The Potential of Puree Jamblang (Syzygium cumini) in Improving the Quality of Acidophilus Milk as a Probiotic

Yurliasni¹, Zuraida Hanum², and Yunasri Usman³
¹Milk Processing Science and Technology Laboratory, Faculty of Agriculture, Syiah Kuala University, Darussalam- Banda Aceh, Indonesia
Email: yurliasni@unsyiah.ac.id

Abstract. This research on the use of jamblang puree in fermented milk has been carried out in Milk Processing Science and Technology Laboratory. The aim is to investigate the function of jamblang puree in enhancing acidophilus milk as a probiotic drink. This research uses a completely randomized factorial pattern design, consisting of two factors, namely the percentage of jamblang puree (A) with 4 levels (0, 10, 20 and 30)% and the percentage of starter (B) with 3 levels (2.5, 5, 7.5)% . The starter used is Lactobacillus acidophilus. Changes in the quality of acidophilus milk are known through the measurement of pH, syneresis value, and total lactic acid bacteria. The results showed that the addition of jamblang puree significantly decreased the pH value and total lactic acid bacteria but the interaction of the two factors significantly increased syneresis. In conclusion, the addition of jamblang puree creates products whose quality is still in the SNI range so it can be categorized as a product that is suitable for consumption.

1. Introduction
Acidophilus milk is a fermented milk product using lactic acid bacteria (LAB) Lactobacillus acidophilus which is a probiotic that has a beneficial effect on health [1]. Besides containing good nutritional value, as a functional food, the acidophilus milk is also expected to have other characteristics by adding natural fruits that have health benefits such as jamblang (Syzygium cumini). The active compounds contained in jamlang fruit include rafinose, fructose, citric acid, malic acid, and anthocyanin [2]. Moreover, since it can increase the nutritional value, the addition of jamblang fruit is also expected to be able to support the level of consumer acceptance on pH, syneresis and total probiotic cells contained in acidophilus milk. As revealed by [3] that the probiotic that has a healthy effect and inhibits pathogens is $10^6$ cfu / mL.

2. Materials and Methods
2.1 Material.
Materials used in this study include UHT milk, jamblang fruit and Lactobacillus acidophilus as a starter. Some supporting materials that also used are agar media, NaOH, phenolphthalein and BPW (buffer pepton water), and Man deRagosa Sharp Agar (MRS)

2.2 Methods
2.2.1 Preparation of Bulk culture. The preparation of bulk culture includes the process of rejuvenation of stock culture, making of mother culture, intermediate culture to ready-bulk culture.
The process of making bulk culture was carried out by piping as much as 2.5, and 5% intermediate culture into UHT milk, homogenized, and incubated at 37 °C for 18 h, then it is and ready to be used as a bulk starter.

2.2.2 Preparation of Acidophilus Milk. There are 2.5 and 5%, Lactobacillus acidophilus, respectively as a starter, which is added to UHT milk based on the design, then homogenized. The last step is the addition of pasturized jamblang puree by 0, 10, 20 and 30 % is which is then incubated at 37 °C for 18 h

2.2.3 pH measurement. The pH is measured using a calibrated pH meter (ToA). A total of 10 ml of the sample is poured into a test tube, then a pH meter electrode is inserted into the sample. The results can be read and monitored by the pH meter

2.2.4 Total lactic acid bacteria. A total of 1 mL sample is pipetted into a sterile test tube containing 9 mL of NaCl 0.9% as 10⁻¹ dilution, followed by the same procedure until reaching a 10⁻⁶ dilution. Furthermore, 1 mL of 10⁻⁶ dilution sample s pipetted into a sterile petridish and the MRS is poured, homogenous and allowed to stand until it hardens. The petridishes containing microbes are incubated upside down at 37°C for 48 h. The growing colonies are calculated based on the dilution factors, by the formula: number of colonies (cfu / mL) = number of growing cells x 1 / rate of dilution [4]

2.2.5 Syneresis. The sample was weighed as much as 10 grams, then put into a test tube and then left in the refrigerator for 1 hour at 5 °C. The sample was then centrifuged for 10 minutes at 3000 rpm. The supernatant (whey liquid) was obtained from each centrifuged sample. The value of syneresis is calculated using the formula: syneresis = (Supernatant weight) / Sample weight x 100%

3. Results and Discussion

3.1 pH measurement

The result of the analysis of variance on the data obtained shows that the addition of jamblang puree has a very significant effect on the pH of acidophilus milk and there are differences between treatments. Table 1 illustrates that the higher the level of jamblang puree, the lower the average pH produced.

| Treatments | pH value |
|------------|----------|
| B₁         | 4.15₄    |
| B₂         | 4.05₅    |
| B₃         | 3.98₆    |
| B₄         | 3.95₇    |

Note: Means in the same column with different superscript differ significantly at (P<0.01)
B₁: 0; B₂: 10%; B₃: 20%; B₄: 30% (Jamblang puree level)

It is clearly seen that based on Duncan’s Multiple Range test, there is a very significant difference between treatments shown by different superscripts. The highest level of jamblang puree (30%) produces acidophilus milk with a very low pH (3.95). However, on the contrary, without and addition of 10% of the puree, the pH value produced is relatively higher. The low of pH value is strongly related to the acid production during the milk fermentation process. Besides, the decrease in pH value is also caused by the acid components contained in jamblang fruit such as citric acid, malic acid and galik acid [2]
3.2 Total Lactic Acid Bacteria (LAB)
The results of the total LAB analysis by ANOVA obtained are shown in Table 2. It is very obvious that the two treatment factors do not affect the total of LAB, but it is only the level of jamblang puree which has a very significant effect on the total LAB. Based on the jamblang level, it can be concluded that there is a very significant difference between the treatments. At the highest level of jamblang puree, the total LAB is reduced very significantly.

| Treatments | Total LAB (log cfu/mL) |
|------------|-----------------------|
| B₁         | 8.80⁰                 |
| B₂         | 8.38⁰                 |
| B₃         | 8.16⁰                 |
| B₄         | 8.05⁰                 |

Note: Means in the same column with different superscript differ significantly at (P<0.01)
B₁:0 ; B₂: 10 %; B₃ : 20%; B₄: 30% (Jamblang puree level)

This condition can occur due to the accumulation of metabolite compounds produced by starter and jamblang puree during the fermentation process which have the ability to suppress the growth of LAB and consequently the number of cells decreased ultimately. The LAB growth is also influenced by various factors such as nutrition, temperature, humidity, oxygen, pH and inhibiting substances [6]. The reduced number of LAB cells can also occur due to the high concentrations of metabolites which are compounds that have antimicrobial properties [7]. Being a probiotic product the acidophilus milk is expected to have an optimal cell count about 10⁸ to 10⁹ cfu/mL as stated by [8], that probiotics are used to stimulate the human immune system and balance the number of beneficial and harmful bacteria.

3.3 Syneresis Value
Syneresis is a situation in which the release of liquid from the gel during the fermentation process, occurs in this case, due to the production of acidophilus milk. Based on the analysis of variance, it is found that the level of puree jamblang has a very significant effect on syneresis value. At the same time the starter level has no effect at all on the syneresis.

| Treatments | Syneresis value (%) |
|------------|---------------------|
| B₁         | 29.68⁰              |
| B₂         | 33.60⁰              |
| B₃         | 53.73⁰              |
| B₄         | 54.47⁰              |

Note: Means in the same column with different superscript differ significantly at (P<0.01)
B₁:0 ; B₂: 10 %; B₃ : 20%; B₄: 30% (Jamblang puree level)

Based on the results shown in the Table 3, the high levels of jamblang puree cause high values of syneresis so the texture of acidophilus milk is broken and unstable. Furthermore, without the addition of jamblang puree and the addition of 10% of the jamblang puree results in lower syneresis values. This syneresis value is an indicator that shows a better texture of fermented milk. The same research conducted by [9] states that the low ability of proteins to bind water can cause syneresis. In addition, according to the [10] report, syneresis can also occur by agitation during the fermentation process. The potential to form a gel and decrease the viscosity of the solution will decrease along with the decrease in pH, because H⁺ ions help the hydrolysis of glycosidic bonds. According to [10], the viscosity of yogurt describes the nature of the liquid that has resistance to a flow that can provide an increase in the strength which withstands the relative movement.
4. Conclusion
The best level of jamblang puree that can be added to acidophilus milk to keep the minimum prebiotic properties is 10%

References
[1] Tamime AY and Robinson R K 2007 Yoghurt science and technology. 3rd ed. Abington, Cambridge, England: Woodhead Publishing Ltd, CRC Press, LLC, NW, USA
[2] Ayyanar M and Subash-Babu 2012 Syzygium cumini(L) Skeels A review of its phytochemical constituent and traditional uses. Asean Pacific of Journal of Tropical biomedicine 2 (3) pp 240-246
[3] Kailasapath K and Chin J 2000 Survival and therapeutic potential of probiotic acidophilus and Bifidobacterium sp Immunology and Cell Biology
[4] Cappuccino G J and Natalie S 2005 Microbiology Laboratory Manual 7th Edition Benjamin Cummings Inc publishing
[5] Karinawatie S J, Kusnadi and Martati E 2008 Efektifitas Konsentrat protein Whey dan Dextrin untuk mempertahankan Viabilitas Bakteri Asam Laktat dalam Starter Keringbeku Yoghurt Jurnal Teknologi Pertanian. Vol 9, No 2 pp: 121-130
[6] Rastina S M, Wientarsih I 2015 Aktivitas Antibakteri Ekstra Etanol Daun Kari (Murraya koenigii) terhadap Staphylococcus aureus, Escherichia coli, dan Stduomonas sp. Jurnal Kedokteran Hewan. Vol 9 (2) pp: 185-189
[7] Pryantoro S T 2015 Gut Microbiota and Irritable Bowel Disease 28 (2) pp: 43-48
[8] Setiadi 2007 Proteolityc activity of Lactobacillus acidophilus in fermented cow milk Jurnal Ilmu Peternakan 7 pp 69-72
[9] Harwalkar V R and Kalab 1983 Suceptibility of Yogurt to syneresis comparison ofcentrifugation and drainage methods 38 pp 517-521
[10] Manab A 2008 Kajian sifat fisik yogurt selama penyimpanan pada (Study of the physical properties of yogurt during storage at 4°C