

Production of acidophilus milk enriched with purees from colored sweetpotato (*Ipomoea batatas* Linn.) varieties

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ABSTRACT

Perez, R. H. and J. D. Tan. 2006. Production of acidophilus milk enriched with purees from colored sweetpotato (*Ipomoea batatas* Linn.) varieties. *Ann. Trop. Res.* 28(1):70-85

Probiotic foods and drinks are becoming more popular nowadays. Probiotic foods are foods that contain health-promoting microorganisms. The beneficial effects of probiotics may be mediated by a direct antagonistic effect against specific groups of organisms, resulting in a decrease in their metabolism or by stimulation of immunity.

Acidophilus milk enriched with purees from *kinampay* and RC-2000 sweetpotato varieties was developed. Acidophilus milk is a probiotic drink, which is a product of milk fermentation by the bacteria *Lactobacillus acidophilus*. The fermented milk has been reported to have therapeutic value for suppressing toxin-producing organisms in the intestine of human.

The sugar level and product acceptability of the developed product was evaluated through sensory evaluation, and a proximate composition of the product was determined by proximate analysis.

It was found out that at 1% and 10% levels of inocula of the starter culture, the change in Total Titratable Acidity (TTA) of acidophilus milk was the same, hence, 1% inoculum was found practical in the production of acidophilus milk. Findings from previous experiments show that the maximum number of viable cells can be maintained

Keywords: acidophilus milk, probiotics, sweetpotato puree, fermented milk

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at TTA and pH of 0.65% and 4.7 respectively (Reed, 1982). Basing on that research finding, those acidity values were used as the target optimum conditions. The optimum incubation time was found to be at 16 hours at 37°C for plain acidophilus milk and 14 hours for acidophilus milk enriched with *kinampay* and RC-2000 purees before incubation.

The developed acidophilus milk was found to be more acceptable using 6.25% sugar level.

The addition of sweetpotato puree to the acidophilus milk generally improved the sensory qualities and proximate composition of the product. It reduced the moisture content and increased the ash and protein contents of the product.

INTRODUCTION

Probiotic was first used in 1965 to describe the growth-promoting effect of one microorganism against another, but soon afterward, the term was applied primarily to animal feed supplements (Hoover, 1993 as cited by Davide, 1995). Recently, according to Davide (1995), probiotics are used with reference to human consumption of live microorganisms as food additives for nutritional health and well being.

Acidophilus milk is a probiotic drink, which is a product of milk fermentation by the bacteria *Lactobacillus acidophilus*. The fermented milk has been reported to have therapeutic value for suppressing toxin-producing organisms in the intestine of man especially in infants. The acidophilus bacteria contained in milk are able to pass through the stomach and gain predominance in the intestine and in the process lessen putrefaction that takes place in the intestinal tract due to the presence of other types of bacteria (Van den berg, 1997). *L. acidophilus* is known to have beneficial effects on the maintenance of normal intestinal microflora by producing inhibitors, stimulating the host immune system and reduction of serum cholesterol levels. It also helps in nutritional enhancement by increasing calcium absorption and alleviation of effects of renal malfunction by reducing the levels of toxic substances (Varnam and Sutherland, 1994).

L. acidophilus is a lactose-fermenting bacterium that produces lactic acid as a major product of fermentation (Reed, 1982). These bacilli are considered probiotic bacteria and are added to a variety of food, like milk, in an attempt to enhance human health. Beneficial effects of probiotics may be mediated by a direct antagonistic effect against specific groups of organisms,

resulting in a decrease in their metabolism or by stimulation of immunity.

Owing to such beneficial effects, probiotic foods such as acidophilus milk are often prescribed for regular consumption of individuals suffering from intestinal disturbances. However, the market of acidophilus milk is still low due to its unattractive color and its sour taste. Therefore, there is a need to improve the taste and color of this product to enhance its acceptability. The quality of appearance and taste of food govern to a large extent its consumer acceptability and highly colored foods are generally attractive and appealing to consumers.

Beta-carotene is a provitamin-A, which plays an important role in the nutrition of man. It is essential for normal growth, good eyesight and teeth development and for maintenance of integrity of epithelial tissues in humans (Guthrie, 1979). According to Olson (1986) as cited by Hendry and Houghton (1996), betacarotene has also been used successfully for the treatment of erythroporetic protoporphyria, a condition in which the patients are extremely sensitive to light. He also added that betacarotene affords protections against cancer and heart disease.

On the other hand, anthocyanin is a natural food color that has demonstrated therapeutic or medicinal properties including antioxidant activity, anti-inflammatory activity, reduce serum cholesterol and reduce serum lipid levels (Hendry and Houghton, 1996).

Hence, considering the attractive purple color of *kinampay* and pleasing orange shade of RC2000, it is believed that it could be a potential colorant and flavorant of acidophilus milk. Knowing the nutritional benefits one can get from these food colorants which is present in these two sweetpotato varieties, the incorporation of sweetpotato to the milk does not only improve its product appearance but also its nutritional value.

This study aimed to produce acidophilus milk enriched with sweetpotato purees, to determine the time required for optimum quality of the product, and to evaluate the physico-chemical and sensory qualities of the resulting product.

This study was conducted at the Food Microbiology Laboratory, PhilRootcrops, LSU, Visca, Baybay, Leyte and Department of Food Science and Technology, LSU, Visca, Baybay, Leyte from April 2003 to February 2004.

This study used only two sweetpotato varieties, namely: RC2000 as the

source of beta-carotene; and, *kinampay* as the source of anthocyanin. The acidophilus milk was prepared from carabao's milk.

MATERIALS AND METHODS

Material preparation

Fresh carabao's milk was purchased from the Philippine Carabao Center (PCC), LSU, Visca, Baybay Leyte. The two-(2) sweetpotato varieties - RC2000 and *kinampay* were purchased from PhilRootcrops, LSU, Visca, Baybay Leyte. The refined sugar was purchased from Baybay Municipal Public Market. The starter culture *L. acidophilus* was requested from the Institute of Food Science (IFST) University of the Philippines at Los Baños, Laguna, (UPLB).

Starter culture preparation

The pasteurized milk was aseptically transferred to a sterile bottle. One (1) mL from the previously incubated GYP broth was aseptically added to the milk. The bottle was swirled to distribute the microorganisms evenly. The mixture, which serves as the starter culture, was then incubated at 37°C for 24 hours.

Determination of optimum level of inoculum

Starter culture consisting of active *L. acidophilus* cells at 1% and 10% level was used. One-hundred (100) mL of pasteurized milk was aseptically dispensed in a 150 mL capacity long-necked bottle. Twelve (12) bottles were inoculated with 1 mL of the starter culture while the remaining twelve (12) bottles were inoculated with 10 mL of the starter culture. The inoculated milk was incubated at 37°C for ten (10) days. After 1 day of incubation, one bottle with 1% and one with 10% inoculum were retrieved from the incubator and were subjected to TTA and pH analyses. Destructive sampling was done every other day for 10 days.

Sweetpotato puree preparation

Freshly harvested sweetpotatoes (RC2000 and *kinampay*) were washed thoroughly and peeled. The peeled roots were sliced (approximately 5mm thick) and steamed for 15 minutes. The steamed-cooked roots were added with the sugar solution in 1:1 weight to volume ratio and osterizer for 2-3min (Figure 1). Sugar levels of sweetpotato puree of 25% and 12% (wt/wt) were used.

Optimum incubation time determination

Pasteurized milk was dispensed in 150-mL long-necked bottles at 100 mL per bottle. The milk was inoculated with 1-% starter culture (1 mL in 100 mL) and incubated at 37°C for 24 hours. Two bottles were taken out every sampling time and analyzed for TTA, pH, and microbial count. Similar experiment was conducted with a variation of the addition of the sweetpotato puree before incubation.

At TTA and pH of 0.65% and 4.7 respectively, the maximum number of viable cells can be maintained (Reed, 1982). Based on these findings, the fermentation process of acidophilus milk was terminated at this TTA and pH level.

Acidophilus milk processing

Acidophilus milk was processed by adding the puree before and after incubation. The first method, which was the addition of the puree before inoculation, was done by aseptically adding the puree to the fresh milk in 25% volume to volume ratio. The 25% and 12% sugar levels of the sweetpotato puree yielded 6.25% and 3.125% (wt/vol) sugar levels of the final products respectively. The mixture was homogenized in a blender for 4-5 seconds. The starter culture was added to the mixture in 1-% volume to volume ratio. The inoculated mixtures were incubated at 37°C for 14 hours. After incubation, the products were homogenized using a blender for 2-3 seconds. The finished product were stored in a sterile container and refrigerated.

In the second method, the puree was added to the milk after it was incubated for 16 hours. The mixture was homogenized in a blender for 2-3

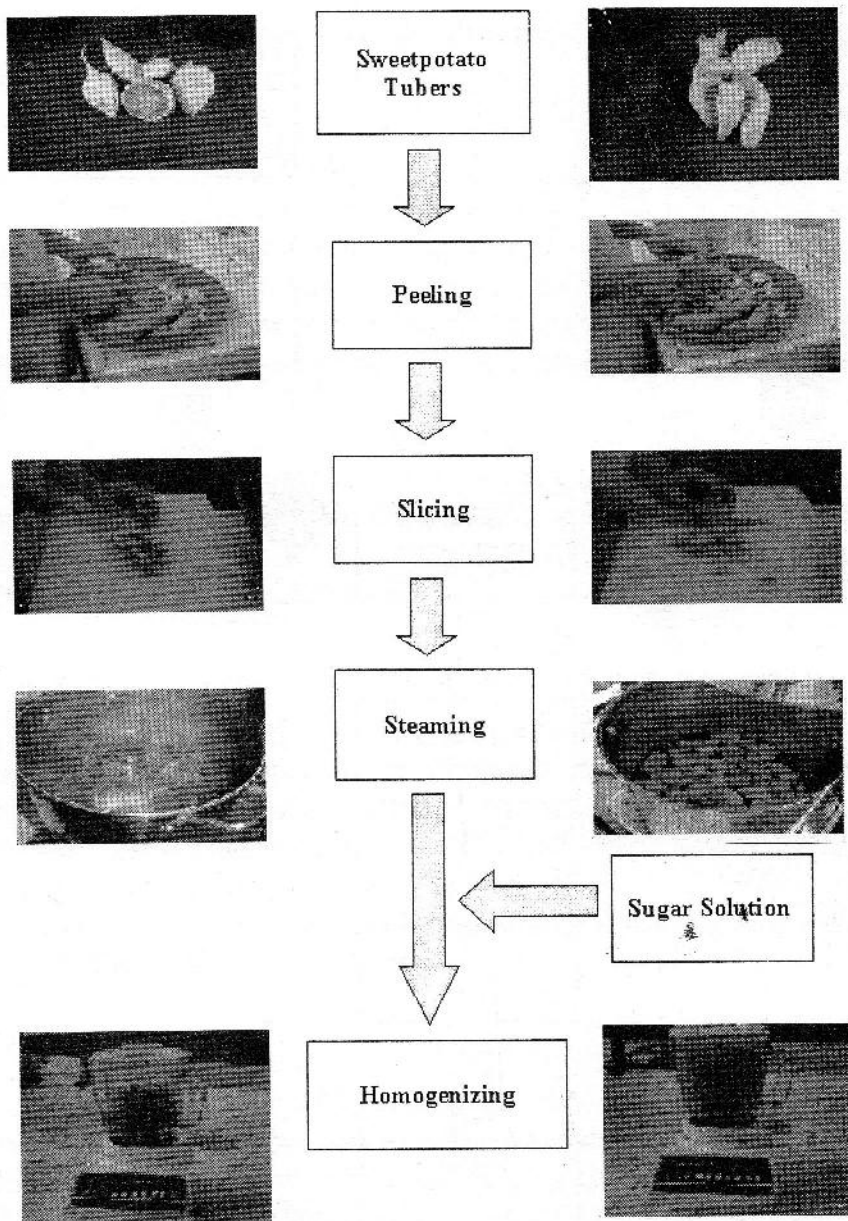


Figure 1. Sweetpotato puree processing

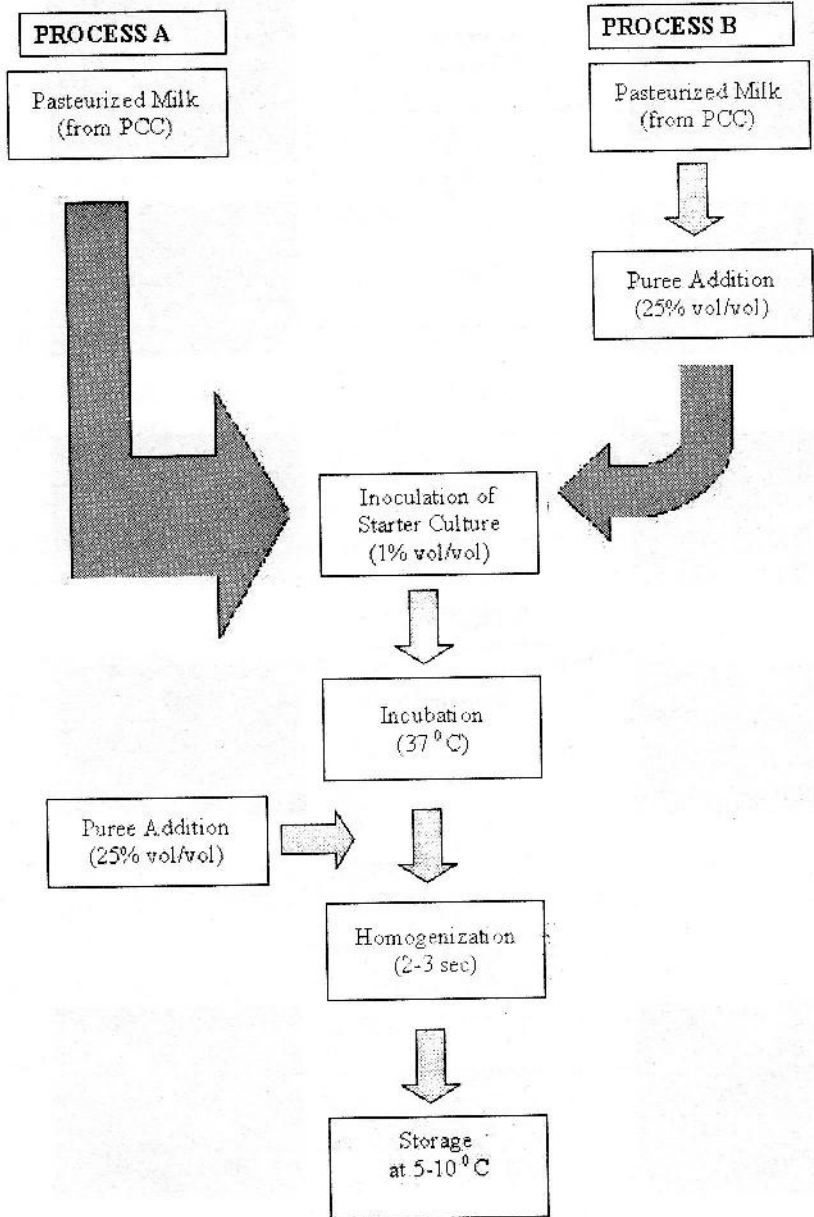


Figure 2. Acidophilus milk processing

seconds. The product was then be stored in a sterile container and refrigerated (Figure 2).

Physico-chemical analyses

The physico-chemical attributes of the product such as total titratable acidity (TTA), pH, microbial count, and proximate analyses (moisture content, ash content, and protein content) were determined.

Ten-(10) mL of the product was poured out in a beaker and was added with distilled water to 50 mL mark. TTA was determined by titrating the product with 0.1 N standard NaOH.

Microbial analyses was done by serial dilution (104, 106, 108) and plated in a GYP plus CaCO_3 medium. The plated agar was incubated at 37°C for two days. The CFU (colony forming unit) of lactic acid-producing cells which showed clearing zones in GYP with CaCO_3 were counted and recorded at their respective level of dilution.

Sensory evaluation

Sensory evaluation was conducted to determine the acceptability of the product in terms of color, flavor, after-taste, texture, taste, and general acceptability. This was done with twenty-five (25) randomly selected staff and students from PhilRootcrops and DFST, LSU, Visca, Baybay, Leyte who regularly participate in sensory evaluation of food products. The product was evaluated following the standard procedure using a quality scoring with a 9-point hedonic scale.

Statistical analysis

The data that were gathered from the sensory evaluation were analyzed using the Friedmann Two-way Test. The data that were obtained from the physical quality measurements were evaluated using Analysis of Variance (ANOVA) following the Randomized Complete Block Design (RCBD). Three treatments with two sub treatments with two replications were used. The treatments were designated as follows:

T_0 – control sample, plain acidophilus milk

T₁ – *kinampay*-enriched acidophilus milk

T_{1a} – puree added before incubation

T_{1b} – puree added after incubation

T₂ – RC2000-enriched acidophilus milk

T_{2a} – puree added before incubation

T_{2b} – puree added after incubation

The parameters that were found to have significant difference was further analyzed using the Duncan's Multiple Range Test (DMRT).

RESULT AND DISCUSSION

Determination of optimum starter culture level

As early as 1-day incubation, TTA of the samples with 1 and 10% inoculum exceeded the optimum conditions of 0.65% and 4.7 TTA and pH respectively. (Figures 3 and 4). For practical reason therefore, 1-day incubation at 1-% inoculum was used in the subsequent experiments.

Determination of optimum time of incubation

In order to determine the specific optimum time within the 24-day incubation, TTA and pH was monitored every 2 hours for 24 hours during incubation. Results show that the optimum TTA and pH of 0.65% and 4.7 respectively were attained at 16 hours of incubation. However, when sweetpotato puree was added during incubation, the optimum TTA was attained at 14 hours while optimum pH was observed at about 15 hours of incubation (Figures 5 and 6). This result clearly showed that fermentation was accelerated in the presence of carbohydrate source, which might hasten the growth and activity of the microorganisms. This result was confirmed in figure 7. The maximum lactic acid bacterial (LAB) counts were attained at 16 hours of fermentation when optimum TTA and pH were attained. However, with puree-enriched acidophilus milk, maximum LAB counts was observed at 14 hours of incubation.

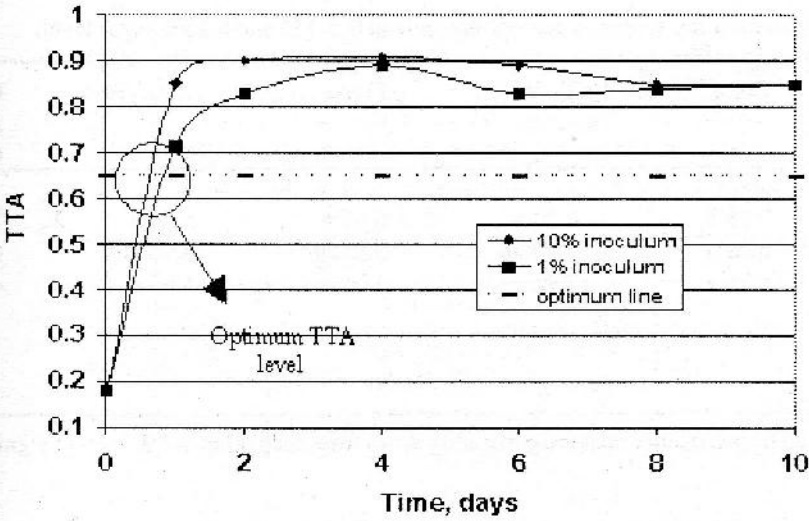


Figure. 3. Change in total titratable acidity (TTA) of acidophilus milk during incubation at 1% and 10% inoculum.

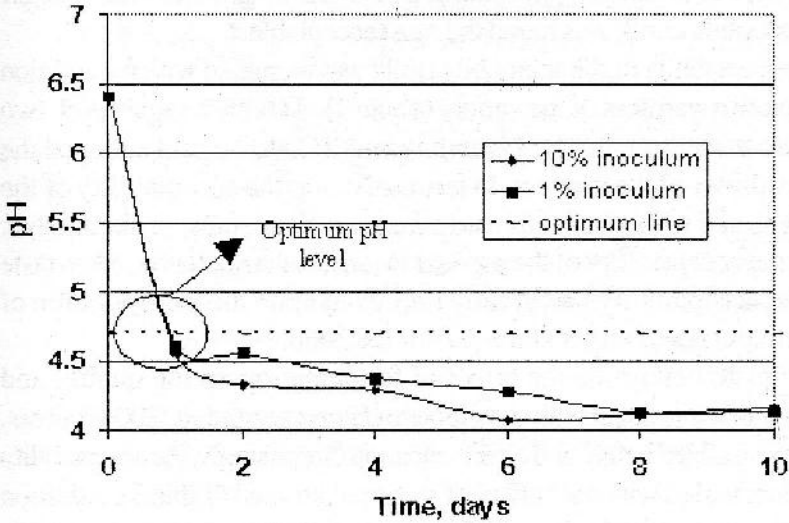


Figure. 4. Change in pH of acidophilus milk during incubation at 1% and 10% inoculum.

Table 1. Mean acceptability scores of acidophilus milk at 0, 3.125 and 6.25% sugar level.

| Sugar Level | Color | Taste | Flavor | After-Taste | Gen. Acceptability |
|--------------------------------------|--------|---------|---------|-------------|--------------------|
| 6.25% | 7.16 a | 6.20 a | 6.36 a | 5.64 a | 7.24 a |
| 3.125% | 6.96 a | 5.40 ab | 5.88 ab | 5.56 ab | 6.24 b |
| 0% (plain acidophilus milk) | 6.96 a | 3.92 c | 4.64 c | 4.84 c | 4.44 c |

Values followed by same letter do not significantly differ from each other at 5% level of significance.

Sensory evaluation

Incorporation of sweetpotato puree with different sugar levels did not show significant difference in the acceptability in terms of color with plain acidophilus milk (Table 1). However, the taste, flavor, after-taste and general acceptability was affected by the addition of sugar. In general, 6.25% sugar level in acidophilus milk was found the most acceptable.

The acceptability of the acidophilus milk was increased with the addition of sweetpotato regardless of the variety (Table 2). At 6.25% sugar level, two sweetpotato varieties namely; *kinampay* and RC2000 could enhance the sensory attributes of the product. In terms of color, the acceptability of the products with and without sweetpotato puree was comparable to one another. However, the acceptability of the product in terms of taste, flavor, after-taste and general acceptability was greatly improved upon the incorporation of sweetpotato purees of either *kinampay* or RC2000.

In order to determine the effect of fermentation on the quality and acceptability of the product with sweetpotato (*kinampay* and RC2000) purees, the purees were added before and after incubation. Surprisingly, the acceptability of the product varied with the variety of sweetpotato used (Table 3). Addition of RC2000 puree either before or after incubation did not show generally affect the acceptability of the product in terms of color, taste, flavor, after-taste and general acceptability. The product added with RC2000 puree added before and after incubation both have color range of light orange to yellowish

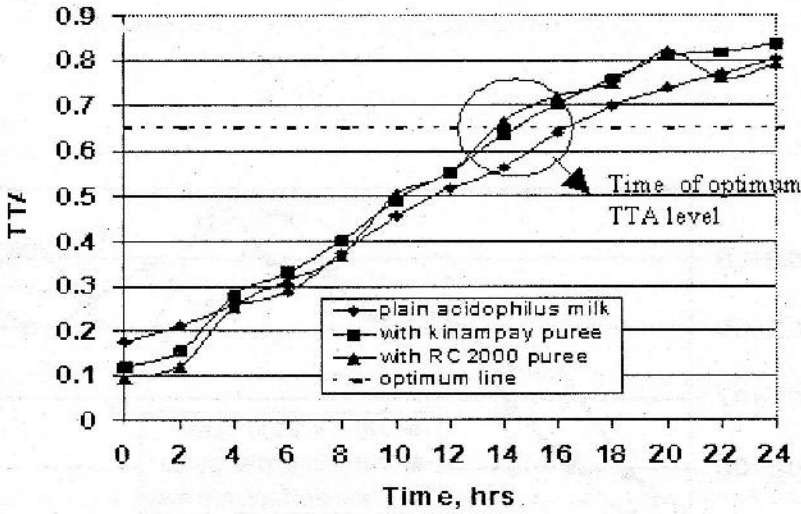


Fig.5. Change in TTA against fermentation time of plain acidophilus milk and product with puree added before incubation.

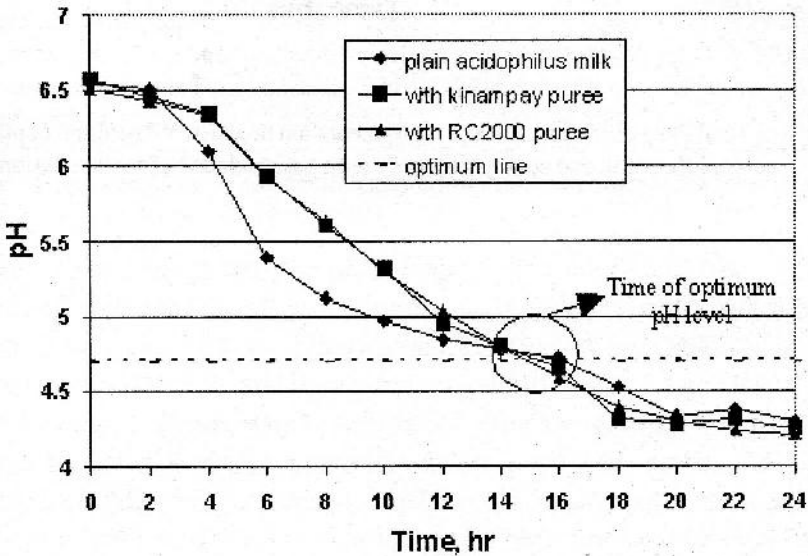


Fig.6. Change in pH against fermentation time of plain acidophilus milk and product with puree added before incubation.

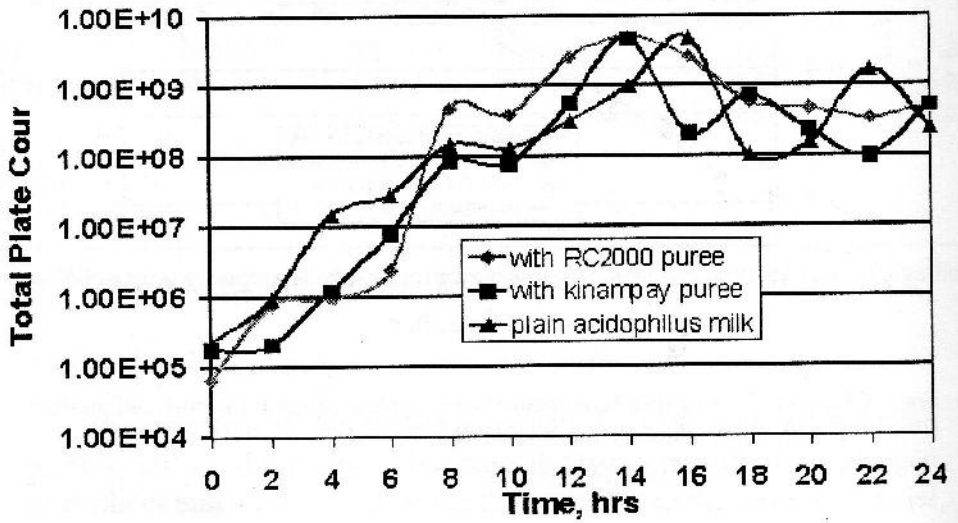


Fig 7. Plot of Total Plate Count of LAB against fermentation time in GYP medium of plain acidophilus milk and acidophilus milk with puree added before incubation.

Table 2. Mean acceptability scores of plain acidophilus beverage and sweetpotato puree-enriched acidophilus beverage.

| Variety | Color | Taste | Flavor | After-Taste | Gen. Acceptability |
|------------------|-------|--------|--------|-------------|--------------------|
| RC2000 | 7.16a | 6.20a | 6.24a | 6.24a | 6.92a |
| <i>Kinampay</i> | 7.04a | 5.64ab | 5.72ab | 5.88ab | 6.30ab |
| Plain | | | | | |
| Acidophilus Milk | 6.84a | 4.16c | 4.00c | 4.64c | 4.20c |

Values followed by same letter do not significantly differ from each other at 5% level of significance.

Table 3. Mean acceptability scores of plain acidophilus beverage and acidophilus beverage added with sweetpotato puree before and after incubation.

| | Color | Taste | Flavor | After-Taste | Gen. Acceptability |
|---|---------|---------|---------|-------------|--------------------|
| RC2000 added before incubation | 7.28ab | 5.96bc | 6.12abc | 5.72bc | 6.12bc |
| RC2000 added after incubation | 6.92abc | 6.28ab | 6.52ab | 6.40ab | 6.56ab |
| <i>Kinampay</i> added before incubation | 6.00cd | 5.40bcd | 5.56bc | 5.44c | 5.44bcd |
| <i>Kinampay</i> added after incubation | 7.84a | 7.40a | 7.20a | 7.00a | 7.40a |
| Plain Acidophilus Milk | 5.92bcd | 3.60 d | 3.52d | 3.80d | 4.00d |

Values followed by same letter do not significantly differ from each other at 5% level of significance.

orange. However, in the case of *kinampay*, the time of addition of puree greatly affect the acceptability of the product. The addition of *kinampay* puree before and after incubation yielded a product with color range falling in pinkish purple to pinkish white and light purple to purple respectively. The product with *kinampay* puree, which was added after incubation was the most acceptable in terms of all sensory parameters, used in the evaluation. While the product added with *kinampay* puree before incubation was less acceptable in terms of color and general acceptability. This is because the acid had an effect on the anthocyanin pigments since these pigments are affected by pH change. The low pH causes the purplish color to fade into pinkish to red (Hendry and Houghton, 1996). In general, the plain acidophilus milk was the

Table 4. Proximate composition of acidophilus beverage as affected by time of addition of puree using 6.25% sugar level.

| | Moisture Content | % Ash | % Protein |
|---|------------------|--------|-----------|
| RC2000 added before incubation | 72.07bc | 0.52cd | 6.54ab |
| RC2000 added after incubation | 72.04bcd | 0.655a | 5.79c |
| <i>Kinampay</i> added before incubation | 70.28bcd | 0.55c | 6.67a |
| <i>Kinampay</i> added after incubation | 70.28bcd | 0.64ab | 5.58cd |
| Plain Acidophilus Milk | 82.70a | 0.48cd | 4.83 e |

Values followed by same letter do not significantly differ from each other at 5% level of significance.

least acceptable of all the products tested in terms of color, taste, after-taste and general acceptability.

Proximate composition of acidophilus milk

Addition of sweetpotato puree to the fermented milk changes the proximate composition of the product. In terms of moisture content, the products added with puree have significantly lower moisture than the product without puree (Table 4). However, the time of addition of puree did not affect the moisture of the product.

In terms of the ash content, the product, which was added with RC2000 and *kinampay*, purees after incubation have the highest amount of ash. This is because some of the minerals are destroyed during the fermentation process. Some water-soluble minerals are lost during the fermentation process because they are utilized by the microorganisms associated with the fermentation process. (Jones, 1975).

In terms of the protein content of the product, it was found out that the addition of puree to the fermented milk significantly increases its protein content. Moreover, the time of addition of puree to the fermented milk also influenced the product protein content. High amount of protein was observed in the product when the sweetpotato puree was added before incubation. These results clearly show that fermentation increases the protein content of the product. L-isomers of amino acids are produced during fermentation (Jones, 1975).

CONCLUSION AND RECOMMENDATION

Based on the results of the study, it can be concluded that the addition of sweetpotato puree to the acidophilus milk improves the sensory qualities and nutritional values of the acidophilus milk.

It is therefore recommended that further study should be conducted to monitor the stability of the pigments especially anthocyanin in acidophilus beverage, shelf life of the product and to incorporate other nutritious varieties of sweetpotato in acidophilus milk.

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