Adopting traditional fermented foods as carriers for probiotics

The case of *Obushera* and *Lactobacillus rhamnosus yoba*

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Abstract

Purpose – Traditional fermented products can be adopted as probiotic carriers. This study was aimed at evaluating the potential of using *Obushera*, a traditional sorghum beverage from Uganda, as a carrier for *Lactobacillus rhamnosus yoba*.

Design/methodology/approach – Probiotic *Obushera* was produced by fermenting sorghum malt with *Lb. rhamnosus yoba* 2012 and Streptococcus thermophilus C106 at 30 °C and at room temperature (21°C-25 °C) for 24 h. Acidity, pH, total soluble solids and microbial counts were monitored. Consumer acceptability and purchase index of probiotic *Obushera* were compared to four commercial non-probiotic brands. Shelf stability of probiotic *Obushera* was determined by monitoring changes in pH, acidity, soluble solids, microbial counts and consumer acceptability during refrigerated storage.

Findings – *Lactobacillus rhamnosus yoba* 2012 multiplied and lowered the pH of *Obushera* from 5.3 to < 4.0 ($p < 0.0001$) whilst increasing acidity from 0.21 to 0.46 per cent ($p < 0.0001$) in 9 h at 30 °C. Consumer acceptability varied with *Obushera* brand ($p < 0.0001$). The overall acceptability score of probiotic *Obushera* (score of 6.4 = like slightly) was similar to that of the two most acceptable commercial brands (scores of 5.8 and 6.6). Acidity, pH and *Lb. rhamnosus* counts of probiotic *Obushera* varied within 0.6 per cent –1.05 per cent ($p < 0.0001$), 3.3–3.4 ($p < 0.0001$), and 8.2-9.2 log cfu/ml ($p < 0.0001$), respectively during two months of

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Conflict of interest: The authors declare that the Yoba for Life foundation is a non-profit organization, with accreditation from the Dutch Tax Authorities as a Public Benevolent Institution (PBI). The Yoba for Life foundation aims at the promotion of local production and consumption of fermented products in Africa through the distribution and sales of ready-to-use sachets with dried bacterial starter cultures through a network of partners and volunteers.

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storage. The overall acceptability of probiotic *Obushera* (scores of 6.9-7.8) did not change significantly during storage ($p = 0.185$).

Practical Implications – Traditional fermented foods such as *Obushera* can be adopted as carriers of probiotic microorganisms.

Originality/value – Use of commercial probiotic strains in traditional fermented foods is a novel approach that can be adopted to improve safety of traditional fermentations and health of consumers.

Keywords Probiotics, Sorghum, Fermented cereal beverage, *Obushera*, *Lb. rhamnosus*

Paper type Research paper

1. Introduction

Probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit to the consumer" (FAO/WHO, 2002). Interest in probiotics has grown in the past two decades (Reid and Dhir, 2019). Growth in the probiotics sector can be attributed to the growth of the functional food industry and increasing global health awareness among consumers (Global Industry Analysts Incorporated, 2016). To date several studies have evaluated and reported on various health benefits associated with the intake of a variety of probiotic organisms (Sarkar, 2013; Sánchez *et al.*, 2017; Kerry *et al.*, 2018).

Current probiotic products and/or supplements predominantly include strains of the bacterial genera *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Bacillus* and the yeast *Saccharomyces boulardii* (Drisko *et al.*, 2003; Parvez *et al.*, 2006; Kerry *et al.*, 2018). Some of the health benefits conferred by different probiotics include: modulating the immune system, reduction of eczema in infants, lowering serum cholesterol, suppression of the growth of pathogenic microbes, alleviating constipation and preventing or managing different gastrointestinal illnesses including different types of diarrhea (Drisko *et al.*, 2003; Parvez *et al.*, 2006; Kerry *et al.*, 2018).

Diarrhea kills more than 800, 000 children every year (UNICEF/WHO, 2009) with several others including adults suffering from related complications that negatively impact on quality of life and development. Diarrhea is also the leading cause of malnutrition in children under five years old (WHO, 2013). It is of great significance in Sub-Saharan Africa since it accounts for about 37 per cent of death of children in the region (Franz *et al.*, 2014). Probiotics can be used (Dunne, 2001) alongside vaccination, promotion of hygiene and treatment of cases to manage or prevent diarrhea (WHO, 2013). There is, however, limited information on the use of probiotics in Africa. As of 2005, only South Africa appeared to have an established market for probiotics mainly in the form of supplements, fortified foods and fermented dairy products containing strains from the developed world (Brink *et al.*, 2005). These products are generally sold at premium prices making them more affordable to the middle and upper income classes. There have been attempts in Africa to develop locally sourced strains into probiotics. However, most research on probiotics has largely involved *in vitro* studies aimed at evaluating the probiotic potential of isolates or traditional products (Franz *et al.*, 2014). The potential health benefits of these isolates and products have not been subjected to intensive randomized control studies as is recommended (FAO/WHO, 2002). This is largely due to the relatively large expenses involved in randomized control studies and starter culture development. Fortunately, Africa can circumvent these costs by adopting probiotic strains from the developed world whose health benefits are already proven (Franz *et al.*, 2014). One of the promising candidate strains is *Lactobacillus rhamnosus* GG.

Lactobacillus rhamnosus GG (LGG) is one of the most widely studied probiotics with evidence showing that it is beneficial in the treatment of gastrointestinal diseases (De Roos and Katan, 2000; Guandalini, 2011; Horvath *et al.*, 2011; Kerry *et al.*, 2018). Clinical studies

have demonstrated that LGG reduces the duration of rotavirus-associated diarrhea (De Roos and Katan, 2000; Guandalini, 2011) which is a major concern, in particular among children in the Ugandan society (Nakawesi *et al.*, 2010). LGG also prevents antibiotic induced diarrhea, travelers' diarrhea and *Clostridium difficile* colitis (De Roos and Katan, 2000; Drisko *et al.*, 2003; Guandalini, 2011). LGG also prevents the growth of pathogens such as *Cronobacter sakazakii* in products and also increases the concentrations of vitamins especially vitamin B1 during fermentation (Kort *et al.*, 2015). LGG, in the form of *Lactobacillus rhamnosus* yoba 2012, is now more accessible in Africa following the introduction of the concept of "generic probiotics" (Kort and Sybesma, 2012). *Lb. rhamnosus yoba* can now be incorporated in traditional food products since these are readily available as part of the normal diet and are equally affordable (Franz *et al.*, 2014). Africa has a diversity of traditional fermented foods from milk, meat, legumes, roots and tubers as well as cereal (Franz *et al.*, 2014). Cereal fermented foods offer a good opportunity for use as probiotic carriers because they are common throughout the continent.

Lb. rhamnosus yoba 2012 has been evaluated for use in African traditional products like *Uji, Mutandabota, Zomkom, Kwete* and *Obushera* (Kort *et al.*, 2015; Mpofu *et al.*, 2014; Wacoo *et al.*, 2019). However, emphasis was mainly on growth parameters of the starter in these products. Inclusion of probiotic starters in a product may affect its sensory properties and hence acceptability (Gomes and Malcata, 1999; Nyanzi *et al.*, 2010; Rathore *et al.*, 2012). Additionally, to ensure efficacy of probiotics a minimum probiotic content of 10^6 cfu per ml or per g is recommended in the product at the time of consumption (Tripathi and Giri, 2014). It is therefore not only vital to ensure growth of the probiotic but to also evaluate their effect on consumer acceptability and survival in the product during storage.

The purpose of this study was to evaluate the ability of *Lb. rhamnosus* yoba 2012 to produce a shelf stable and acceptable probiotic *Obushera* product. The study also evaluated survival of the probiotic during storage. *Obushera* is an extensively studied popular sorghum and or millet based beverage from Uganda which has also been commercialized (Mukisa, 2012; Muyanja *et al.*, 2003). This study therefore illustrates the potential of utilizing locally available traditional foods as carriers for probiotics.

2. Materials and methods

2.1 Probiotic culture

The yoba 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. This culture was propagated by re-suspending 1 g of dried culture into 100 ml of Man-Rogosa-Sharpe (MRS) broth (Pronadisa Laboratories Conda S.A Madrid Spain) and incubated at 30°C for 24 h. Cells were recovered from the broth (centrifuging at 7500 × g for 10 min at 4°C) rinsed in sterile quarter strength Ringer's solution (Ringer's tablets from Merck KGaA, Darmstadt, Germany). The resulting pellets were re-suspended in 10 ml of Ringer's solution and used as starters for production of probiotic *Obushera*.

2.2 Sorghum malt flour and honey

Obushera containing honey was produced following procedures described by Mukisa *et al.* (2010). Black wattle honey was purchased from Green and White Enterprises, Kampala Uganda and stored in a jerry can at room temperature (about 25°C) prior to use. Sorghum grain (Seso 3 variety) was obtained from National Semi-Arid Resources Research Institute (NASARRI), Serere, Soroti District in Uganda. Sorghum malt flour was prepared as described earlier (Mukisa *et al.*, 2012).

2.3 Preparation of probiotic Obushera

Obushera was prepared by dissolving 63 g of sorghum malt flour and 135 g of honey per liter of water (Mukisa, 2012). The mixture was heated with constant stirring up to 90°C, held for 10 min and then cooled to 30°C prior to inoculation. The slurry was inoculated with the yoba starter to a concentration of 6 log cfu/ml. Fermentation was carried out at 30°C. Titratable acidity, pH, and total soluble solids were determined at $t = 0$, 3, 6, 9, 12 and 24 h while microbial counts were taken at $t = 0$, 12 and 24 h.

2.4 Evaluating the shelf stability of probiotic Obushera

The shelf stability of probiotic *Obushera* was evaluated based on pH, titratable acidity, total soluble solids, microbial counts and consumer acceptability scores. The probiotic products were stored under refrigeration (mean temperature of 4.8°C) and analyzed at weekly intervals for up to two months.

2.5 Evaluating alternative inoculation approaches for probiotic Obushera

This study also evaluated the possibility of initiating the fermentation of probiotic *Obushera* by: using pre-fermented milk, directly inoculating *Obushera* base with the dry starter or pre-fermented *Obushera* (back-slopping from the former or using a second passage of *Obushera*). The idea was to develop a procedure which could be easily adopted at the local household level to produce probiotic *Obushera*. In using pre-fermented milk, the method described by Yoba for Life for the production of yoghurt was slightly modified. One liter of milk was pasteurized at 85°C for 15 min, cooled to 45°C and inoculated with 1 g of Yoba starter culture. The milk was then incubated at room temperature 21-25°C overnight instead of using a vacuum flask as is recommended by Yoba for Life. One liter of the *Obushera* base was then prepared and divided into two 500 ml portions. Each portion was inoculated with 5 ml of the fermented milk. In the second scenario of inoculation, 1 liter of the *Obushera* base was prepared and subdivided into two 500 ml portions. One gram of dry Yoba starter culture was aseptically added to each portion. In the third scenario of inoculation by using pre-fermented *Obushera*, 1 liter of *Obushera* based was prepared and divided into two 500 ml portions. Each portion was then inoculated with 1 per cent of *Obushera* samples prepared by using the dried Yoba starter cultures and fermented for 24 h. All inoculated *Obushera* bases were fermented at room temperature (21 – 25°C). Samples were taken at $t =$ 0 and 24 h for determination of pH, titratable acidity and *Lb. rhamnosus* counts.

2.6 Microbiological analyses

Obushera samples were serially diluted in ¼ Strength Ringer's solution. *Lb. rhamnosus* yoba 2012 counts were determined by pour plating selected dilutions in sterile MRS agar and incubating anaerobically at 30°C for 48 h. Yeast counts were determined by surface spreading on Potato Dextrose Agar and incubating at 30°C for 2-5 days. Coliform counts were determined by pour plating selected serial dilutions in Violet Red Bile Lactose Agar and incubating at 30°C for 48 h. Microbiological media were obtained from Laboratorios, CONDA (Madrid, Spain).

2.7 Physicochemical analyses

The pH was determined using a digital pH meter (pH 98107, USA). Total soluble solids (°Brix at room temperature) were determined using a refractometer (KH0294067, USA). Titratable acidity was determined by titrating 10 mL of the sample against a standardized solution of 0.1 M NaOH with phenolphthalein as the indicator (AOAC, 1990).

2.8 Sensory evaluation

An untrained panel (n = 62) consisting of students, staff members and other people from the surrounding area was used to evaluate the acceptability of the probiotic *Obushera* a day after fermentation. The acceptability of the probiotic *Obushera* was compared to that of four other commercial *Obushera* products on the market in Kampala, Uganda. 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 *Obushera* brands by responding to the statement: "I would regularly purchase this product". A five-point Likert scale (with the anchors 1 – strongly disagree, 2 – disagree, 3 – not decided, 4 – agree and 5 – strongly agree) was used for evaluating willingness to purchase. For determining shelf stability, a mini panel of 8 members was used to determine the acceptability of the samples during storage. Bottled water was provided for rinsing the palate in between tasting of samples.

2.9 Statistical analysis

Results for consumer acceptability scores, purchase index and changes in physicochemical attributes during storage were was analyzed using one-way Analysis of Variance (ANOVA). Mean comparisons were made using the Tukey's HSD test. Results for changes in pH, acidity and microbial counts (at time $t = 0$ and $t = 24$) during the evaluation of inoculation approaches were analyzed using a student's t-test. A value of $\alpha = 0.05$ was used to detect significant differences in means. All statistical analyses were performed using XLSTAT software (version 2012.4.03, Addinsoft, France).

3. Results

3.1 Fermentation of Obushera using Lb. rhamnosus yoba

Lb. rhamnosus yoba grew in, and fermented, *Obushera* with its cell counts increasing from 7.2 log cfu/ml to 8.8 log cfu/ml in 24 h ($p < 0.0001$). The changes in pH, acidity and soluble solids during fermentation of probiotic *Obushera* with *Lb. rhamnosus* yoba are shown in Figure 1. Acidity of probiotic *Obushera* increased with a simultaneous reduction in pH during the fermentation process. The pH decreased ($p < 0.0001$) from 4.9 to 3.9 while titratable acidity increased ($p < 0.0001$) from 0.31 per cent to 0.74 per cent after 24 h (Figure 1). The product attained a pH $\leq$ 4.5 within 4 h and a pH $\leq$ 4.0 within 8 h corresponding to acidity values of about 0.4 per cent and 0.45 per cent respectively. Total soluble solids remained constant (15.2 °Brix) within the first 3 h then rapidly reduced to 14.5 within 12 h (Figure 1).

3.2 Acceptability of probiotic Obushera

A comparison of the consumer acceptability and purchase index of probiotic *Obushera* with four commercial brands of *Obushera* (designated as Com-1, Com-2, Com-3 and Com-4) is shown in Table I. The acceptability (color, aroma, taste and overall acceptability) and purchase index of *Obushera* varied with brand ($p < 0.0001$). The acceptability scores and purchase index of probiotic *Obushera* was similar to that of the three most acceptable commercial brands (Comm-1, Com-2 and Com-3). Probiotic *Obushera* had acceptability scores for all attributes ranging approximately from 6 to 7 ('like slightly' to 'like moderately') and a purchase index of 3.6 (roughly equivalent of 'I can regularly purchase this product').

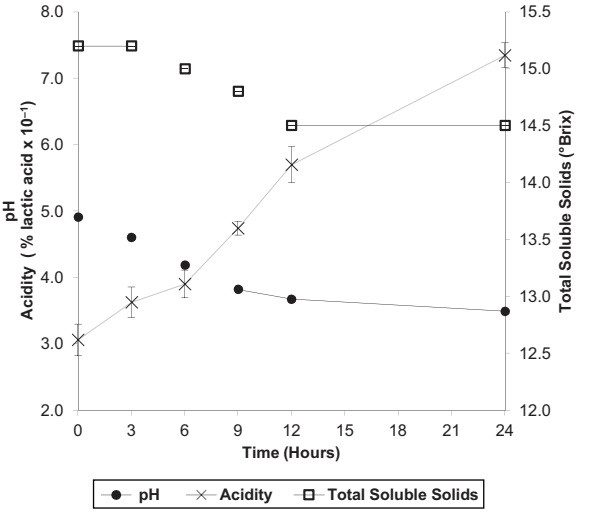


Figure 1.
Changes in pH (●), acidity (×) and total soluble solids (□) during fermentation of *Obushera* with *Lb. rhamnosus yoba* at 30°C

Note: Data points represent means of three independent fermentations. Error bars represent standard deviations

Table I.
Comparison of the consumer acceptability scores and purchase index of probiotic *Obushera* produced by *Lb. rhamnosus* Yoba with commercial *Obushera* brands

	Acceptability Score					
Sample*	Appearance	Color	Aroma	Taste	Overall acceptability	Purchase index
Probiotic	$6.7^{a} \pm 2.0$	$6.8^{a} \pm 1.8$	$6.2^{ab} \pm 2.0$	$6.3^{a} \pm 2.2$	$6.4^{ab} \pm 2.0$	$3.6^{a} \pm 1.3$
Com – 1	$5.7^{bc} \pm 2.2$	$6.3^{ab} \pm 1.9$	$6.4^{a} \pm 1.8$	$6.9^{a} \pm 1.8$	$6.6^{a}+1.7$	$3.7^{a} \pm 1.0$
Com – 2	$5.1^{cd} \pm 2.3$	$5.4^{b} \pm 2.2$	$5.6^{abc} \pm 2.4$	$5.9^{ab} \pm 2.1$	$5.8^{ab} \pm 2.3$	$3.2^{ab} \pm 1.5$
Com – 3	$6.3^{ab} \pm 1.8$	$6.2^{ab} \pm 1.7$	$5.4^{bc} \pm 1.9$	$5.3^{b} \pm 1.8$	$5.7^{b} \pm 1.7$	$2.9^{b} \pm 1.3$
Com – 4	$4.1^{d} \pm 2.1$	$4.2^{c} \pm 2.0$	$4.9^{c} \pm 2.1$	$3.8^{c} \pm 2.1$	$4.2^{c} \pm 1.9$	$2.2^{c} \pm 1.1$
p-value	*< 0.0001*	*< 0.0001*	*0.000*	*< 0.0001*	*< 0.0001*	*< 0.0001*

Notes: Values are means ± standard deviations (n = 62 respondents). Data were subjected to analysis of variance. Values in the same column with the same superscripts ($^{a, b, c, d}$) are not significantly different ($p > 0.05$). *The commercial brands of *Obushera* are represented by the labels Com −1, 2, 3 and 4. 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 five-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)

3.3 Shelf stability of probiotic Obushera

Shelf stability of probiotic *Obushera* was evaluated by monitoring changes in pH, acidity, total soluble solids, viability of *Lb. rhamnosus yoba* and consumer acceptability during refrigerated storage for a period of 8 weeks (Figure 2). The pH of the probiotic *Obushera* ranged between 3.2 and 3.5 ($p < 0.0001$) while the acidity increased ($p = 0.000$) gradually from 0.6 per cent to 1.05 per cent. The total soluble solids content remained constant ($p < 0.05$) at 13°Brix. Counts of *Lb. rhamnosus* yoba remained above 8 log cfu/ml during the

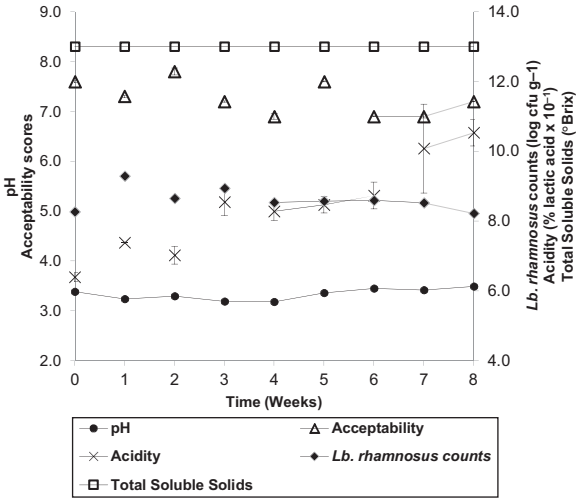
entire storage period. Coliforms, yeasts and molds were not detected (< 1 cfu/ml) during the entire period of storage. Probiotic *Obushera* was acceptable throughout the storage period with overall acceptability scores of 7-8 ($p = 0.185$).

3.4 Alternative inoculation and fermentation approach

Three approaches for initiating the fermentation of probiotic *Obushera* were evaluated: use of pre-fermented milk, directly inoculating *Obushera* base with dry yoba starter and pre-fermented *Obushera* (blackslopping by using 24 hour fermented *Obushera* inoculated with dry starter). Table II shows the changes in pH, acidity and *Lb. rhamnosus* counts in probiotic *Obushera* produced with the different inoculation approaches after fermenting at room temperature (21-25°C) for 24 h. Generally, all the three inoculation approaches could be used to produce *Obushera* after fermenting at room temperature for 24 h. *Lb. rhamnosus yoba* grew from 6.5-7.0 log cfu/ml to 8.1-8.96 log cfu/ml. The pH dropped from 5.0-5.2 to 3.8-4.2 while acidity increased from 0.19-0.22 per cent to 0.28-0.46 per cent. Sorghum malt slurry inoculated with pre-fermented *Obushera* (backslopping) had the highest *Lb. rhamnosus yoba* counts ($p = 0.009$), highest acidity ($p < 0.0001$) and lowest pH ($p < 0.0001$).

4. Discussion

To improve access to probiotics in Africa, Franz *et al.* (2014) recommend the incorporation of probiotic strains with well-documented health benefits in locally popular traditional foods. Recently Gharbi Yahyaoui *et al.* (2017) evaluated the use of *Bsissa*, a traditional fermented food made from wheat, chickpea, fenugreek seeds and majoram as a carrier of the probiotic *Lb. rhamnosus* GG. Parker *et al.* (2018) and Wacoo *et al.* (2019) also evaluated the use of lait caillé a fermented milk from Senagal and *Kwete a fermented maize beverage* from Uganda as vehicles for the probiotic *Lb. rhamnosus* GG, respectively. *Lb. rhamnosus* GG was selected for this study given its various proven benefits in the treatment of gastrointestinal diseases



Note: Data points are means of three independent fermentations. Error bars represent standard deviations

Figure 2. Shelf stability of probiotic *Obushera* containing *Lb. rhamnosus* during storage at 4.8°C: changes in pH (●), acidity (×), total soluble solids (□), *Lb. rhamnosus yoba* counts (◆) and consumer acceptability (Δ)

Table II.
Comparison of inoculation methods (starter culture growth and acidification) for initiating the fermentation of probiotic *Obushera* with *Lb. rhamnosus* yoba

Parameter	Time	Inoculation method: Pre-fermented milk	Direct dry starter addition	Back slopping with *Obushera**	*p*-value
Lb rhamnosus Yoba (Log cfu/ ml)	0	$7.20^{a} \pm 0.03$	$7.02^{a} \pm 0.36$	$6.50^{a} \pm 0.08$	
	24	$8.13^{bx} \pm 0.13$	$8.45^{by} \pm 0.07$	$8.90^{bz} \pm 0.07$	0.009
p-value		0.011	0.031	0.001	
pH	0	$5.2^{a} \pm 0.10$	$5.0^{a} \pm 0.10$	5.3 ± 0.00	
	24	$4.1^{bx} \pm 0.00$	$4.0^{bx} \pm 0.00$	$3.8^{y} \pm 0.10$	<0.0001
p-value		<0.0001	<0.0001	<0.0001	
Acidity (% lactic acid)	0	$0.22^{a} \pm 0.05$	$0.19^{a} \pm 0.02$	$0.21^{a} \pm 0.02$	
	24	$0.28^{ax} \pm 0.03$	$0.31^{by} \pm 0.03$	$0.46^{by} \pm 0.02$	<0.0001
p-value		0.064	0.001	<0.0001	

Notes: Values are means ± standard deviations of two independent fermentations. Fermentations were carried out at room temperature (21-25°C); *One liter of *Obushera* base was inoculated with 10 ml of *Obushera* fermented directly with the yoba dry starter cultures. Means of 0 h and 24 h, for each parameter were compared using a t-test. Means in a column, for each parameter, with different superscripts (a and b) are significantly different ($p < 0.05$). Means in a row, for each parameter at 24 h, with different superscripts (x, y, and z) are significantly different ($p < 0.05$)

(De Roos and Katan, 2000; Drisko *et al.*, 2003; Guandalini, 2011; Horvath *et al.*, 2011). *Obushera* was chosen as the candidate vehicle for *Lb. rhamnosus yoba* because it is one of the popular traditional fermented foods in the country (Mukisa, 2012; Muyanja *et al.*, 2003). *Obushera* was originally commonly consumed in households within south and southwestern part of Uganda (Muyanja *et al.*, 2003). It has, however, gradually become popular even in the capital city and among consumers of all walks of life after introduction of well-packaged commercial versions of the product on the market (Mukisa, 2012). There are currently more than 30 commercial brands on the market in the capital city alone (Byakika *et al.*, 2019).

The probiotic organism chosen should be able to grow in the product (Franz *et al.*, 2014). *Lb. rhamnosus yoba* inoculated in *Obushera* grew by 2-3 log cycles reaching a maximum of 8.9 log cfu/g during fermentation at temperatures between 21-30°C. Maximum counts of *Lb. rhamnosus* in *Obushera* were similar to those reported for total lactic acid bacteria and specific starters such as *Lb. plantarum, Lb. fermentum, W. confusa* and *Lc. lactis* in *Obushera* (Mukisa, 2012; Muyanja *et al.*, 2012). *Lb. rhamnosus* grew in *Kwete* and maintained concentrations of greater than 8 log cfu/g during storage for four weeks (Wacoo *et al.*, 2019). Similar counts were also reported of *Lb. rhamnosus* were also reported for *Bsissa*, a plant-based fermented food. (Gharbi Yahyaoui *et al.* (2017) wheat, chickpea, *Lb. rhamnosus* was able to grow in *Obushera* because the product contained fermentable sugars such as glucose, fructose and maltose (Mukisa, 2012; Muyanja *et al.*, 2012) and free amino nitrogen from sorghum malt (Byakika, 2015).

A rapid pH drop to ≤ 4.5 or even much lower than 4.0 and a titratable acidity of about 0.7 per cent lactic acid are recommended for ensuring the microbiological safety and stability of lactic acid fermented beverages (Kunene *et al.*, 1999; Nout, 1992; Nout *et al.*, 1989; Steinkraus, 1995). Traditional fermentations can take between 24 h to 120 h to attain similar pH and acidity values but with use of starter cultures and fermentation at 30°C such values can be attained within 12 - 24 h (Mukisa, 2012; Wacoo *et al.*, 2019). *Lb. rhamnosus yoba* was also

able to attain similar values of pH and acidity within 24 h during fermentation at 30°C thus demonstrating its ability to produce microbiologically safe *Obushera*. The maximum acid levels observed during storage of probiotic *Obushera* (0.70 – 1.05 per cent) were similar to those reported for *Kwete* when using the same starter culture, *Lb. rhamnosus* (Wacoo *et al.*, 2019). These values are also in agreement with maximum levels of acid production observed when using starters containing *Lb. plantarum* for *Obushera* production (Ntaate, 2014).

It is important to evaluate the effect of adding probiotics on the acceptability of traditional fermented foods since probiotic starters may affect the flavor profile, sensorial properties and acceptability of products (Salmerón *et al.*, 2015; Nyanzi *et al.*, 2010). Furthermore, novel probiotic products should be compared with existing related traditional products to evaluate the market potential of the former (Nyanzi *et al.*, 2010). This study shows that the *Lactobacillus rhamnosus* yoba starter did not significantly affect the acceptability of *Obushera*. Similar observations were made for *Kwete produced* using *Lactobacillus rhamnosus* (Wacoo *et al.*, 2019). Consumers also indicated their willingness to purchase the probiotic products to the same extent as the non- probiotic commercial counterparts. Therefore, probiotic *Obushera* produced using the yoba culture can be readily accepted and frequently purchased by consumers thus increasing accessibility of probiotics in Uganda.

A probiotic product should contain a minimum of 6 log cfu per ml or gram at the time of consumption (Tripathi and Giri, 2014). Daily Intake of 100-1,000 ml of the product provides the recommended daily dose (8 – 9 log cfu) sufficient for realizing probiotic effects (Knorr, 1998; Tripathi and Giri, 2014). Probiotic *Obushera* contained 7.6 – log 8.9 log cfu/ml of *Lb. rhamnosus yoba* during the entire eight weeks of storage. *Lb. rhamnosus* counts in *Kwete* also remained above 8 log cfu/g during storage for four weeks (Wacoo *et al.*, 2019). Considering the *Lb. rhamnosus* counts observed in stored *Obushera*, consuming 10 ml/day of the probiotic *Obushera* product would be more than sufficient to meet the recommended daily intake of probiotics.

Probiotic *Obushera* generally remained stable and acceptable during the entire study period (eight weeks or 56 days). The shelf life of unpasteurized-unrefrigerated, unpasteurized-refrigerated and pasteurized *Obushera* is four days, seven days and over 30 days, respectively (Byaruhanga and Ndifuna, 2002; Mukisa *et al.*, 2010). Pasteurization destroys the fermenting flora (Byaruhanga and Ndifuna, 2002) thus preventing over acidification and alcohol production. Traditional production of *Obushera* generally does not involve pasteurization and the beverage is stored at room temperature (Mukisa *et al.*, 2010). The product is, therefore, consumed within a few days before it becomes too acidic and alcoholic (Mukisa *et al.*, 2010). A few processors or sellers of *Obushera* currently store the unpasteurized product under refrigeration leading to a shelf life extension of three days or more. Fermenting *Obushera* with *Lb. rhamnosus* and storing under refrigeration without pasteurizing extends the shelf life by at least 52 days. This is possible due to the cold temperatures retarding microbial growth hence preventing over-acidification of the product.

The *Lb. rhamnosus yoba* starter was primarily produced for use in dairy fermentations. It is, therefore, not surprising that the propagation instructions require first growing the culture in milk and using this as inoculum for the fermentation. This study shows that sorghum malt slurries can be used to propagate the culture for purposes of inoculating a non-dairy fermentation like *Obushera*. This is not only cost effective as it eliminates the cost of milk as a propagation media but it also ensures that the use of the culture fits well in the local production process of *Obushera* thus ensuring ease of adoption by processors.

5. Conclusions

This study illustrates the potential of utilizing locally available traditional foods as carriers for probiotics. The *Lb. rhamnosus yoba* starter was able to ferment sorghum malt to produce *Obushera* with a pH < 4.0 in 9 h at 30°C (or in 24 h at room temperature). The product was acceptable and remained stable during storage under refrigeration for two months. Therefore, traditional fermented food products and processes can be adapted to produce probiotic foods that are readily acceptable and accessible to the local consumer.

References

AOAC (1990), *Official Methods of Analysis of the Association of Analytical Chemists*, Association of Official Analytical Chemists Arlington.

Brink, M., Senekal, M. and Dicks, I. (2005), "Market and product assessment of probiotic/prebioticcontaining functional foods and supplements manufactured in South Africa", *South African Medical Journal*, Vol. 95 No. 2, pp. 114-119.

Byakika, S. (2015), "Evaluation of sorghum malt as a growth and carrier medium for Lactobacillus plantarum MNC 21 biomass", MSc Thesis. Makerere University, Kampala.

Byakika, S., Mukisa, I.M., Byaruhanga, Y.B., Male, D. and Muyanja, C. (2019), "Influence of food safety knowledge, attitudes and practices of processors on microbiological quality of commercially produced traditional fermented cereal beverages, a case of Obushera in Kampala", *Food Control*, Vol. 100, pp. 212-219.

Byaruhanga, Y. and Ndifuna, M. (2002), "Effect of selected preservation methods on the shelf life and sensory quality of Obushera", *Muarik Bull*, Vol. 5, pp. 92-100.

De Roos, N.M. and Katan, M.B. (2000), "Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998", *The American Journal of Clinical Nutrition*, Vol. 71 No. 2, pp. 405-411.

Drisko, J.A., Giles, C.K. and Bischoff, B.J. (2003), "Probiotics in health maintenance and disease prevention", *Alternative Medicine Review*, Vol. 8 No. 2, pp. 143-155.

Dunne, C. (2001), "Adaptation of bacteria to the intestinal niche: probiotics and gut disorder", *Inflammatory Bowel Diseases*, Vol. 7 No. 2, pp. 136-145.

FAO/WHO (2002), "Guidelines for the evaluation of probiotics in food", Food and Agriculture Organization of the United Nations and World Health Organization Working Group Report London, Ontario, Canada.

Franz, C.M., Huch, M., Mathara, J.M., Abriouel, H., Benomar, N., Reid, G., Galvez, A. and Holzapfel, W. H. (2014), "African fermented foods and probiotics", *International Journal of Food Microbiology*, Vol. 190, pp. 84-96.

Gharbi Yahyaoui, A., Bouzaiene, T., Aouidi, F., Aydi, A. and Hamdi, M. (2017), "Traditional cereal food as container of probiotic bacteria, 'Lb. Rhamnosus GG': optimization by response surface methodology", *Journal of Food Quality*, Vol. 2017, pp. 1-12.

Global Industry Analysts Incorporated (2016), "Probiotics – a global strategic business report", available at: www.strategyr.com/pressMCP-1084.asp (accessed 22 July 2017).

Gomes, A.M. and Malcata, F.X. (1999), "Bifidobacterium spp. and Lactobacillus acidophilus: biological, biochemical, technological and therapeutical properties relevant for use as probiotics", *Trends in Food Science and Technology*, Vol. 10, pp. 139-157.

Guandalini, S. (2011), "Probiotics for prevention and treatment of diarrhea", *Journal of Clinical Gastroenterology*, Vol. 45, pp. S149-S153.

Horvath, A., Dziechciarz, P. and Szajewska, H. (2011), "Meta-analysis: Lactobacillus rhamnosus GG for abdominal pain-related functional gastrointestinal disorders in childhood", *Alimentary Pharmacology and Therapeutics*, Vol. 33 No. 12, pp. 1302-1310.

Kerry, R.G., Patra, J.K., Gouda, S., Park, Y., Shin, H.S. and Das, G. (2018), "Benefaction of probiotics for human health: a review", *Journal of Food and Drug Analysis*, Vol. 26 No. 3, pp. 927-939.

Knorr, D. (1998), "Technology aspects related to microorganisms in functional foods", *Trends in Food Science and Technology*, Vol. 9, pp. 295-306.

Kort, R. and Sybesma, W. (2012), "Probiotics for every body", *Trends in Biotechnology*, Vol. 30 No. 12, pp. 1-3.

Kort, R., Westerik, N., Serrano, L.M., Douillard, F.P., Gottstein, W., Mukisa, I.M., Tuijn, C.J., Basten, L., Hafkamp, B. and Meijer, W.C. (2015), "A novel consortium of Lactobacillus rhamnosus and Streptococcus thermophilus for increased access to functional fermented foods", *Microbial Cell Factories*, Vol. 14 No. 1, pp. 1-14.

Kunene, N., Hastings, J. and Von Holy, A. (1999), "Bacterial populations associated with a sorghum-based fermented weaning cereal", *International Journal of Food Microbiology*, Vol. 49 Nos 1/2, pp. 75-83.

Mpofu, A., Linnemann, A.R., Sybesma, W., Kort, R., Nout, M. and Smid, E.J. (2014), "Development of a locally sustainable functional food based on mutandabota, a traditional food in Southern Africa", *Journal of Dairy Science*, Vol. 97 No. 5, pp. 2591-2599.

Mukisa, I., Nsiimire, D., Byaruhanga, Y., Muyanja, C., Langsrud, T. and Narvhus, J. (2010), "Obushera: descriptive sensory profiling and consumer acceptability", *Journal of Sensory Studies*, Vol. 25, pp. 190-214.

Mukisa, I.M. (2012), "Sensory characteristics, microbial diversity and starter culture development for obushera, a traditional cereal fermented beverage from Uganda", PhD Thesis, Norwegian University of Life Sciences.

Mukisa, I.M., Muyanja, C.M., Byaruhanga, Y.B., Schüller, R.B., Langsrud, T. and Narvhus, J.A. (2012), "Gamma irradiation of sorghum flour: effects on microbial inactivation, amylase activity, fermentability, viscosity and starch granule structure", *Radiation Physics and Chemistry*, Vol. 81 No. 3, pp. 345-351.

Muyanja, C., Narvhus, J. and Langsrud, T. (2012), "Organic acids and volatile organic compounds produced during traditional and starter culture fermentation of Bushera, a Ugandan fermented cereal beverage", *Food Biotechnology*, Vol. 26 No. 1, pp. 1-28.

Muyanja, C., Kikafunda, J., Narvhus, J., Helgetun, K. and Langsrud, T. (2003), "Production methods and composition of Bushera: a Ugandan traditional fermented cereal beverage", *African Journal of Food, Agriculture, Nutrition and Development*, Vol. 3 No. 1, pp. 10-19.

Nakawesi, J.S., Wobudeya, E., Ndeezi, G., Mworozi, E.A. and Tumwine, J.K. (2010), "Prevalence and factors associated with rotavirus infection among children admitted with acute diarrhea in Uganda", *BMC Pediatrics*, Vol. 10 No. 1, p. 15.

Nout, M. (1992), "Accelerated natural lactic fermentation of cereal-based formulas at reduced water activity", *International Journal of Food Microbiology*, Vol. 16 No. 4, pp. 313-322.

Nout, M.R., Rombouts, F. and Havelaar, A. (1989), "Effect of accelerated natural lactic fermentation of infant good ingredients on some pathogenic microorganisms", *International Journal of Food Microbiology*, Vol. 8 No. 4, pp. 351-361.

Ntaate, D. (2014), "Evaluation of starter cultures for the production of enturire, a sorghum-based alcoholic beverage", BSc Thesis. Makerere University, Kampala.

Nyanzi, R., Jooste, P.J., Abu, J.O. and Beukes, E.M. (2010), "Consumer acceptability of a synbiotic version of the maize beverage mageu", *Development Southern Africa*, Vol. 27 No. 3, pp. 447-463.

Parker, M., Zobrist, S., Donahue, C., Edick, C., Mansen, K., Nadjari, M., M., Heerrikhuisen, M., Sybesma, W., Molena, D., Diallo, A., Milani, P. and Kort, R. (2018), "Naturally fermented milk from Northern Senegal: bacterial community composition and probiotic enrichment with Lactobacillus Rhamnosus", *Frontiers in Microbiology*, Vol. 9, pp. 1-12.

Parvez, S., Malik, K., Ah Kang, S. and Kim, H.Y. (2006), "Probiotics and their fermented food products are beneficial for health", *Journal of Applied Microbiology*, Vol. 100 No. 6, pp. 1171-1185.

Rathore, S., Salmerón, I. and Pandiella, S.S. (2012), "Production of potentially probiotic beverages using single and mixed cereal substrates fermented with lactic acid bacteria cultures", *Food Microbiology*, Vol. 30 No. 1, pp. 239-244.

Reid, G. and Dhir, R. (2019), "Probiotics: Reiterating what they are and what they are not", *Frontiers in Microbiology*, Vol. 10, p. 424.

Salmerón, I., Thomas, K. and Pandiella, S.S. (2015), "Effect of potentially probiotic lactic acid bacteria on the physicochemical composition and acceptance of fermented cereal beverages", *Journal of Functional Foods*, Vol. 15, pp. 106-115.

Sánchez, B., Delgado, S., Blanco-Míguez, A., Lourenço, A., Gueimonde, M. and Margolles, A. (2017), "Probiotics, gut microbiota, and their influence on host health and disease", *Molecular Nutrition and Food Research*, Vol. 61, pp. 1600240-1600243.

Sarkar, S. (2013), "Probiotics as functional foods: documented health benefits", *Nutrition and Food Science*, Vol. 43 No. 2, pp. 107-115.

Steinkraus, K. (1995), *Handbook of Indigenous Fermented Foods, Revised and Expanded*, Marcel Dekker, New York, NY.

Tripathi, M. and Giri, S. (2014), "Probiotic functional foods: Survival of probiotics during processing and storage", *Journal of Functional Foods*, Vol. 9, pp. 225-241.

UNICEF/WHO (2009), *Diarrhoea: Why Children Are Still Dying and What Can Be Done*, The United Nations Children's Fund (UNICEF), New York, NY, World Health Organization (WHO), Geneva.

Wacoo, A.P., Mukisa, I.M., Meeme, R., Byakika, S., Wendiro, D., Sybesma, W. and Kort, R. (2019), "Probiotic enrichment and reduction of aflatoxins in a traditional African maize-based fermented food", *Nutrients*, Vol. 11 No. 2, p. 265.

WHO (2013), *Diarrhoeal Disease. Fact Sheet No 330*, World Health Organization, Geneva.

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