

PROXIMATE COMPOSITION, ACCEPTABILITY AND STABILITY OF PROBIOTIC DAIRY YOGHURT CONTAINING COOKING BANANA/MATOOKE PUREE AND *Lactobacillus rhamnosus* yoba

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doi: 10.15414/jmbfs.2018.7.4.343-347

ARTICLE INFO

Received 20. 6. 2017
Revised 19. 10. 2017
Accepted 20. 11. 2017
Published 1. 2. 2018

Regular article



ABSTRACT

Cooking bananas/*matooke* are eaten in the unripe state but quickly ripen leading to postharvest losses. The purpose of this study was to develop fruit-based probiotic dairy yoghurt using ripe *matooke* and *Lactobacillus rhamnosus* yoba. Yoghurt mixtures containing varying proportions of *matooke* (0%, 5%, 7.5% and 10%) were fermented at room temperature for 32 hours. Acidity, pH and counts of *Lactobacillus rhamnosus* were determined during fermentation and storage (4°C for three weeks). Consumer acceptability was determined at weekly intervals. *Lactobacillus rhamnosus* counts increased from 7 – 9 log cfu.g⁻¹ after 24 hours of fermentation. Banana accelerated the acidification rate with banana yoghurts attaining pH = 4.4 – 4.5 and acidity of 0.60 – 0.67% in 24 hours. The control took 32 hours to attain a pH = 4.5. Ripe cooking banana introduced a slight banana aroma but did not significantly ($p > 0.05$) affect consumer acceptability. Yoghurt containing 7.5% banana was most acceptable and contained: 148 kcal 100 g⁻¹, 76.81% moisture, 12.37% carbohydrates, 6.18% protein, 3.35% fat, 0.77% ash and 1.04% calcium. Yoghurt with 7.5% banana was stable at 4°C for 21 days and maintained viable counts above the target of log 6 cfu.g⁻¹. This study showed that an acceptable fruit based probiotic yoghurt can be produced by adding ripe banana to the yoghurt mix.

Keywords: Fruit yoghurt; cooking banana; *matooke*; *Lactobacillus rhamnosus* yoba; Probiotic

INTRODUCTION

Probiotics are ‘live microorganisms which when administered in adequate amounts confer a health benefit to the consumer’ (FAO/WHO, 2002). There is increasing global interest in the use of probiotics owing to the range of associated health benefits. *Lactobacillus rhamnosus* GG (LGG) is a widely studied probiotic that is beneficial in the treatment of gastrointestinal diseases (Guandalini, 2011; Horvath *et al.*, 2011). LGG reduces the duration of rotavirus-associated diarrhea, a major health concern in children in Uganda and several developing countries (Guandalini, 2011; Nakawesi *et al.*, 2010). However, access to affordable probiotics and acceptable food carriers are major challenges for adoption of probiotics in Africa (Franz *et al.*, 2014). Generic forms of probiotics, such as *Lactobacillus rhamnosus* yoba (a generic form of LGG), can be used to increase access to probiotics in Uganda and other developing countries (Kort and Sybesma, 2012). Locally consumed foods in Uganda, such as yoghurt and banana, can be evaluated as probiotic carriers for *Lactobacillus rhamnosus* yoba. Yoghurt is a product of the lactic fermentation of milk by starter cultures, resulting in a pH drop to ≤ 4.6 (Tamime, 2002). *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* are the major symbiotic starter cultures used (Codex Alimentarius, 2003). A mixed starter culture of *Lactobacillus rhamnosus* yoba and *Streptococcus thermophilus* C106 has also been used to ferment dairy yoghurt, *Mutandabota* (baobab pulp and milk), and cereal beverages such as *Obushera*, *Uji* and *Zom Kom* (Kort *et al.*, 2015). *Lactobacillus rhamnosus* yoba is unable to degrade lactose and casein in milk while *Streptococcus thermophilus* C106 has the ability and thus inclusion of the latter facilitates growth of the probiotic in dairy products (Kort *et al.*, 2015). Yogurt is among the most common dairy products consumed around the world (Saint-Eve *et al.*, 2006) and is also commonly used as a probiotic carrier (Franz *et al.*, 2014). In Uganda, yoghurt is popular amongst all social classes (Balikowa, 2011) and is thus a potentially acceptable carrier for probiotics targeting the Ugandan market. The Ugandan market has two types of yoghurt: set and stirred yoghurts, although the latter is more common (Mukisa and Kyoshabire, 2010). Most of the brands are flavored with synthetic flavorants while very few contain dried fruit pieces.

The demand for fruity yoghurts with different flavours has been on the rise because fruits and fruit flavours significantly increase yoghurt consumption among all age groups (Chandan, 2011). Fruit preparations are commonly added to yoghurt formulations at levels of 10 – 15%, which should ideally cause minimal or no negative effects on the product’s aesthetic appeal (Chandan, 2014).

The cooking banana/*matooke* (*Musa* spp. AAA-EA group) is extensively grown in Uganda (Karamura *et al.*, 1998). It is highly perishable and is generally eaten in cooked form before ripening (Karamura *et al.*, 1998). About 22 – 45% of *matooke* is lost during peak production seasons due to the rapid ripening, inadequate storage and transportation, and lack of alternative food processing options (Muranga *et al.*, 2010). Raw *matooke* has been evaluated for use in bread making (Muranga *et al.*, 2010) and in development of complementary foods (Muranga *et al.*, 2009; Muranga *et al.*, 2011). However, there are no known studies on the utilization of ripe *matooke* in any value addition processes such as production of fruit based dairy yoghurts. *Lactobacillus rhamnosus* yoba has previously been used to produce an acceptable fruit yoghurt-like product called *Mutandabota*, which contains 14% baobab fruit in milk (Mpofu, 2015).

The objective of this study was to utilize ripened *matooke* in the production of probiotic dairy yoghurt containing *Lactobacillus rhamnosus* yoba. We evaluated the effect of banana concentration on growth of *Lactobacillus rhamnosus* and acceptability of the yoghurt. Proximate composition and shelf stability of the most acceptable banana yoghurt formulation were also determined. Using ripe *matooke* in fruit yoghurt production can reduce postharvest losses of *matooke*. The probiotic banana yoghurt developed can be targeted at children to help prevent or manage diarrhea.

MATERIALS AND METHODS

Milk, sugar and banana

Pasteurized milk (Jesa Dairy Ltd, Uganda) and brown sugar (Kakira Sugar Ltd, Jinja Uganda) were purchased from Tusky’s supermarket, Makerere, Kampala. Fresh unripe *matooke* were purchased from the Kasubi market, Kampala and allowed to ripen at room temperature for five days.

Starter culture

The probiotic starter culture comprising of *Lb. rhamnosus* yoba 2012 and *Streptococcus thermophilus* C106 (Kort et al., 2015) was obtained from the Yoba for Life Foundation (Amsterdam, The Netherlands) and stored at -40°C prior to subsequent use. The starter culture was prepared by following the manufactures instructions. One liter of milk was pasteurized at 85°C for 15 minutes, cooled to 45°C, inoculated with 1 g of starter culture and incubated for 12 hours in a thermos flask.

Production of probiotic fruit yoghurt containing ripened cooking banana

Control yoghurt and banana yoghurt were produced by mixing milk, ripe cooking banana and sugar in different proportions (Table 1). The ingredients were blended to a uniform mixture using a blender (Sayona, Patisseries, China). The mixture was then pasteurized at 85°C for 15 minutes and cooled to 45°C using a cold water bath prior to prior to inoculation with 2% freshly prepared starter culture. Incubation was carried out at room temperature. Samples were taken at t = 0, 6, 12, 24 and 32 hours during fermentation for analysis. At the end of fermentation the probiotic yoghurt was stored at 4°C for a period of 3 weeks. Samples were taken weekly intervals to determine product stability and viability of the probiotic starter. Two independent fermentations were carried out for each formulation.

Table 1 Formulations used for preparing banana yoghurts containing ripe cooking banana

Yoghurt formulation	Amount of different raw materials (g)		
	Milk	Ripe cooking banana	Sugar
0% banana – control	900	0	100
5% banana	850	50	100
7.5% banana	825	75	100
10% banana	800	100	100

Analyses

Physico-chemical analyses

The pH of yoghurt was determined using a digital pH meter (Dr. Meter, model pH-100, Yantai Dongrun Instrument Co. Ltd, Shandong, China) calibrated with standard buffers of pH = 4.00 and 6.86. Total soluble solids (°Brix at room temperature) were determined using a hand refractometer (RSG-100/ATC, Sino Science and Technology Co. Ltd, Zhangzhou, China). Total titratable acidity was determined by titrating 10 ml of each sample against standardized 0.1 M sodium hydroxide (AOAC, 1995).

Proximate composition

Proximate composition was only determined for the most acceptable formulation and the control yoghurt. Dry matter was determined using the oven method at 100°C for 24 hours (AOAC, 2000). Ash content was determined by igniting in a muffle furnace at 550 °C for 6 hours (AOAC, 2000). Crude protein was determined by the Kjeldahl method (AOAC, 2000) using a conversion factor of

6.38 (Codex Alimentarius, 2003). Total Carbohydrate was determined using the phenol-sulfuric acid for total carbohydrate (AOAC, 2000). Crude fiber was determined following acid and alkali digestion (AOAC, 2000). Crude fat was determined using Soxhlet extraction while gross energy content was determined by oxygen bomb calorimeter (Gallenkamp Autobomb, UK) (AOAC, 2000).

Microbiological analyses

Serial dilutions of the yoghurt samples were prepared using ¼ strength ringer’s solution (Merck KGaA, Darmstadt, Germany). *Lactobacillus rhamnosus* counts were determined by plating selected dilutions in De Man Rogosa Sharpe agar (Laboratorios Conda, Asiagel, Spain) and incubating anaerobically at 37°C for 2 days. Yeasts and molds counts were determined after plating in Potato Dextrose Agar (Laboratorios Conda, Asiagel, Spain) and incubating at room temperature for 5 days. Microbial counts were determined at 0 and 24 hours of fermentation and at 0, 1, 2 and 3 weeks during refrigeration storage at 4°C.

Sensory evaluation

An untrained consumer panel (n=40) was used to determine the consumer acceptability of the four different formulations of banana yoghurt. Sensory evaluation was carried out in individual booths in the sensory evaluation laboratory at the Department of Food Technology and Nutrition, Makerere University. Panelists ranked acceptability of various sensory attributes using a 9 point hedonic scale (9 =like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely). Panelists also expressed their willingness to regularly purchase each of the yoghurt formulations using a 5 point Likert scale (5 = strongly agree, 4 = agree, 3 = not decided, 2 = disagree, 1 = strongly disagree). To evaluate the shelf life, a panel of 10 members was used to assess any changes in score the acceptability of different attributes and willingness to purchase the yoghurt as described earlier.

Statistical analysis

Means of two treatments were subjected to a two sample t-test while means of more than two treatments were subjected to one way analysis of variance (ANOVA) to test for significant differences at a 5% level of significance. The least significant difference test (Fisher’s LSD) was used to determine means that were significantly different from one another after the ANOVA test. All statistical analyses were performed by XLSTAT (2010, Addinsoft, Paris, France).

RESULTS

Fermentation of banana yoghurts by *Lactobacillus rhamnosus* yoba

Lactobacillus rhamnosus yoba propagated well and fermented all four banana yoghurt formulations used in this study. Cells counts increased significantly (p < 0.05) from about 7 log cfu.g⁻¹ to 9 log cfu.g⁻¹ after 24 hours of fermentation (Table 2). Yeasts and moulds were not detected in any of the samples.

Table 2 Changes in *Lactobacillus rhamnosus* yoba counts during fermentation of yoghurt containing different concentrations of ripe cooking banana/matooke

Time (h)	Cell counts (Log cfu.g ⁻¹ in different formulations of banana yoghurt)			
	0% banana – Control	5% banana	7.5% banana	10% banana
0	7.22 ± 0.04 ^a	7.25 ± 0.18 ^a	7.13 ± 0.18 ^a	7.21 ± 0.27 ^a
24	9.81 ± 0.16 ^b	9.50 ± 0.58 ^b	9.94 ± 0.03 ^b	9.56 ± 0.59 ^b
p-value	0.002	0.034	0.002	0.036

Results are means ± standard deviations of two independent fermentations. Means in the same column with different superscripts (a and b) are significantly different (p < 0.05).

Growth of *Lb. rhamnosus* yoba in banana yoghurts was accompanied by a decrease in pH from 6.13 to 4.32 – 4.49 and increase in acidity from 0.22 to 0.64 – 0.66% after 32 hours of fermentation (Figure 1). Increasing banana concentration in the formulation significantly (p<0.05) increased the rate of acidification. However, the final acidity of the four yoghurt formulations was not

significantly (p>0.05) different. Yoghurt with 10% banana attained a pH of less than 4.5 in 24 hours.

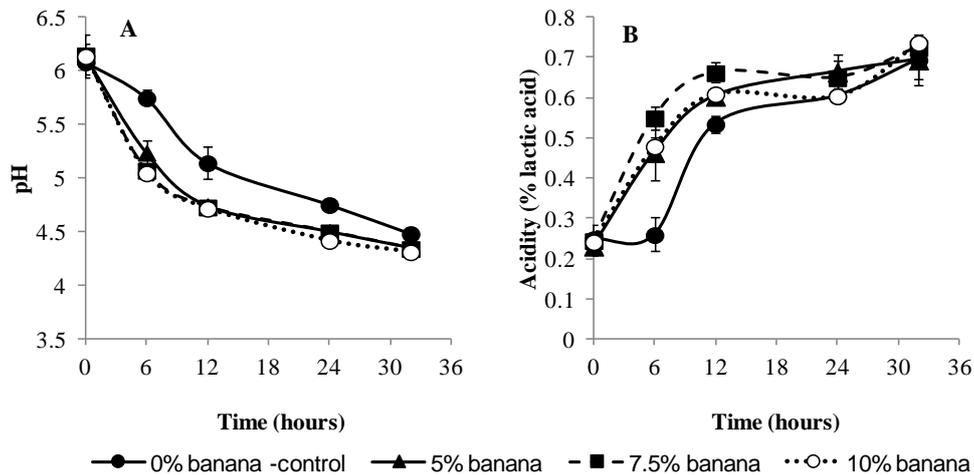


Figure 1 Changes in pH (A) and acidity (B) during the fermentation of banana yoghurts. Error bars show standard deviations of two independent fermentations.

Effect of banana concentration on acceptability of banana yoghurts

Generally all the banana yoghurt formulations were accepted by the consumers (Table 3). Banana concentration significantly ($p < 0.05$) affected the acceptability scores of appearance, taste, overall acceptability and the purchase index. Yoghurt with 7.5% banana received the highest scores for all parameters evaluated. The

purchase index, appearance and taste scores of yogurts containing 5% and 7.5% banana were not significantly different from those of control yoghurt. Addition of banana had no significant ($p < 0.05$) effect on the acceptability of aroma and mouthfeel. Panelists generally noted that the banana yogurt samples had a slight banana aroma, were relatively thick and smooth.

Table 3 Effect of ripe banana on the acceptability and purchase index of yoghurt

Yoghurt formulation	Acceptability scores					Purchase Index
	Appearance	Taste	Aroma	Mouthfeel	Overall Acceptability	
0% banana- Control	5.8±1.8 ^b	6.2±1.5 ^{ab}	6.3±1.4 ^a	5.8±1.8 ^a	6.1±1.5 ^{ab}	3.2±1.2 ^{ab}
5% banana	6.6±1.4 ^{ab}	6.3±1.9 ^{ab}	6.3±1.5 ^a	6.4±1.7 ^a	6.6±1.4 ^{ab}	3.4±1.1 ^{ab}
7.5% banana	7.1±1.2 ^a	6.7±1.6 ^a	6.3±1.8 ^a	6.7±1.7 ^a	6.8±1.5 ^a	3.8±1.2 ^a
10% banana	6.1±1.8 ^b	5.7±1.8 ^b	5.6±2.0 ^a	5.9±1.8 ^a	5.8±1.4 ^b	3.0±1.4 ^b
p-value	0.002	0.053	0.205	0.091	0.018	0.037

Values are means ± standard deviations (n = 40 panelists). Values in the same column with different superscripts (a, b) are significantly different ($p < 0.05$). For acceptability scores the interpretation of the anchors on the 9 point hedonic scale used are as follows: 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely. A 5 point Likert scale was used for the purchase index/willingness to buy (anchors: 1 – strongly disagree, 2 – disagree, 3 – not decided, 4 – agree and 5 – strongly agree).

Effect of addition of ripe banana on the nutritional composition of yoghurt

The nutrition composition of the most acceptable banana yoghurt formulation (7.5% banana) was compared to that of control yoghurt (Table 4). Addition of banana up to 7.5% did not significantly ($p > 0.05$) affect the energy content, carbohydrate, protein, fat, fiber, ash and calcium content of yoghurt but caused a slight and significant ($p < 0.05$) reduction in moisture content.

Table 4 Effect of ripe banana on the nutrient composition of yoghurt

Component	Content of nutrients in yoghurt		p-value
	0% banana – control	7.5% banana	
Energy (Kcal.100 g ⁻¹)	129.19±7.27 ^a	148.00±0.65 ^a	0.068
Moisture (%)	77.63±0.10 ^a	76.81±0.09 ^b	0.013
Carbohydrates (%)	10.73±0.75 ^a	12.37±0.06 ^a	0.092
Protein (%)	5.95±0.001 ^a	6.18±0.14 ^a	0.142
Fat (%)	3.71±0.18 ^a	3.35±0.11 ^a	0.145
Fiber (%)	0.00 ^a	0.07±0.05 ^a	0.194
Ash (%)	0.73±0.08 ^a	0.77±0.09 ^a	0.731
Calcium (%)	1.07±0.00 ^a	1.04±0.02 ^a	0.127

Results are means ± standard deviations of two independent fermentations. Values in the same row with the same superscripts (a, b) are not significantly different ($p > 0.05$).

Shelf stability of probiotic yoghurt containing 7.5% ripe banana

Probiotic banana yoghurt was generally stable during storage at 4°C for three weeks in terms of pH, acidity and overall consumer acceptability (Table 5). Values of pH (4.32 – 4.49), acidity (0.61 – 0.81) and overall acceptability (7.3 – 7.7) did not vary significantly ($p > 0.05$). Cell counts of *Lb. rhamnosus* yoba

reduced significantly (9.95 – 6.25 log cfu.g⁻¹) during storage but remained above 6.0 log cfu.g⁻¹. Yeasts and moulds were not detected in any of the samples.

Table 5 Changes in pH, titratable acidity, microbial counts and overall acceptability of probiotic yoghurt containing 7.5% ripe banana during storage at 4°C

Storage time (weeks)	pH	Acidity (% lactic acid)	<i>Lb. rhamnosus</i> cell counts (Log cfu.g ⁻¹)	Overall acceptability score
0	4.49±0.01 ^a	0.65±0.04 ^a	9.95±0.03 ^a	7.7±0.9 ^a
1	4.32±0.06 ^a	0.81±0.10 ^a	7.34±0.28 ^c	7.6±0.9 ^a
2	4.34±0.01 ^a	0.70±0.04 ^a	8.07±0.03 ^b	7.4±1.0 ^a
3	4.38±0.01 ^a	0.61±0.03 ^a	6.25±0.15 ^d	7.3±0.8 ^a
p-value	1.000	1.000	< 0.0001	0.438

Results are means ± standard deviations of two independent fermentations. Values in the same column with different superscripts (a, b, c and d) are significantly different ($p < 0.05$).

DISCUSSION

The objective of this study was to develop acceptable and shelf stable probiotic fruit yoghurt containing ripened banana (*matooke*) and *Lactobacillus rhamnosus* yoba. *Matooke* was chosen because it is one of the major food crops grown in Uganda with post-harvest losses of up 22 – 45% (Muranga et al., 2010). There is increased interest in adding value to the cooking banana so as to minimize postharvest losses. The probiotic *Lb. rhamnosus* GG was primarily selected for two reasons: accessibility and health benefits. LGG is currently readily accessible in Uganda, and other countries in a generic form, *Lb. rhamnosus* yoba 2012 (Kort et al., 2015). Clinical studies have confirmed that LGG is beneficial in the treatment of gastrointestinal diseases including: rotavirus diarrhea, *Chlostridium*

difficile diarrhea, antibiotic induced diarrhea and travelers' diarrhea among others (Drisko *et al.*, 2003; Guandalini, 2011; Horvath *et al.*, 2011).

Growth of *Lactobacillus rhamnosus* yoba in banana yoghurts

Effectiveness of probiotic foods requires that the probiotic organism should be able to grow and survive in the selected carrier during processing, handling and storage (Franz *et al.*, 2014). *Lactobacillus rhamnosus* yoba inoculated in banana yoghurts grew by 2 log cycles reaching a maximum of log 9.94 cfu.g⁻¹ after 24 hours of fermentation at room temperature (21 – 25°C). *Lactobacillus rhamnosus* yoba also grew in *Mutandabota*, a dairy product containing 14% baobab pulp from 5.8 – 8.8 log cfu.ml⁻¹ in 24 hours at temperature of 23 – 37°C (Mpofu, 2015). *Lb. rhamnosus* grew in banana yoghurt because it was cultured with *S. thermophilus* C106 which has the ability to digest casein and lactose thus providing fermentable sugars to the former (Kort *et al.*, 2015). *Lb. rhamnosus* is also able to utilize sucrose, glucose and fructose (Hedberg *et al.* 2008) which are the major sugars in ripened banana (Marriott *et al.*, 1981).

With regards to effect of banana concentration on growth of *Lactobacillus rhamnosus*, all banana concentrations used in this study facilitated growth of the probiotic to the same extent as the control yoghurt. However, significantly faster rates of acidification were observed in samples containing banana puree. The increased rate of acidification could have resulted from an increase in concentration of fermentable sugars with increasing proportion of banana in the mixture. As a result, the banana yoghurts prepared in this study attained a pH < 4.6 and acidity of > 0.6% within 24 hours of fermentation at room temperature compared to the 32 hours taken by the control. An optimum pH of 4.1 – 4.4 and minimum acidity of 0.6% is recommended for yoghurts since these levels of acidity facilitate coagulum development and also prevent growth of spoilage and pathogenic microorganisms (Codex Alimentarius, 2003; Downes and Ito, 2001; Kroger, 1976).

Acceptability of banana yoghurt formulations

Yoghurt containing 7.5% banana received the highest acceptability and purchase index scores although these were generally not significantly different from the control. Yousef *et al.* (2013) also noted that yoghurt containing 7.5% banana pulp received higher acceptability scores than that containing 10%. The fact that acceptability scores for taste, aroma were not significantly different may be due to the fact that the banana flavor was 'slightly faint' as most panelists pointed out. This implies that ripened cooking bananas can be incorporated in yoghurt up to a level of 10% without negatively impacting on sensory appeal. However, in this study a concentration of 7.5% banana was selected for having had the highest acceptability score for appearance ($p < 0.05$). At 10% concentration of banana the yoghurt became too thick to drink.

Composition of banana yoghurt containing 7.5% banana puree

Addition of ripened cooking banana at a level of 7.5% did not significantly affect the proximate composition of yoghurt. The fat, ash and carbohydrate contents of banana yoghurts were similar to values previously reported for yoghurts on the Ugandan market (Mukisa and Kyoshabire, 2010). Protein content was twice as high as values previously reported by Yousef *et al.* (2013) but met the Codex requirement of a minimum value of 2.7% (Codex Alimentarius, 2003). The energy content of the yoghurt was higher than values of 79 Kcal per 100 g reported by McCance (2002). The high energy content in this study could be attributed to addition of up to 10% sugar in the yoghurts developed in this study. Banana yoghurt had a significantly lower moisture content than the control because inclusion of ripe cooking banana increased the total solids thus resulting in lower moisture content of yoghurt as previously observed by Yousef *et al.* (2013).

Shelf stability of probiotic banana yoghurt

The shelf life of yoghurt is determined by changes in physical, chemical and microbiological characteristics (Memiši *et al.*, 2014). Consumer acceptability, which is an important determinant of shelf life of yoghurt, is influenced by changes in the acidity, pH and sweetness (Cruz *et al.*, 2010; Zanzi and Jideani, 2012). These characteristics should remain relatively unchanged as was observed in the current study during the three weeks of storage at 4°C. Storage under refrigeration slows down biochemical processes thus ensuring product stability (Chandan 2014; Jay *et al.*, 2008). More importantly, for probiotics, the products should contain a minimum of log 6 cfu of the probiotic organism per ml or gram at the time of consumption (Tripathi and Giri, 2014). Consuming 100 – 1000 ml per day of such a product provides the recommended daily dose (log 8 – 9 cfu) essential for realizing the health benefits of probiotics (Knorr, 1998; Tripathi and Giri, 2014). A daily dose of 100 ml of the banana yoghurt developed in this study would provide between log 8 – log 11 cfu per day thus meeting the recommended daily dosage. The viable counts of *Lactobacillus rhamnosus* obtained in this study after three weeks of storage were below log 8 cfu.g⁻¹ that was reported by Hekmat, Soltan and Reid (2009) in yoghurt after 28 days of storage at 4°C. Hekmat *et al.*, (2009) attributed the high survival in their study to the addition of 0.33% yeast extract in the yoghurt. Yeast extract contains

high level of carbohydrates and can thus be used as a prebiotic agent to help promote bacterial growth (Hekmat *et al.*, 2009; Yousef *et al.*, 2013).

CONCLUSION

Lactobacillus rhamnosus yoba can be used to produce acceptable probiotic banana yoghurt at room temperature within 24 hours. The product remains stable for at least 3 weeks under storage at 4°C. Consumption of 100 ml of this yoghurt meets the recommended daily probiotic intake. Although addition of banana in concentration between 5 – 10% introduced a slight banana aroma the composition and acceptability of the yoghurt were not affected. This study illustrates the potential of reducing postharvest losses of tropical fruits by incorporating them into other value added products.

Acknowledgements: This research was funded by the Food Technology and Business Incubation Center (FTBIC), Makerere University.

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