

Production of a Probiotic Soy-Soursop Yogurt Containing *Lactobacillus rhamnosus* yoba 2012

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ABSTRACT

There is an increasing demand for functional food products developed from local resources. In this work, *Lactobacillus* (*Lb.*) *rhamnosus* yoba 2012 and *Streptococcus thermophilus* were used to produce a soy-soursop probiotic yogurt. Soy milk was supplemented with soursop pulp in amounts of 0%, 5%, 10%, and 15%. The mixtures were pasteurized at 85 °C for 15 s, cooled, inoculated with a starter and incubated at 45 °C for 24 h. Samples were taken at 0, 2, 4, 6 and 24 hours for analysis of pH, acidity, and *Lb. rhamnosus* yoba 2012 counts. Consumer acceptability and willingness to purchase were determined after 24 h of fermentation. The most acceptable sample was analyzed for nutritional composition. *Lb. rhamnosus* grew in the soy-soursop yogurt up to 8.1-9.3 log cfu/mL. The highest cell growth was observed in yogurt containing 5% soursop whereas the lowest was observed in yogurt containing 15% soursop. Yogurt pH reduced to 3.9-4.4, with 15% soursop having the lowest pH ($p < 0.05$). Titratable acidity increased to 0.6%-0.9% lactic acid. All the yogurts were accepted although those containing 15% soursop had the lowest scores (5 = *neither like nor dislike*). There were no yeasts, molds or coliforms detected during the 21 days of cold storage (4 °C) of all the yogurt. Therefore, adding soursop to soymilk produces a satisfactory probiotic soy-soursop yogurt.

Keywords: *Lb. rhamnosus*, Probiotic, Soursop, Soy, yogurt.

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I. INTRODUCTION

Probiotics are live microorganisms that, when ingested in sufficient quantities, provide health benefits to the host [1]. Probiotic strains include bacteria of the genera *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Bacillus*, and the yeast *Saccharomyces boulardii* [2]. These probiotics provide health benefits such as immune system modulation, infant eczema reduction, lowering serum cholesterol, suppressing the growth of pathogenic microbes, relieving constipation, and preventing or managing various gastrointestinal illnesses, including different types of diarrhea [2].

Lactobacillus (*Lb.*) *rhamnosus* GG is a well-studied probiotic bacteria. It has been shown to help treat gastrointestinal disorders such as rotavirus-associated diarrhea, travelers' diarrhea, and *Clostridium difficile* colitis [2]-[4]. Kort and Sybesma [5] used the concept of "generic probiotics" to make LGG, in the form of *Lb. rhamnosus* yoba 2012, available in Africa. Since dairy products are commonly used to deliver probiotic microbial strains to consumers [6], *Lb. rhamnosus* yoba 2012 is conventionally mixed with *Streptococcus thermophilus* C106. The latter helps in lactose hydrolysis for easy utilization by the *Lb. rhamnosus* strain

[7]. However, plant-based alternative products are gaining popularity due to their availability, cost-effectiveness, lactose-free nature, and appeal to vegans. In addition to dairy, *Lb. rhamnosus* yoba 2012, has been successfully tested for fermentation of various products like *Uji*, *Mutandabota*, *Zomkom*, *Kwete*, and *Obushera* [7]-[9].

This study evaluated the use of a plant-based soy-soursop yogurt as a potential vehicle for *Lb. rhamnosus* yoba 2012 for a number of reasons. Soybean (*Glycine max*) (L) Merr, is economically the most important bean in the world, providing vegetable protein for millions of people and is a source of bioactive peptides [10]. Soybean contains about 40% protein and has saponins which enhance immune function whilst binding cholesterol to limit its absorption in the intestine [10]. Soy milk is a suitable economical substitute for cow's milk in addition to being an ideal nutritional supplement for lactose-intolerant people [11]. Fermenting soy improves the bioavailability of isoflavones, assists in the digestion of protein, provides more soluble calcium, enhances intestinal health, and supports the immune system [12]. Isoflavones have several health benefits, including cardiovascular protection, osteoporosis prevention, lowering the risk of some cancers, antioxidant properties, antiviral and hepatoprotective capabilities, and hepatoprotective activity

[13]. Soursop (*Annona muricata* L.) is a fruit native to tropical North and South America [14]. The fruits have a unique pleasant, subacid and aromatic flavor, but in their fresh form are not as popular as other tropical fruits [15]. Soursop fruit is a good source of fiber and bioactive compounds such as polyphenols possessing nutraceutical properties [16]. The ripe fruits are highly perishable [15]. Soy milk and soursop can be combined and fermented into a yogurt using the *Lb. rhamnosus* yoba 2012 thus tapping into the health benefits of these three. However, the ability of *Lb. rhamnosus* yoba 2012 to ferment the product, its effect on acceptability and stability ought to be evaluated.

Therefore, the purpose of this study was to develop a probiotic yogurt from soy and soursop. The study also evaluated the survival of the probiotic *Lb. rhamnosus* yoba 2012 during a storage period of 21 days. This study illustrates the potential of incorporating soursop in soy milk and utilizing the blend as a carrier for probiotics.

II. MATERIALS AND METHODS

A. Materials

Fresh fully ripe soursop (*Annona muricata*) fruits and soybeans (local Ugandan variety) were purchased from Owino market, Kampala, Uganda.

B. Preparation of Soursop

The fruits were washed, hand-peeled, deseeded, cored and pulped. Pulp was added to water in a ratio of 1:4 [17] and blended using a blender (Saachi, model: NL-BL-4361, UAE) at highest speed.

C. Preparation of Soy Milk

Soybeans were sorted to remove the diseased, defected and extraneous matter. These were then soaked in water at room temperature (25 °C) for 12 h in a volume which was twice that of the soybeans. The soaked beans were drained and blanched at 95 °C for 15 min to reduce the beany flavor [18]. Soy milk was extracted by adding five parts of boiling water and subsequently sieving with a muslin cloth [19].

D. Preparation of Soy-soursop Yogurt

The soy-soursop yogurt was processed in four different batches with each having a varying concentration of soursop pulp (0%, 5%, 10% and 15%) as shown in Table I. Corn Starch (Bholenath, India), was added as a stabilizer at a rate of 1%, to a mixture containing 10% sugar (Kakira Sugar, Uganda) and 1L of the soy-soursop milk. The mixture was heated to 85 °C and held for 15 s [20]. The soy-soursop mixture was thereafter cooled to 45 °C gradually for over 15 min. The cooled soy-soursop blend was then inoculated with the starter culture comprising of *Lb. rhamnosus* yoba 2012 and *Streptococcus thermophilus* C106 (Yoba for Life Foundation, Amsterdam, The Netherlands) following manufacturer's instructions. One gram of the probiotic starter

was added to 1 L of soy-soursop milk followed by incubating at 45 °C for 24 h. Fermentations were carried out in triplicate.

E. Evaluating Fermentation Characteristics of *Lb. Rhamnosus* Yoba 2012

Soy-soursop yogurt was serially diluted in sterile quarter Strength Ringer's solution. The *Lb. rhamnosus* yoba 2012 counts were determined by pour plating selected serial dilutions in de Man Rogosa and Sharpe agar (Merck KGaA, Darmstadt, Germany). Petri dishes were incubated at 30 °C for 48 h. Titratable acidity, expressed as percentage lactic acid, was determined by titrating 10 mL of the sample against a standardized solution of 0.1 M NaOH with phenolphthalein as the indicator [21].

F. Sensory Evaluation and Purchase Intent of Soy-soursop Yogurt

An untrained panel (n = 30) consisting of students from the School of Food Technology, Nutrition and Bioengineering, Makerere University, Kampala, Uganda was used to evaluate the acceptability of the yogurt. The yogurt had previously been stored at 4 °C for 2 h prior to sensory evaluation. Panelists ranked the acceptability of various attributes using a nine-point hedonic scale (9-like extremely, 5-neither like nor dislike and 1-dislike extremely). Panelists were also asked to indicate their willingness to purchase the different soy-soursop yogurt by responding to the statement: "I would regularly purchase this product". A five-point Likert scale (1 – strongly disagree, 2 – disagree, 3 – not decided, 4 – agree and 5 – strongly agree) was used to evaluate willingness to purchase. Bottled water was used to rinse the palate in between sample tastings.

G. Evaluating the Shelf Stability of Probiotic Soy-soursop Yogurt

The shelf stability of soy-soursop yogurt was evaluated based on pH and microbial counts (total coliforms, yeasts and molds). The yogurt was stored at a mean temperature of 4°C and analyzed at weekly intervals for up to 3 weeks. Samples were serially diluted in sterile quarter Strength Ringer's solution. Coliform counts were determined by pour plating selected serial dilutions in Violet Red Bile Lactose Agar and incubating at 37 °C for 24 h [22]. Yeast and mold counts were determined by surface spreading selected serial dilutions on pre-poured Potato Dextrose Agar containing 1% lactic acid. Petri dishes were then incubated at 30 °C for 3 days [23]. Microbiological media were obtained from Merck KGaA (Darmstadt, Germany). The pH of the yogurt was measured using a pH meter (Cyberscan pH 110 with RS232, Thermo Fisher Scientific Inc. Monza, Italy).

H. Evaluating the Nutritional Composition of the Probiotic Soy-soursop Yogurt

Moisture, ash, crude fiber, carbohydrate, crude fat, and protein content of the most acceptable yogurt were analyzed. All analyses were carried out in triplicate. Moisture, ash, crude protein (N (%) × 6.25), dietary fiber and crude fat content were determined using the oven drying method [21], muffle furnace [21], Kjeldahl method [24], Acid Detergent Fiber method [21] and Soxhlet method [21], respectively. Total carbohydrate content was calculated as the difference between 100 and the sum of the percentages of crude protein,

TABLE I: FORMULATIONS USED FOR MAKING PROBIOTIC SOY-SOURSOP YOGURT

Treatment	Soursop (mL)	Soy milk (mL)
0%	0	1000
5%	50	950
10%	100	900
15%	150	850

fat, moisture, and ash [25].

I. Statistical Analysis

Results were expressed as means $\pm$ standard deviation. Results for consumer acceptability scores, purchase index and changes in growth of *Lb. rhamnosus* yoba 2012 in the different yogurt samples were analyzed using one-way Analysis of Variance. Differences were considered significant at $p < 0.05$. The Least Significant Different was used for mean separation. SPSS software version 29.0.0.0, IBM, New York, USA was used.

III. RESULTS AND DISCUSSION

A. Fermentation Characteristics of *Lb. rhamnosus* yoba 2012 in the Soy-soursop Mixture

The counts of *Lb. rhamnosus* yoba 2012 are shown in Fig. 1. The probiotic propagated well (starting from 3 log cfu/mL) in all treatments with 5% soursop treatment having the highest cell counts while 15% soursop had the lowest cell counts. Addition of soursop affected the growth of *Lb. rhamnosus* yoba 2012. Yogurt containing 15% soursop had a significantly lower growth of the *Lb. rhamnosus* yoba 2012 than that containing 0% soursop and 5% soursop. However, it was not significantly different from the yogurt containing 10% soursop.

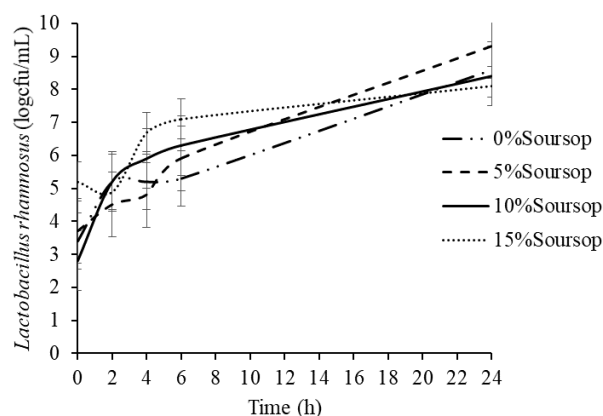


Fig. 1. Growth of *Lb. rhamnosus* yoba 2012 during fermentation of soy-soursop. Error bars show standard deviations of three independent fermentations.

The addition of low volumes of soursop positively influenced the growth of *Lb. rhamnosus* yoba 2012 possibly due to the prebiotic properties of the soursop [26]. However, an increase in the amount of soursop reduced the growth of *Lb. rhamnosus* yoba 2012 after 24 h probably due to increased acidity. This is in agreement with Yáñez *et al* [27] who reported that increased acidity, especially resulting from the fermentation processes, can reduce the survival and viability of probiotic bacteria. According to Hayakawa *et al*. [28], the accumulation of lactic acid in batch fermentation limits the maximal growth of *Lactobacillus*. The exponential growth phase of the *Lb. rhamnosus* yoba 2012 (Fig. 1) coincided with the sharp drop in pH and a correspondingly sharp increase in titratable acidity (Fig. 3).

According to Luckow and Delahunty [29], it is important to have a significant number of viable probiotic microorganisms in the product for maximum health benefits. At the end of the

fermentation, all the yogurt samples had more than the recommended probiotic microbial cells (minimum of 6 log cfu per mL or gram) required for products to offer probiotic benefits [30]. Daily Intake of 100-1,000 mL of such a product provides the recommended daily dose (8-9 log cfu) sufficient for realizing probiotic effects [31, 30].

B. Changes in Acidity of the Soy-soursop Drink during Fermentation

The pH reduced during fermentation from 6.0-6.4 to 3.9-4.3 (Fig. 2). Titratable acidity increased from 0.3-0.4% to 0.6-0.9% lactic acid (Fig. 3). Lactic acid production was concomitant with the reduction in pH and the samples attained a pH of ≤ 4.5 in 12-16 h.

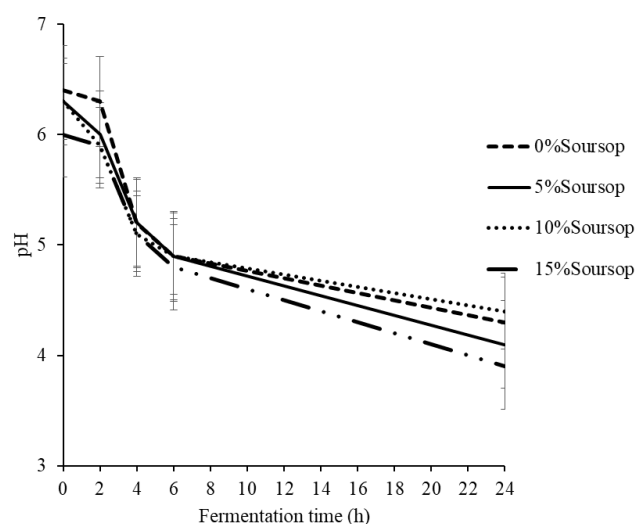


Fig. 2. Changes in pH during fermentation of the soy-soursop drink with *Lb. rhamnosus* yoba 2012. Error bars show standard deviations of four independent fermentations.

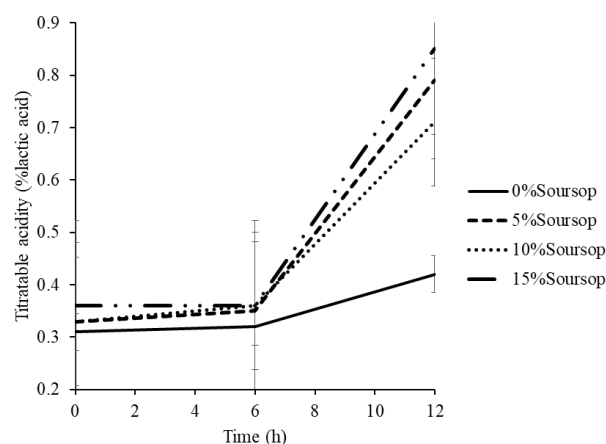


Fig. 3. Changes in titratable acidity during the first 12 hours of fermentation of soy-soursop drink.

The changes in pH and acidity were due to the fermentation that led to conversion of sugars by *Lb. rhamnosus* yoba 2012 to lactic acid [32]. These changes were similar to trends observed by Mukisa and Birungi [33] in dairy yogurt containing bananas. The changes in pH were also in agreement with Kort *et al*. [7] who reported a reduction in pH during fermentation by *S. thermophilus* and *Lb. rhamnosus* yoba 2012 in dairy milk. After 24 hours of fermentation, the pH of all the soursop yogurt treatments had reduced to values less than 5 with 15% soursop yoghurt having the lowest pH.

The soursop pulp before addition of soy had a pH of 3.8. The pH of the yogurt containing 15% soursop after 24 h was lower than 4.2, which is the minimum recommended pH by the Uganda Standards and East African Standards for yogurts [34]. Other researchers also reported an increase in titratable acidity and a decrease in pH in yogurt with added fruits [33], [35].

The acidity of soy-soursop yogurt increased to more than 0.6% lactic acid which meets the acidity requirements for East African Standards for yogurt [34]. The acidity values are in agreement with the maximum levels of acid production observed when using *Lb. rhamnosus* yoba 2012 in the fermentation of dairy yogurt containing banana [33].

C. Consumer Acceptability and Purchase Indices of Probiotic Soy-soursop Yogurt

The sensory acceptability scores of the yogurt are summarized in Table II. It is important to determine the consumer acceptability of novel probiotic products because probiotic starters may affect their sensorial properties and acceptability [36]. The overall acceptability scores varied from 5 (*neither like nor dislike*) in 15% soursop yogurt to 7 (*like moderately*) in plain soy yogurt. According to Hasimah and Faridah [37], average scores for each quality attribute above 5.0 are acceptable. Therefore, all the soy-soursop yogurt treatments were accepted by panelists. However, the acceptability of 5%, 10% and 15% soursop yogurt were not significantly different from that of plain soy yogurt with respect to appearance, taste, mouthfeel, and overall acceptability. All samples had a purchase index of 3 indicating that the consumers were not decided on whether they would regularly purchase the products. This is possibly due to the fact that most yogurt consumers in Uganda are more familiar with dairy yogurt.

The appearance, taste and acceptability of all the yogurt treatments were not significantly different which agrees with Lutchmedial *et al.* [15] who reported that the addition of varying levels of soursop did not affect the acceptability of yogurt.

However, aroma scores were not significantly different with an increase in soursop concentration. This differs from Lutchmedial *et al.* [15] who reported higher scores in aroma with an increase in soursop concentration. This is also the same for the overall acceptability where the plain soy yogurt had the highest acceptability scores. This could be due to the difference in varieties, harvesting period and ecology of the soursop fruits [38].

D. Shelf Stability of the Probiotic Soy-soursop Yogurt

Coliforms, yeasts, and molds were not detected in the soy-soursop yogurt during the three weeks of storage at 4 °C. The soy-soursop yogurt thus met the microbial quality standards

as stated by the ISO standards for yogurt [39]. The trend in pH of the yogurt during storage is shown in Table III.

TABLE III: CHANGES IN pH VALUES OF SOY-SOURSOP DURING STORAGE AT 4°C

% Soursop	Week 0	Week 1	Week 2	Week 3
0	4.4 ^a ±0.0	4.4 ^a ±0.0	4.4 ^a ±0.0	4.4 ^a ±0.0
5	4.3 ^b ±0.0	4.3 ^b ±0.0	4.3 ^b ±0.0	4.3 ^b ±0.0
1	4.2 ^c ±0.0	4.1 ^c ±0.0	4.1 ^c ±0.0	4.1 ^c ±0.0
15	3.9 ^d ±0.0	3.9 ^d ±0.0	3.9 ^d ±0.0	3.9 ^d ±0.0

Values are means ± standard deviation. Mean values in the same column with similar superscripts are not significantly different ($p > 0.05$).

The slight drop in pH during storage could have been due to continued production of lactic acid by the *Lb. rhamnosus* yoba 2012 [40]. The change was, however, slight due to the effect of refrigeration in slowing microbial growth and biochemical processes [41]. A pH less than 4.5 is recommended for ensuring the microbiological safety and stability of lactic acid-fermented beverages [42]. All products met this requirement.

IV. CONCLUSION

This study illustrates the potential for developing a functional probiotic beverage from soy and soursop using *Lb. rhamnosus* yoba 2012. The probiotic culture was able to ferment soy-soursop juice to produce a yogurt with pH <4.5 at 45 °C in 12-16 h. The product was acceptable and remained stable during refrigerated storage for three weeks. Considering the *Lb. rhamnosus* yoba 2012 counts observed in the study (above 8 log cfu/mL), consuming 10 mL/day of the probiotic soy-soursop yogurt would be sufficient to meet the recommended daily intake of probiotics.

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DISCLOSURE OF CONFLICT OF INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article. All the authors have consented to the publication.

DECLARATIONS

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TABLE II: CONSUMER ACCEPTABILITY SCORES AND PURCHASE INDICES OF THE PROBIOTIC SOY-SOURSOP YOGURT

% Soursop	Appearance	Aroma	Taste	Mouthfeel	Overall acceptability	Purchase index
0	7 ^a ±2	6 ^a ±2	6 ^a ±2	7 ^a ±2	7 ^a ±2	3 ^a ±1
5	7 ^a ±2	5 ^b ±3	5 ^a ±2	5 ^a ±2	6 ^a ±2	3 ^a ±1
10	7 ^a ±2	5 ^b ±2	5 ^a ±2	5 ^a ±2	6 ^a ±2	3 ^a ±1
15	6 ^a ±2	5 ^b ±2	5 ^a ±2	5 ^a ±2	5 ^a ±2	3 ^a ±1

Data are presented as means ± standard deviations ($n=30$ panelists). Values in the same column with similar superscripts are not significantly different ($P > 0.05$).

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