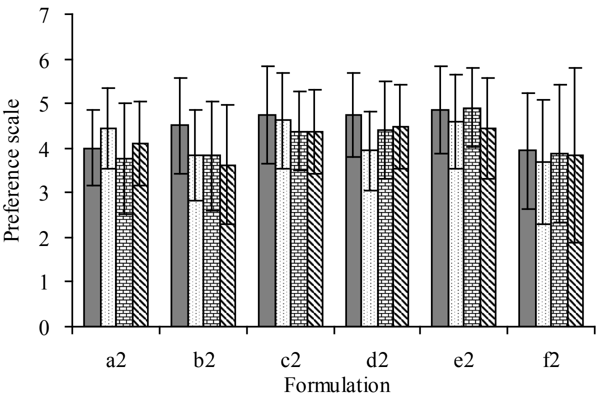

Figure 2. Results of hedonic assay on taste, color, aroma, and viscosity of corn milk yogurt in step II (n = 30). ■ taste, ▨ color, ▧ viscosity, ▦ aroma.

Table 5. Total score of six formulation in step 2 using weighing method

| Formulation | Ingredient addition | Total score |
|---|---|---|
| a2 | 6% sugar 5% full cream milk | 2.2 |
| b2 | 8% sugar 5% full cream milk | 2.31 |
| c2 | 10% sugar 5% full cream milk | 4.3 |
| d2 | 6% sugar 5% skim milk | 4.93 |
| e2 | 8% sugar 5% skim milk | 5.62 |
| f2 | 10% sugar 5% skim milk | 1.83 |

Tabel 6. Physical properties of the best formula of corn milk yoghurt

| Description | Physical properties |
|---|---|
| Viscosity | Heavy viscous |
| Color | Light yellow |
| Taste | Sour |
| Aroma | Acetaldehyde |

**Total Acid.** In the initial week (week 0) the pH value was 4.2 ± 0.02. The weekly observation showed that the pH value of corn milk yoghurt was decreasing (Figure 3) or its acidity level was increasing (Figure 4). The observation of the total titrated acid of corn milk yoghurt for 4 weeks at refrigerator temperature showed that the lactic acid percentage of corn milk yoghurt continually increased. In the initial week (0 week) the lactic acid percentage was 0.99% and after 4-week observation it increased to 1.22% ± 0.01 (Figure 4).

**Total Soluble Solid.** The observation of the total soluble solid of corn milk yoghurt for 4 weeks showed that the total soluble solid continually decreased from week to week. In the initial week (0 week) the total soluble solid was 17.5 °Brix, and

Table 7. Chemical composition of the best formula of corn milk yoghurt

| Chemical properties | Amount (%) |
|---|---|
| Moisture | 79.80 |
| Ash | 0.67 |
| Fat | 1.80 |
| Protein | 2.72 |
| Carbohydrate | 15.00 |
| Total acid | 0.99 |

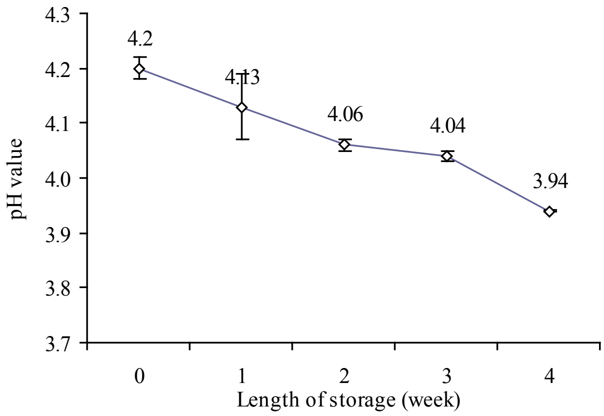

Figure 3. The pH value of corn milk yogurt.

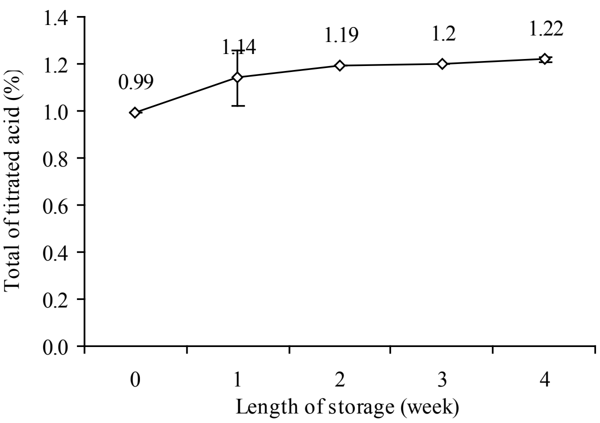

Figure 4. Total titratable acid of corn milk yogurt.

after 4-week observation it plunged to 16.5 °Brix (Figure 5).

**Total Lactic Acid Bacteria.** The total number of lactic acid bacteria increased during the first two weeks of observation and reached its maximum growth. On the third week, the number started to plunge and finally reached 1.1 x $10^9 \pm 0.14$ cfu/ml on the fourth week (Figure 6).

**Viscosity.** The viscosity value of corn milk yoghurt was decreasing during the 4-week storage from 2550 to 2400 cp. On the first week, the viscosity of corn milk yoghurt was recorded at 2550 ± 70.71 cp. The decline continued until the third week to reach 2400 ± 70.71 cp, but it then increased on the fourth week until 2450 ± 70.71 cp (Figure 7).

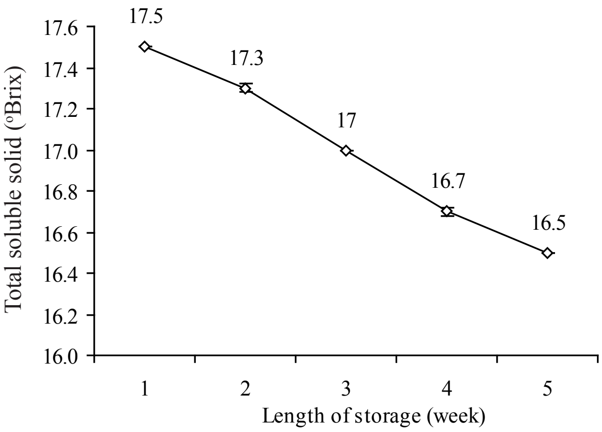


Figure 5. Total soluble solid of corn milk yogurt.

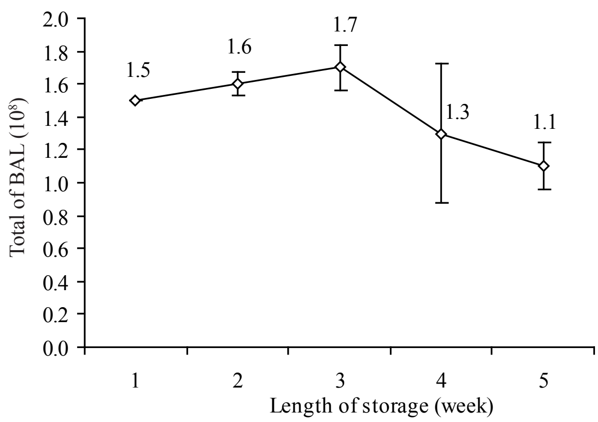


Figure 6. Total lactic acid bacteria of corn milk yogurt.

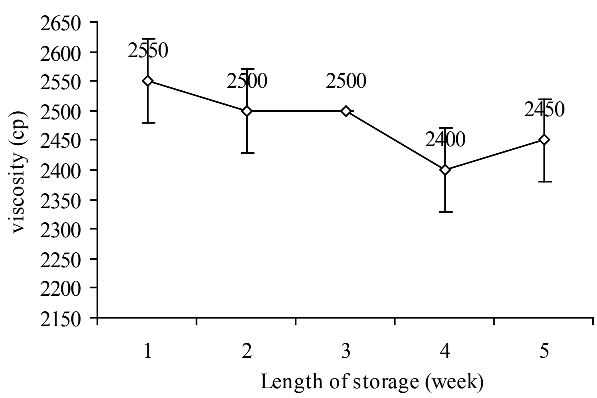


Figure 7. The viscosity of corn milk yoghurt.

## DISCUSSION

The first step of corn yoghurt making process was intended to determine the heating process in term of the amount of evaporation of the corn milk mixture since heating up milk is considered to be an essential process in preparing yoghurt (Lee & Lucey 2010). During this experiment, heating process contributed to starch gelatinization and increase of total soluble solid in the mixture which will affect the consistency of the product. Meanwhile, heating process in the second step was carried out to allow desirable formation of gels and rheological properties of yoghurt (Lucey *et al.* 1998). Besides, it was also conducted to meet pasteurization purposes i.e. eliminating spoilage and pathogenic microorganisms (Lee & Lucey 2010).

In general, corn contains high amount of starch. The average content of starch in corn is estimated around 10-11%, yet the content of starch in sweet corn is lower than other varieties of corn. The starch content in corn is expected to play a role as natural stabilizer in product. Based on the sensory evaluation results, different temperatures of heating affected the overall acceptance towards the product, including aroma, taste, and viscosity (Kalviainen *et al.* 2003; Jaworska *et al.* 2005). This was confirmed by experiment results that formula g1, which was processed in shorter period of heating, showed the highest mean of hedonic score in term of taste; while formula d1, which was heated relatively longer than formula a1, had lower mean of hedonic score in term of taste. The length of heating process also affected the color of end product. The longer duration of heating will result into a darker colored product; thus formula a1 which was heated longer than formula d1 and g1 seemed to appear darker in term of color. Lastly, the length of heating period also had an observable effect on viscosity; longer heating period increased water absorption by the corn starch, so that viscosity also increased. This phenomenon was also affected by the proportion of water which was added previously. The presence of water and starch in a heated condition will trigger gelatinization, and the gelatinized starch will increase the viscosity of the solution.

Sweet corn was selected as raw material due to its high sugar content compared to other varieties of corn. The major sugar contained in sweet corn is sucrose and normal sweet corn contains 9-16% sugar in general (Cobbledick 2012). Moreover, sweet corn has been popular for its aroma and sweet taste (Supavititvatana *et al.* 2008). The distinctive taste of corn milk yoghurt lies on its authentic corn aroma. However, the strong aroma of corn might also nullify the typical cow milk yoghurt aroma. For this reason, the addition of fresh milk in Step I was conducted to maintain the typical yoghurt flavor.

In the Step II, there was an addition of reconstituted milk and sugar. The addition was conducted to improve the aroma, taste and total soluble solid properties of product. The low total soluble solid content in product might result into slow acid production and this will lead to syneresis once the product is stored at refrigerator temperature (Tamime & Robinson 1989). According to Needs *et al*. (2000), in order to obtain desired viscosity and texture, skim milk can be added to the product. The addition of reconstituted milk increased the total soluble solid content in the product. Based on the results, the addition of reconstituted milk and sugar was proven able to improve the hedonic scores of the product. This could be seen from higher mean of hedonic scores from sensory evaluation in Step II compared to Step I. Furthermore, all the tested properties such as taste, aroma and viscosity were evaluated better by the panelist in Step II compared to Step I assessment.

On the other hand, milk is also the major source of lactose and contains various amino acids that can prosper the growth of lactic acid bacteria during fermentation period (Chairunnisa 2009), thus by enhancing the growth factor of bacteria, it is excepted that the acid production can be accelerated. The ratio of 1:1:1 of *L. delbruekii*: *S. thermophilus*: *L. casei* is the optimum ratio for bacterial growth (Walstra *et al.* 1999).

The chemical composition of corn milk-based yoghurt was successfully meet the Indonesian National Standard for yoghurt product, except the protein content which was slightly lower than the standard. The corn milk based yoghurt contains 2.72% protein while the standard of protein in yoghurt product is 3.5%. The low protein content in corn milk based yoghurt was caused by the natural properties of corn which is rich in carbohydrate and contains less protein and fat. However, the effort to increase the fat and protein content was done through the addition of fresh milk and reconstituted milk. Beside, the addition of milk especially full cream milk resulted into a creamy sensation of the product. The total fat content in this product was 1.8% while for standard the minimum is 0.5% for yoghurt made by addition of skim milk.

Generally, the pH value of yoghurt product falls between the ranges of 4.0-4.5 (Aswal *et al*. 2012). This product pH value measured at 4.2 ± 0.02, so that the pH value of this product can be considered appropriate. During 4-week storage the pH value declined. The decline in pH during storage was also found by Kim *et al*. (2009). The organic acid composition during storage condition undergoes continuous change, thus it affects the pH (Fernandez-Garcia *et al.* 1994). The decreasing pH value might be caused by lactic acid bacteria that have fermented most of the sugar sources (sucrose, glucose, and lactose) into lactic acid or other form of organic acids. The total titrated acid is represented by lactic acid as a result of the fermentation of milk into yoghurt, so the decreasing pH value can increased the total titrated acid (Usmiati *et al*. 2011).

The corn milk based yoghurt can be classified as probiotic beverage because it has the total lactic acid bacteria of 1.5 x $10^9$ CFU/ml. According to Tammime and Robinson (1989), probiotic beverage is required to contain ≥ $10^6$ CFU/ml of total lactic acid bacteria. During the first 2 weeks of storage, the total lactic acid bacteria was increased. However, the number of total lactic acid bacteria decreased from the third week to the fourth week of storage. The growth follows the microbial growth curve where the growth during the lag phase will be exponential. The exponential growth continues until it reaches the maximum growth. From this point, further growth will start to decline. The decline might be caused by the decreasing nutrition sources such as sugar, amino acids or fat, which have been taken up by the bacteria during their exponential growth period. The insufficient nutrition will weaken the viability of microbes to continue growing (Gilliland *et al*. 2002).

During the observation period, the viscosity of product was related to the total soluble solid content and pH value. As low pH value caused the formation of more coagulant, the total soluble solid will be increased, and the observed viscosity will be higher (Walstra 1990).

From week 0 to 3, the total soluble solid content was decreasing, thus, the viscosity was also decreasing. This decreasing total soluble solid content was due to the decreasing amount of simple sugars as a result of the activities of lactic acid bacteria in breaking down the sugars through fermentation. The total soluble solid content also affected the texture of corn milk yoghurt. As the total soluble solid content increases, the perceived thickness of yoghurt also increases (Sodini *et al.*

2004). The total soluble solid content of corn milk yoghurt was 17.5 °Brix. This value was slightly higher than the total soluble solid content of yoghurt product as defined by Tammime and Robinson (1989), which is 14-16°Brix. However, this is not a problem because the maximum total soluble solid content in yoghurt product cannot exceed 25% (Tammime & Robinson 1989).

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