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RESEARCH

Effect of Formulation of Jackfruit Seed Extract, Soybean Juice and Moringa Leaf Extract on the Amount of Lactic Acid Bacteria, Total Acid, and pH of Kalelo Yogurt as Probiotics & Supplementary Feeding for Stunting Prevention

Nur Hatijah[1(corresponding author)], Melina Sari[2], Atika Nuswantari[3]
[1]Nutrition Department, Health Polytechnic of Surabaya, Indonesia (nurhatijah@gmail.com)
[2]Nutrition Department, Health Polytechnic of Surabaya, Indonesia (melinasari6@gmail.com)
[3]Nutrition Department, Health Polytechnic of Surabaya, Indonesia (nuswantariatika78@gmail.com)

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ABSTRACT

Introduction: Efforts to improve nutrition for the prevention of stunting include specific interventions with specific targets, namely pregnant women in the form of supplementary feeding for pregnant and lactating mothers. Micronutrient food sources that are still not maximized are processed including jackfruit seeds (Arthocarphus heterophilus), Soybean (Glycine max L. Mer) and Moringa oleifera leaves. **Objective:** Determine the right formulation in making kalelo yogurt as a probiotic and PMT to prevent stunting. **Methods:** Post-test only design method with yogurt formula treatment. The resulting yogurt was seen as having an effect on the amount of lactic acid bacteria, total acid and pH. The statistical analysis used was Complete Randomized Design (CRD) 3 X 2 followed by Duncan test to see the difference in mineral content of each formulation with α 0.05. **Results:** The number of lactic acid bacteria (LAB) ranged from 5.8 x108 to 1.8 x109, fulfilling the SNI Yoghurt standard with the highest number of F3 formulas. Total acid ranged from 11-1.2% calculated as lactic acid, fulfilling standard SNI yogurt with the highest amount of formula F2. Total solids of yoghurt formula ranged from 16.29% -16.79%, fulfilling standard SNI yogurt with the highest value of Formula F1 & F3. The pH value of Kalelo yogurt is 3.8 with a formula that has the best pH, namely F2 formula. **Discussion**: LAB can develop during fermentation because it uses sugar from carbohydrates in the formula. During the fermentation process, lactose derived from skim milk will be hydrolyzed by the enzyme β-galactosidase to glucose and galactose, then converted to lactic acid. The high amount of solids is caused by the addition of soybeans, jackfruit seeds which are high in sugar content. Organic acids cause a decrease in pH during the fermentation process. In addition to increasing incubation time, microbial activity is increasing and the number of microbes is increasing, resulting in pH decreasing. **Conclusion**: The number of lactic acid bacteria (LAB), the amount of acid calculated as lactic acid, the amount of solids and the value of PH calelo yogurt fulfills the SNI standard of yoghurt.

Keywords: Provision of additional food, Yoghurt, Pregnant women, Breastfeeding mothers, *Arthocarphus heterophilus, Glycinem ax L. Mer, Moringa oleifera*

INTRODUCTION

Stunting is a condition that is caused due to lack of nutritional intake and not in accordance with the needs in a long enough time that causes chronic malnutrition. In 2013 the percentage of short (very short and very short) nutritional status in Indonesia was 37.2%, compared to 2010 (35.6%) and in 2007 (36.8%) did not show a significant decline / improvement. Based on the results of the 2015 PSG, 29% of Indonesian toddlers were in the short category, with the highest percentage in the provinces of East Nusa Tenggara and West Sulawesi[(1)].

Activities that the government seeks to improve nutrition for the prevention of stunting or short toddlers are intervention in the health sector through activities such as immunization, Provision of Supplementary Foods for pregnant women (PMT Bumil), Toddler PMT, and monitoring the growth of Toddlers at the Posyandu[(1)]. In addition to giving PMT from the government, to meet the nutritional needs, it can be in the form of additional food or drinks that are high in macro nutrients and micro-nutrients. Additional food recovery development is prioritized based on food ingredients or local food [(2)]. Material selection is based on community interest. Food that will be processed into additional food for pregnant women should be the same as the food habits of pregnant women everyday. The choice of type of food can be based on preferences, gestational age, local habits and considering the presence of restrictions in the local area. These foods are a source of energy, protein, vitamins and minerals. The type of food used in making additional food for pregnant women should be selected from locally available ingredients and at affordable prices. [(3)]

The use of local materials and local food into a formula product that is rich in nutrients has been widely used. Research conducted by Utami et al.[4] shows that the nutritional status of pregnant women who get peanut formula drinks is higher than the provision of kidney beans, soybeans, and formula milk for pregnant women. Addition of tempeh flour to the sausage substitution sausage as much as 60% has levels of protein, iron, and β-carotene which are in accordance with SNI and can meet the additional nutritional needs of pregnant women, 68% protein, 12% iron, and β-carotene 35%[5].

Indonesia is rich in micronutrient sources that are still not maximally processed. Local foods that can be developed into PMT products include jackfruit seeds (*Arthocarphus heterophilus*), soybeans (*Glycine max L. Mer*) and Moringa oleifera leaves. Jackfruit seeds have a high phosphorus content of 200 mg in 100 g. Indonesian people, including in Java, often consume jackfruit, while the seeds are often dumped just like that. There are some who use it by boiling it and then eating it, but the percentage is very small. Though jackfruit in Indonesia is found everywhere [6].

Jackfruit seeds contain polysaccharides that cannot be digested by the body so they can cause gas or what is commonly referred to as flatus (farting). One method of solving the polysaccharide can be done by fermentation. Based on the research of Wichienchot, et al. (2011) in Manurung, et al.[7] Jackfruit seeds contain high oligosaccharides which are confirmed as selective prebiotics in microflora fermentation in an intestinal system and can suppress pathogenic microbial growth.

Soybean is one type of beans which has a high enough protein content that can be processed into various types of processed products, one of which is soy milk. In 100 g of soybeans it contains energy of 381 kcal, protein 40 g, fat 16.7 g and carbohydrates 24.9 g. The interaction between the amount of water and the type of soybean did not significantly affect organoleptics[8]. Soy milk is currently being used as an alternative substitute for cow's milk. In addition to containing calcium, soybeans also contain phytoestrogens which are almost the same as estrogen which helps to absorb calcium in the blood. Respondents who were given soy milk had a higher increase than control respondents who were not given soy milk. Control increased by 0.05 mg / dl (0.58%) while those giving soy milk increased by 1.72 mg / dl (17.85%)[9].

Moringa is known throughout the world as a nutritious plant and WHO has introduced Moringa as one of the alternative foods to overcome nutritional problems (malnutrition)[10]. All parts of Moringa plants have nutritional value, efficacious for health and benefits in the industrial sector. The high nutritional value, efficacy and benefits caused the moringa to be nicknamed Mother's Best, but in Indonesia the use of moringa is still not widely known, generally only known as one vegetable menu. Moringa leaves are rich in minerals and amino acids after each antioxidant content.

The content of micronutrients is 7 times vitamin C orange, 4 times vitamin A carrots, 4 glasses of calcium milk, 3 times potassium bananas, and protein in 2 yogurts. Therefore moringa has the potential as a probiotic drink for health drinks, or added to nutritional food [10]. Research of Zakaria et al.[11] showed that there was an increase in the quantity of breast milk in the group of breastfeeding mothers, both in the form of extracts and moringa flour, but did not provide a significant change in the levels of iron, vitamin C and Vita-min before and after intervention in both groups. Giving Moringa extracts increases the volume of Asi higher than Moringa flour. In addition, the administration of local Moringa leaf powder NTB can improve the physical state of PEM condition to lead to a normal physical condition in KEP model mice[12].

One type of health food / drink that has been developed is food products that contain species of intestinal bacteria (probiotics). Preparations of microbial cell components in probiotics have been reported to have beneficial effects on the health of their hosts [13]. Yoghurt can be made by adding 1.5 - 3% bacterial culture by incubation at a temperature of 42-45 oC for 3 hours. Changes in chemical properties, especially the levels and types of sugar, lactic acid and the amount of acid and changes in pH occur in a certain incubation period[14].

Based on the information data above, researchers felt that further research was needed to see "Effect of Jackfruit Seed Extract Formulation, Soybean Juice and Moringa Leaf Extract on the Amount of Lactic Acid Bacteria, Amount of Acid, and pH of Kalelo Yogurt as Probiotics & PMT to prevent stunting"

The purpose of this study was to determine the appropriate formulation in making calelo yogurt as probiotics and stunting PMT prevention by measures comparing the number of microbes of kalelo yogurt as probiotics & stunting PMT prevention with SNI 2981: 2009, comparing the amount of kalelo yoghurt formula with SNI 2981: 2009, determine the amount of kalelo yogurt formula solids, determine the pH value of the kalelo yoghurt formula.

METHODS

The type of research was pre-experimental with the Posttest only design method. The treatment used in this study was yogurt formula. The resulting yogurt will be seen to affect the amount of microbial lactic acid, the

amount of acid, sugar content and pH. The design of the main formulation of the main functional instant drinks is as shown in Table 1.

Table 1. Yogurt formula in 100% kalelo

Bahan	Formula		
	F1	F2	F3
Soybean Juice (1:6)	40	35	50
Jackfruit Juice (1:6)	40	50	35
Moringa exctract	20	15	15
Skim Milk	10	10	10
Sugar	10	10	10
Starter	10	10	10

Laboratory test results on nutrition were tested statistically using a Completely Randomized Design (CRD) 3 X 2 followed by Duncan test to see the difference in mineral content of each formulation significantly α level of 0.05. Variables of this study included: Kalelo yogurt formula, number of lactic acid microbes, amount of acid, sugar content and pH. The material used in this study were jackfruit seeds, soybeans, Moringa leaves, skim milk, sugar, starter microbes. The equipment used included blenders, pans, as light as 60 mesh, basins, analytical scales, stoves, stirrers, bottle storage, refrigerators, fermenters. Testing for the amount of LAB was carried out using the cup count method with de Man Ragosa and Sharpe (MRS) medium so that it was sterile, galactose and cysteine HCl as much as 660 µl which was cooled to 50^{o}C = 15 ml and the results of microbiological analysis used a standard called Plate Standards Count. Analysis of the amount of acid was determined by the acid-base titration method with steps: 10 mL of the sample was diluted with 50 mL of water and added 5 drops of phenolftalein indicator, the sample was titrated with 0.1 N NaOH solution until a stable pink color was formed and then the amount of acid determined by formula:

$$\% \text{ acid} = \frac{ml\ titer\ xN\ titer\ x\ 0.09}{weight}\ x\ 100.$$

PH measurement was done using a pH meter. The pH meter is calibrated first with a buffer for pH 4 and pH 7 according to the pH range of yogurt. Measurements were made by dipping the pH meter electrode into 10 ml samples (AOAC, 1995).

RESULTS

Lactic Acid Bacteria (LAB)

Data of the average analysis of LAB with 2 replications in three types of kalelo yogurt formulas can be seen in Table 2.

Table 2. Average of BAL content (CFU / ml) in three types of kalelo yogurt formula

	F1	F2	F3
Average	7.4 x10^{8}	5.8 x10^{8}	1.8 x10^{9}

Based on the data above, it can be seen that Formula F3 has the largest number of BAL, which is around 1,8 x10^{9} CFU/ml and the smallest is formula F2 which is 5.8 x10^{8} CFU/ml. Statistical tests use annova at the level 5% (p-value = 0.453406) indicates that there are no significant differences between the formulas with each other.

pH

Data of the average pH value with 2 replications in three types of kalelo yogurt formulas can be seen in Table 3.

Tabel 3. The average of pH for the three types of kalelo yogurt formula

	F1	F2	F3
Average	3.852^{a}	3.8875^{b}	3.8275^{a}

Based on the data above it can be seen that Formula F2 has the greatest pH value which is around 3.8875 and the smallest is the F3 formula which is 3.8275. The statistical test using Annova at the level of 5% (P value = 0.04053) showed that there were significant differences, the results of further testing using duncan showed that Formula F2 was significantly different compared to the others.

Lactic Acid

Data of the average of lactic acid with 2 replications in three types of kalelo yogurt formula can be seen as in Table 4.

Tabel 4. The average of lactic acid (%) in three types of kalelo yogurt formula

	F1	F2	F3
Average	1.2634[a]	1.15655[b]	1.00915[c]

Based on the data above, it can be seen that Formula F1 has the largest amount of lactic acid, which is around 1.2634% and the smallest is F3 formula which is 1.00915%. Statistical tests using annova at the level of 5% (p-value = 0.0489) indicate that there are significant differences, the results of further tests using duncan indicate that Formula F1, F2 and F3 differ significantly from each other.

Total dissolved solids

Data from the average analysis of the amount of solids dissolved with 2 replications in three types of kalelo yogurt formulas can be seen in Table 5

Table 5 Average Dissolved Solids (%) in three types of kalelo yogurt formulas

	F1	F2	F3
Average	16.79	16.29	16.79

Based on the data above, it can be seen that Formula F1 & F3 has the highest amount of dissolved solids which is around 16.79% and the smallest is F2 formula, which is 16.29%. Statistical tests using annova at the level of 5% (p-value = 0.509865) showed that there were no significant differences in the amount of dissolved solids in the three kalelo yogurt formulas..

DISCUSSION

Kalelo Microbes as Probiotics & PMTs Prevent Stunting

The amount of kalelo yogurt microbes is calculated as the amount of lactic acid bacteria (LAB) that can help the yogurt fermentation process. The results of the analysis of kalelo yogurt show that the amount of BAL ranges from 5.8 x108 to 1.8 x109. This amount when compared with the yogurt standard (SNI 2981: 2009) the number of starter bactarians Min 107 is in accordance with the standard SNI yogurt. The bacteria used in making yogurt are S. Thermophillus, L. Bulgaricus, L acidophilus, Bifidobacteria which are derived from culture starters with an addition of 10% of the weight of the amount of material. LAB lactic acid can develop during fermentation because it uses sugar derived from carbohydrates found in the formula.

The use of starter inoculation (Lactoacillus bulgaricus and Streptococcus thermophillus) allows for changes in lactose and lactic acid production which results in a decrease in pH, so that the acid level of yogurt is relatively high and the formation of lumps of yoghurt.

Acid Kalelo Yogurt Formula

The amount of kalelo yogurt formula acid was calculated as the amount of lactic acid. Based on yogurt standards (SNI 2981: 2009) the amount of yoghurt acid ranges from 0.5-2%. The results of the Kalelo product analysis of the amount of acid ranged from 11 to 1.2% according to SNI yogurt standards.

Lactic acid contained in kalelo yogurt is obtained from the content of oligosaccharides found in kalelo juice which can help the growth of LAB. During the fermentation process, lactose derived from skim milk will be hydrolyzed by the enzyme β-galactosidase to glucose and galactose, then converted to lactic acid.

Kalelo Yogurt Formula Solids

The amount of solids is a solid part consisting of ingredients that are mixed and the nutrients contained therein are fat, protein, carbohydrates, minerals, vitamins, and soluble fiber[15].

The number of yoghut solids is in accordance with the standards (SNI 2981: 2009), namely min 8.2%. The results of the measurement of calelo yogurt the amount of yogurt formula solids ranged from 16.29% -16.79%. This shows that the amount of solids is above the SNI standard. The high amount of solids is caused by the addition of soybeans, jackfruit seeds which are high in sugar content. According to Osundahusi[16] the addition of fruit that is high in the sugar content in making yogurt will contribute to a higher component of dissolved solids.

According to Fardiaz[17] the fermentation process of BAL which produces lactic acid will be excreted out of the cell and will accumulate in the fermentation liquid. The remaining amount of sugar, lactic acid and organic acids formed counts as the amount of dissolved solids.

PH Value of the Kalelo Yogurt Formula

The pH value of the kalelo yogurt formula ranges from 3.8, indicating that kalelo yogurt has a low pH (acid). Leblanc et al (2004) stated that enzym invertase will hydrolyze sucrose to glucose and fructose, raffinose and stakiosa will be hydrolyzed by alpha-galactosidation enzymes and invertase to glucose, fructose and galactose. The result of gule metabolism by BAL bacteria is the energy needed for cell growth and other organic acids such as succinic acid and citric acid. These organic acids cause a decrease in pH or an increase in the amount of acid during the fermentation process. In addition to increasing incubation time, microbial activity is increasing and the number of microbes is increasing, resulting in pH decreasing.

CONCLUSION

The amount of lactic acid bacteria (LAB), the amount of acid, the amount of yogurt formula solids and the pH value of kalelo yogurt have met the SNI Yogurt standard.

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