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THE POTENTIAL OF *Uapaca kirkiana* FRUIT JAM FOR THE DELIVERY OF *Lactobacillus rhamnosus* yoba AS A PROBIOTIC FOOD

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ABSTRACT

Probiotics are important in enhancing food quality, reducing incidences of diarrhoea and promoting good health. A fruit jam is an ideal food to deliver probiotics because it is easy to produce, a good source of sugar, and most rural population consume it. A probiotic jam was developed using an underutilised fruit, *U. kirkiana*, to benefit the resource-poor population in southern Africa. *U. kirkiana* fruit is found abundant in most semi-dry rural areas of Zimbabwe. Ripe *U. kirkiana* fruits were obtained from preferred domesticated trees by households residing in semi-dry rural areas of Zimbabwe. The fruits were pulped by removing seeds, mashing and sieving through an 800 µM sieve. Pectin content of the pulp was determined. A probiotic jam was developed using the formulation 55 % (wt/vol) pulp, 43 % (wt/vol) sugar, 1.5 % (wt/vol) pectin, and 0.5 % (wt/vol) citric acid. The fruit pulp was mixed with sugar in a stainless steel pot and cooked at 110 °C. Citric acid was added and stirred whilst cooking until it reached 55 °Brix. Pectin was added and the jam was continuously stirred until it reached 68 °Brix. The jam was inoculated with 0.25 % *L. rhamnosus* yoba and left to propagate for 24 hours, while bacterial growth was monitored. The physicochemical and functional properties (pH, total soluble solids, sugars, total titratable acidity, iron content, zinc content, and vitamin C), and *L. rhamnosus* yoba viability in the probiotic jam was analysed. The probiotic jam had vitamin C, total titratable acidity, total soluble solids, and moisture content of 0.34 ±0.02 mg /100 g FW, 2.2 ± 0.11 g / L FW, 68.5 ± 0.2 % FW, and 34.8 ± 1.2 % FW, respectively; iron and zinc content of 4.13 ± 0.52 mg /100 g FW and 0.36 ± 0.02 mg /100 g FW, respectively; high fructose and sucrose content of 12.84 ± 0.21 g /100 g FW and 24.61 ± 0.12 g /100 g FW, respectively; and a total titratable acidity content of 2.2 g / L at day 0 (after production), 2.37 ± 0.01 g / L FW at day 4, and 2.48 ± 0.02 g / L FW at day 7 of storage (25 °C). The probiotic jam had 6.2 ± 0.2 Log CFU / mL viable cells on point of consumption. *U. kirkiana* fruit jam can potentially deliver live *L. rhamnosus* yoba cells as a probiotic food.

Key words: Probiotic food, vitamin C, fruit jam, *L. rhamnosus* yoba, pectin, *U. kirkiana* fruit, sub-Saharan Africa

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INTRODUCTION

A probiotic bacterium is defined as a live microorganism that is able to move through the gastrointestinal tract passage in its active form, and in adequate viable numbers to positively affect the health of the host [1]. The probiotic must be consumed as part of food in order to confer its health benefits [2]. Probiotic uses have an estimated world market share of 15 billion USD [3] and take 30 % of the world market for 'functional foods' [4]. 'Functional foods' contain ingredients that improve physiological and health condition, such as probiotics, prebiotics, minerals, and vitamins [5].

The mode of action of probiotics, especially health improving properties, still lack complete understanding, although suggested action relate to immunomodulation, anticarcinogenic and antimutagenic processes, fighting pathogens, improvement of lactose intolerance symptoms, decreasing blood cholesterol levels, preservation of intestinal mucosa conditions, and improved periodontal health [6]. Many meta-analyses have shown positive effects of specific probiotic strains on treatment of diarrheal disease among children [7].

The use of probiotic strains, such as *Lactobacillus rhamnosus* GG, in food products in sub-Saharan African is not well-documented and these products are yet to be sold [8]. Sub-Saharan Africa has the highest percentage of chronically malnourished people in the world [9] and highest under-five mortality rate of 98 deaths per 1000 live births [10]. Diarrhoea is one of the leading causes of poor health and childhood mortality in sub-Saharan Africa and accounts for 37 % of childhood deaths [11]. More studies on the lactic acid bacterium *L. rhamnosus* GG have reported recognizable health benefits of the probiotic bacterium once consumed, including the treatment of diarrhoea in children [8]. Currently the use of effective probiotics is limited in sub-Saharan Africa because most people are poor and cannot afford to buy them [8]. *L. rhamnosus* yoba was used to ferment a local Zimbabwean food, *mutandabota* as a way to enable resource-poor communities in Southern Africa to benefit from a probiotic food [12]. Furthermore, *L. rhamnosus* strain was used to prevent and reduce the occurrence and duration of diarrhoea in Tanzania and Uganda [13]. *L. rhamnosus* strain is now being promoted across Tanzania and Uganda, where it is used to ferment milk for distribution to over 150,000 people in response to an appeal for assistance in the AIDS epidemic [14]. There is no reason to doubt that such benefits would not assist other African communities.

U. kirkiana (Euphorbiaceae) also known as wild loquat is an underutilised wild fruit that is well-adapted to the miombo woodlands which is located in southern sub-humid tropical zone of Africa and is consumed as part of the diet [15]. The utilization of local food materials such as wild fruits and incorporating probiotics may be a possible way to improve child health in Africa [5]. According to Food and Agriculture Organisation (FAO) [16], priorities for food safety in Africa included studies on lactic acid bacteria strains involved in food safety. This was supported by Mpofu *et al.* [17], who reported the effective suppression of five food pathogens in fermented dairy products with *L. rhamnosus* yoba in Africa.

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The use of indigenous food material as potential vehicles for delivering beneficial probiotics are still limited, notwithstanding their great potential for prophylactic and beneficial use to most underprivileged people in poor countries. Lately, generic probiotics are being studied as a practical solution to gain access for use in food processing by many people in the rural parts of Africa [18]. *L. rhamnosus* yoba 2012 is a generic probiotic obtained after isolation of *L. rhamnosus* GG using a commercial food product [18]. *U. kirkiana* fruit pulp contains a total sugar content of 89 g / 100 g fresh weight (FW) with glucose being the degradable sugar [16]. The sugars in the fruit support the propagation of most probiotic strains and can potentially be a good carrier for *L. rhamnosus* yoba strain. The probiotic jam would benefit most rural populations with a sustainable functional food with potential to improve health and their livelihoods. This study was aimed at determining the *L. rhamnosus* yoba viability, functional, and nutritional properties of the probiotic jam and indicated the benefits of using *L. rhamnosus* yoba in the probiotic fruit jam.

MATERIALS AND METHODS

Sampling Area

The fruits were obtained in Gokwe (semi-dry region located 18.22°S 28.93°E in Agro farming region 3), Kazangarare (semi-hot area located 16.30°S 29.56°E in Agro farming region 2b and part of 3) and Bikita (dry area located 20. 5 S° 31.37° E in Agro farming region 4) in Zimbabwe (**Figure 1**). *U. kirkiana* fruit trees are widely distributed in the forests and have adapted well to the conditions of the areas.



Figure 1: Sampling sites (Bikita, Gokwe, Kazangarare) in Zimbabwe

Pulp extraction

From each area, domesticated fruit trees, highly preferred by households, were chosen using a randomised design to collect samples of 100 ripe fruits that had fallen to the ground from the trees. The edible portion of the fruit was obtained by cutting the fruit, removing seeds, mashing (in mortar and pestle), and sieving. The crude pulp mixture

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was sieved through an 800 µM sieve in the laboratory to obtain a composite pulp sample, which was used to analyse the biochemical and functional properties (pH, total soluble solids (TSS), % pectin, total titratable acid (TTA), moisture content, antioxidant activity (AOA), and vitamin C. The data were expressed per 100 g fresh weight (FW). The composite pulp sample was used for the production of jam.

Pectin extraction

Extraction of pectin was carried out according to a method described by Tang *et al.* [19]. Ripe fruit was cut with a knife, the seeds were removed, and the pulp was collected and dried. Forty grams of dried fruit was weighed using an electrical balance and placed into a beaker. Acid-water was prepared by mixing 40 g citric acid with 200 mL of water in a beaker until the pH reached 3. The acid water was added to the beaker and mixed. Extraction was done using water at 90 °C for 3 hours, cooling to approximately 55 °C and filtered into a beaker using a muslin cloth. Isolation of pectin was carried out using 95 % ethanol as the precipitating agent. One volume of extract was added to ethanol in a ratio of 1:1. Pectin was filtered through a Whatman filter paper (No 1) and washed with excess 96 % ethanol and cold water to further remove any remaining impurities. Finally, the precipitate was dried at 50 °C in a hot oven (UL 40, Memmert, Schwabach, Germany) for 10 hours. After drying, the precipitate was placed in a desiccator for cooling. The pectin was ground into, sieved, and stored in a cool and dry place. The experiments were performed in triplicate to ensure accuracy. The percentage yield of extracted pectin was calculated as follows:

$$\text{Pectin yield (\%)} = \frac{P}{Q} \times 100 \qquad \text{(Equation. 1)}$$

where P = the amount of extracted pectin in grams (g), Q = the initial amount of fruit sample (40 g).

Moisture content determination

The moisture content was determined according to Association of Official Analytical Chemistry (AOAC) [20] standard method, using the following formula:

$$\text{Moisture content (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \qquad \text{(Equation 2)}$$

where W_1= weight of crucible (g); W_2 = weight of crucible (g) + fresh sample (g); W_3 = weight of crucible (g) + dried sample (g).

pH measurement

The pH of the pulp and jam were determined according to AOAC [20] standard method using a digital pH meter (BT-675, BOECO, Hamburg, Germany).

Total Soluble Solids

Total soluble solids content of the fruit pulp and jam was determined according to AOAC [20] standard method using a bench brix refractometer (MA871, North Carolina, Milwaukee Instruments, USA) and distilled water was used to calibrate and rinse-off residual sample after each reading.

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Total Titratable Acid
Pulp and jam acidity (expressed as total titratable acidity) was determined according to AOAC standard [20].

Production of jam
The production process was carried out using a standard process adopted from FAO [21], with a new jam formulation comprising 55 % composite fruit pulp, 43 % sugar, 1.5 % pectin, and 0.5 % citric acid. The fruit jam was inoculated with a fresh probiotic culture with > 5 Log CFU / mL *L. rhamnosus* yoba cells, incubated at 37 °C for 24 hours, cooled and stored at room temperature (25 °C). In the control experiment, distilled water was boiled, cooled to 30 ^{0}C, and it was inoculated into the jam. Functional properties (pH, total soluble solids, total titratable acid, moisture content, antioxidant activity, and vitamin C) were analysed.

Medium and inoculum for probiotic jam
An isolate of *L. rhamnosus* yoba [18], was obtained in a sachet from the Yoba for Life Foundation, Amsterdam, Netherlands and stored at −80 °C. The *L. rhamnosus* yoba strain was reactivated by sub-culturing anaerobically in De Man, Rogosa and Sharpe agar (MRS) broth at 37 °C for 18 hours. *U. kirkiana* fruit pulp was mixed with sugar, boiled and subsequently cooled to room temperature (25 °C), and was then used to cultivate *L. rhamnosus* yoba. In order to enable propagation of the *L. rhamnosus* strain, degradable sugar must be added to food matrices [8]. *L. rhamnosus* yoba was then precultured in the medium and incubated at 37 °C for 36 hours until the number of live cells reached > 6 Log CFU / mL. This culture with 5.4 ± 0.1 Log CFU / mL was used for producing the probiotic jam.

Inoculation of probiotic culture into the jam
Sterilised jars (100 g) containing the *U. kirkiana* fruit jam were opened under aseptic conditions, and the jam was inoculated with a (0.25 mL) fresh probiotic culture according to Mpofu *et al.* [12]. The cell suspensions were gently mixed with the jam. The jam was incubated for 37 °C for 24 hours. The jam was then cooled and stored at room temperature (25 °C) for 7 days to reduce fermentation of the jam.

Enumeration of *L. rhamnosus* yoba into the probiotic jam
Enumeration of viable *L. rhamnosus* yoba was carried out by collecting 1 mL of probiotic jam under aseptic conditions. Thereafter, serial decimal dilutions were carried out in a peptone physiological salt solution (pH 7.0, 8.5 g / L NaCl, and 1 g / L neutralized bacteriological peptone from Oxoid). Diluents of 100 µL were plated in triplicate onto de Man, Rogosa and MRS agar (1.2 % agar, bacteriological peptone from Oxoid, added to de Man, Rogosa and Sharpe broth, Merck). MRS agar plates were incubated at 37 °C under anaerobic conditions in Gas Pack anaerobic jars (Becton Dickinson Microbiology Systems, Baltimore, Maryland, USA). All colonies on the MRS agar were counted and results were expressed as colony forming units per millilitre (CFU/mL) of *L. rhamnosus* yoba, taking into account the dilution factors.

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Glucose, fructose, and sucrose analyses

Glucose, fructose, and sucrose contents were determined according to a procedure by Minekus *et al.* [22], using a Sucrose/D-Fructose/D-Glucose assay kit (K-SUFRG, Megazyme International, Ireland). Samples were placed in plastic cuvettes (10 mm light path) and a colorimetric measurement was used to analyse absorbance of all sugars at 340 nm using a UV-vis spectrophotometer (Genesys 10S, Thermo Scientific, Waltham, Massachusetts, USA) This analysis would indicate the total sugars present in the probiotic jam, the type and amount of sugar present to support the growth of *L. rhamnosus* yoba.

Jam zinc and iron analyses

A probiotic jam sample was digested using concentrated solutions of nitric acid (HNO_3), sulphuric acid (H_2SO_4), and ultrapure hydrogen peroxide (H_2O_2). Iron and zinc contents were determined using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (Agilent 5100, Agilent Technologies, Santa Clara, California, USA).

Determination of vitamin C (ascorbic acid)

Ascorbic acid concentration was determined using a standard method by AOAC [20].

DPPH radical scavenging activity of pulp

Radical scavenging activity was determined using a method by AOAC [20]. The absorbance was determined at 517 nm using a Spectronic Genesys Spectrophotometer (Genesys 5, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Ascorbic acid (0.1 M) was used as a reference control.

Statistical analysis

The results of functional, physicochemical properties and probiotic viability were expressed as the mean ± standard deviation (SD), and all experiments were conducted in triplicates. The LSD test was conducted to determine any significant differences at ($P < 0.05$). One-way analysis of variance for F-test, and Student's t-test for comparisons were conducted at $P < 0.05$ significance level using SPSS package version 18.0 (Coakes and Ong, John Wiley & Sons, Queensland, Australia).

RESULTS AND DISCUSSION

Functional properties of *L. rhamnosus* yoba jam and composite pulp

The jam inoculated with *L. rhamnosus* yoba had a vitamin C, TTA, TSS (°Brix), and moisture content of 0.34 ± 0.02 mg / 100 g, 2.2 ± 0.11g / L, 68.5 ± 0.2 %, and 34.8 ± 1.2 % FW, respectively (Table 1). The composite pulp sample had an antioxidant activity of 35 ± 1.02 % FW. Analysis of AOA was conducted because it is an excellent functional benefit the pulp and jam can deliver to the body. The control sample had a low pH of 3.3 ± 0.10. There was no significant difference ($P < 0.05$) between the jam inoculated with *L. rhamnosus* yoba and control jam sample with respect to the TTA and Brix contents after production.

L. rhamnosus yoba jam had a moisture content of 32.8 ± 1.1 % and the control jam had a moisture content of 32.5 ± 1.2 % FW, which did not differ significantly ($P < 0.05$) and

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were within expected limits of 30.9 – 34.4 % to maintain storage quality [23]. The greater the moisture content, the higher the water activity, which tends to promote the growth of spoilage bacteria, fungi, and moulds [25]. In addition, the use of sugar as an ingredient in producing the jam resulted in sugar binding to water molecules, which reduces the amount of available water in the jam, to the point at which it is too low for undesirable microbial growth. This ensures that the jam is tightly packed after production.

The TSS (Brix) level was 68.5 ± 0.2 and 68.0 ± 0.1 in the *L. rhamnosus* yoba and control jams, respectively. These values are in agreement with a study by Ndabikunze *et al*. [24] who reported a TSS of 68.53 in *U. kirkiana* jam made with commercial pectin. The brix measurement is a ratio (weight by weight) of water to sugar (TSS) in the food material. Brix is mainly determined in fruit pulps and their products such as jams, juices, and jellies. The Brix content normally changes due to physiological conditions present in the fruit. In jam making, the TSS is analysed because it is critical for good gel formation and preservation. A good jam must have a final TSS in the range 65 – 68 % [21]. Lower Brix values (< 65 %) have been found to affect shelf life [21]. Furthermore, the jam will have a runny consistency, making it ideal for the growth of bacteria and moulds. Higher TSS contents of > 68 % will cause the sugar to form crystals and a very stiff gel. In order to overcome this effect, the Brix of the jam was monitored regularly during production and the endpoint of boiling/cooking of the jam was reached when the Brix level reached 68 %. High Brix can also be attributed to the presence of natural enzymes (pectinase) and the heat treatment used in processing, which enables the breakdown of the insoluble pectin from the complex polysaccharides into simpler sugars [25]. The TSS levels in *U. kirkiana* pulp might be due to the ripeness of the fruits before processing, which was also reported by Kansci *et al.* [26], for mango (*Mangifera indica*) fruits at different stages of ripening. The rural communities would benefit in consuming the probiotic jam with a high TSS. Total soluble solids impact on the organoleptic characteristics and improve the nutritional quality of the jam is due to the sugar that is used as an ingredient in the jam.

Vitamin C analysis was important because it is essential for immune response and health [27]. The vitamin C content was 0.34 ± 0.02, 0.28 ± 0.03, and 17.4 ± 0.13 mg /100 g FW for the jam inoculated with *L. rhamnosus* yoba, the control jam sample, and composite pulp, respectively. The drastic decrease in vitamin C levels in the *L. rhamnosus* yoba jam could be attributed to the processing temperatures (110 ºC) used during the production of the jam. Vitamin C is highly sensitive to heat, especially above 70 °C, where it tends to leach out into the surrounding solution [27]. Vitamin C is an essential nutrient for the human body and some of its physiological functions include lowering the risk of cancer, healing of wounds, reducing susceptibility to infections, formation of bones and teeth, and iron absorption [28] and acting as an antioxidant [29]. Vitamin C content was low and not significant ($P < 0.05$) in the probiotic jam. At such low concentration in the probiotic jam, vitamin C will not be an effective pro-oxidant in the body. This meant that the jam cannot be an excellent source of vitamin C for the rural population. Furthermore, the low vitamin C would not increase the keeping quality of the probiotic jam as an antioxidant, and, therefore, the need to add an antioxidant to the jam.

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Iron and Zinc content in jam and composite pulp

Iron and zinc were analysed because their deficiencies are widespread in Africa. The jam inoculated with *L. rhamnosus* yoba had an iron and zinc content of 4.13 ± 0.22 mg /100 g FW and 0.36 ± 0.02 mg /100 g FW, respectively after production. This suggests the action of the probiotic as it produced enzymes, lipases and amylases that acted on the food matrix and released the bound iron and zinc. The control jam (inoculated with distilled water) had iron and zinc contents of 4.03 ± 0.11 mg /100 g and 0.34 ± 0.01 mg /100 g fresh weights, respectively. There was a significant difference ($P < 0.05$) in the iron and zinc contents between the inoculated and control jams. This meant that *L. rhamnosus* yoba was able release enzymes that potentially degraded the food matrix and released some bound iron which resulted in a relatively higher iron content in the probiotic jam. Furthermore, the fermentation process could have resulted in the loss of dry matter, which may have increased the mineral content as *L. rhamnosus* yoba degrades sugars and protein [30]. Sripriya *et al.* [31] reported an increase in iron content in jam caused by the degradation of phytates that complex with minerals during fermentation thereby releasing the bound iron and other minerals such as zinc, calcium and phosphorous. The use of *L. rhamnosus* yoba improves the iron content in the probiotic jam and may make it more available for absorption in the human body.

Production of jam

During the production of the jam, gel formation was desirable. This suggests that the presence of high methoxyl pectin in the pulp was complemented by the addition of commercial pectin during production. The addition of citric acid set the pH between 2.8 – 3.2, which affects the viscosity [32] and improves gel formation, flavour, and shelf life [33]. The probiotic jam had a higher pH, which resulted in a firm gel which is desirable by most panellists when they evaluated the jam. There was a significant difference in pH of the jams at $P < 0.05$. The jam inoculated with *L. rhamnosus* yoba and control jam had pH of 3.5 ± 0.12 and 3.3 ± 0.1, respectively. The probiotic jam had a pH that was in an acceptable range of 3.4 –3.6 according to Food and Agriculture Organisation guidelines. The pH of jams is usually dependent on the fruit pulp acidity [32].

During production, the setting or gelling process requires three main ingredients, pectin, acid, and water [32]. Pectin acts as a gelling agent that causes a physical transformation through aggregate bonding changes between sugar and acid while water acts a solvent [33]. Pectin is readily soluble at a concentration of 25 %, which supports the gelling mechanism. The addition of sugar during the production of the probiotic jam allowed the precipitation of natural pectin molecules in the pulp and the commercial pectin that was added, due to the dehydrating effect of the sugar. Because of their negative charge, the pectin molecules in solution repel each other. The concentration of hydrogen ions is higher at low pH, which tends to suppress the ionisation of the galacturonate carboxyl group, and the tendency of the negatively charged carboxyl groups to repel each other. With the addition of citric acid, that lowers the pH through the addition of more hydrogen ions, the negative charge of the pectin molecules is reduced and subsequently enables the hydrogen bonding of adjacent pectin molecules [33]. This results in the precipitation of pectin molecules to form a web that traps water and solutes in the network [33]. More so, this process could have been improved by the presence of lactic acid produced on degradation of sugar into lactate and lactate dehydrogenase catalyses the production of

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the acid in the probiotic bacteria. Furthermore, the production of a satisfactory gel was necessitated by use of 1.5 % pectin since the composite pulp had a relatively low pectin content of 0.25 ± 0.05 %. The optimum pH for the growth of *L. rhamnosus* is 6.4 – 6.9 [34]. At a pH range of 3.4 - 4.4, its growth is low [35]. The pH of the jam after production was not favourable for the active growth of bacteria and suggests a slowed growth rate in *L. rhamnosus* yoba. However, there was still > 6 Log CFU / mL of live cells before consumption. Such a low pH ensures the microbiological safety of the jam as a low pH inhibits the growth and survival of many food pathogens and microbes [36]. The pH of the probiotic jam plays a significant role in ensuring microbiological safety of the jam. This will benefit the rural folk in consuming a potentially safe food product.

Total titratable acid

There was a significant change in the TTA of the jam over time, and it was noted to range from 2.1 – 2.5 g / L over a 7-day storage period, which is in accordance with the standard value associated with good quality jam (**Figure 2**). Total titratable acid (TTA) contents were significantly different in the jam inoculated with *L. rhamnosus* yoba and control jam at ($p < 0.05$). Ndabikunze *et al*. [24] reported a percentage TTA content of 0.05 ± 0.02 in *U. kirkiana* pulp. Acidity of the pulp is an important aspect in jam making as low pH is required for gel formation. Fruits naturally contain acids, mainly citric acid, but other acids such as malic acid and tartaric acid can also be found in a number of fruits. The source of the TTA noted in the jam could be attributed to the presence of natural acids in the fruit, although these are present in too low quantities to support jam making. Vertuani *et al.* [37] reported the presence of citric, malic, tartaric, succinic, and ascorbic acid in fruit pulps. Furthermore, *L. rhamnosus* yoba is lactic acid fermenting bacteria. *L. rhamnosus* yoba was able to ferment sugars, especially glucose and produce lactic acid in the jam. This could suggest the increase in TTA observed in the probiotic jam when it was incubated and stored for 7 days. A more acidic pH ensures that the carboxyl groups present in the jam mixture are not ionised, thereby lowering the repulsive forces and improving gelling [33].

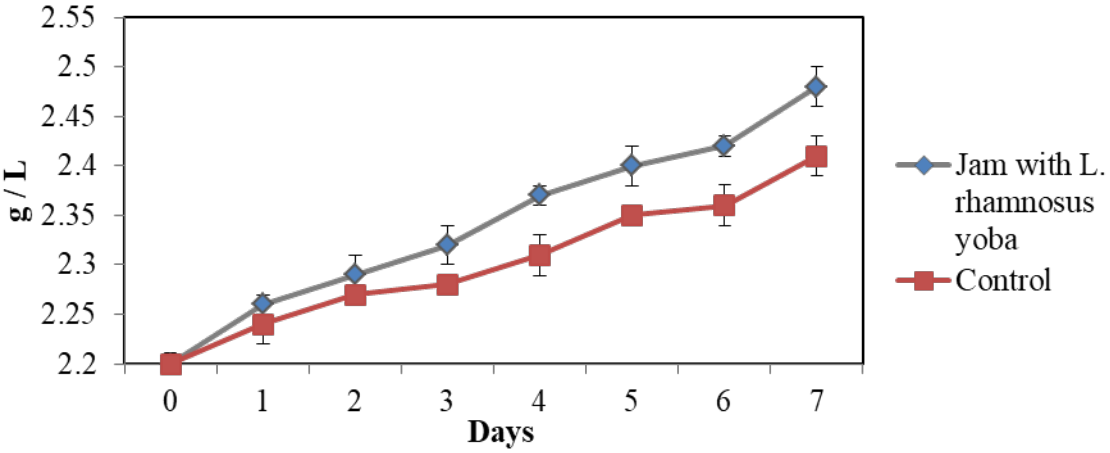


Figure 2: Total Titratable Acid (TTA) content in the jam inoculated with *L. rhamnosus* yoba over 7 days in storage (25 °C)

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Sugars (glucose, sucrose, fructose)

The jam inoculated with *L. rhamnosus* yoba had high fructose and sucrose contents of 12.84 ± 0.21 g / 100 g FW and 24.61 ± 0.12 g / 100 g FW, respectively (**Figure 3**). Fructose and sucrose contents were statistically different at $P < 0.05$ in all the samples. This is explained by the addition of sugar during the production of the jam. In the formulation, 43 % was sucrose. Fructose was the dominant simple sugar. The higher sugar contents could be attributed to the breaking down of the pulp matrix, which releases soluble fractions [25]. Also, the sugar content often differs in fruits due to differences in maturity index and ripening stage [38]. Sugars play a technological function of supporting the growth of *L. rhamnosus* yoba, impart the sweet taste of the jam and providing energy. The sugars (glucose, fructose and sucrose) are responsible for development of colour through Maillard reactions and caramelisation process [39]. This is important in determining the sensorial quality and acceptance of the probiotic jam.

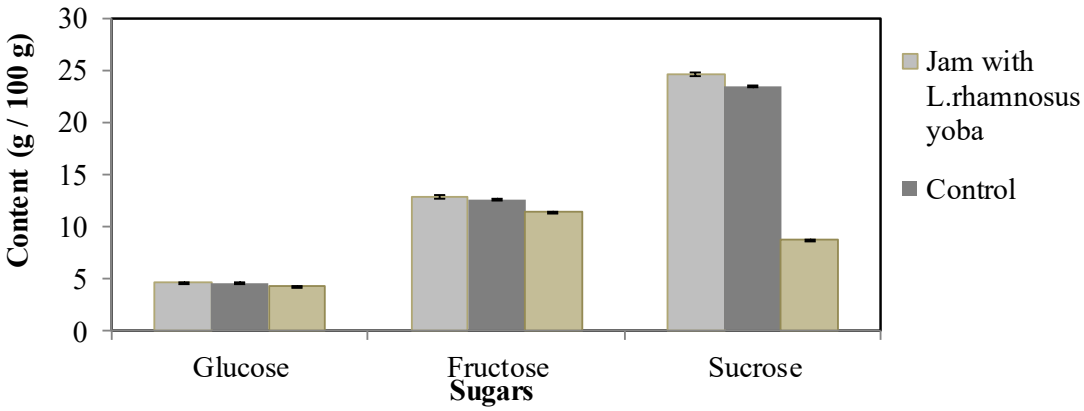


Figure 3: Sugar contents in the jam inoculated with *L. rhamnosus* yoba

Extraction of pectin in composite pulp

The percentage yield of pectin from the composite pulp was 0.24 ± 0.05 % edible portion (EP). Ndabikunze *et al.* [24] reported a pectin content of 0.28 ± 0.05 % EP in *U. kirkiana* fruits collected from the Iringa forest areas in Tanzania. This pectin content was higher compared to that of *V. mombassae* (Lamiaceae) and *S. birrea* (Anacardiaceae) fruits, which had pectin contents of 0.12 ± 0.05 % and 0.17 ± 0.08 %, respectively [24]. The use of heat treatment in pectin extraction, which weakened the structure of the fruits, could have resulted in an increase in the interaction between the acidic solution and the raw material during the extraction, hence resulting in a high pectin yield. An extraction temperature of 90 °C was appropriate because it encouraged the loss of energy through vaporization, but a very high temperature of 100 °C and above can cause degradation of pectin—as pectin is composed of α-1,4-linked units of galacturonic acid—yielding pectin of lower molecular weight, which is unstable [40]. Extraction at lower temperatures (< 80 °C) can result in production of pectin with a low viscosity and poor diffusion between phases, hence resulting in a slow rate of extraction and lower yields of pectin. Studies by Udonne [41] revealed that a low pH produces a high pectin yield irrespective of the plant material, hence a pH of 3 was chosen in this experiment.

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Inoculum

The culture with 5.4 ± 0.1 Log CFU / mL viable *L. rhamnosus* yoba cells was used as an inoculum because the probiotic strain must introduce the probiotic effects into the fruit jam, which will allow for better growth of the bacteria.

Enumeration of probiotic bacteria

Viable counts of *L. rhamnosus* yoba in jam were determined before jam consumption. The viable plate count of *L. rhamnosus* yoba was found to be 6.2 ± 0.2 Log CFU / mL. The jam was able to deliver a live *L. rhamnosus* yoba bacterial cell count that was over 6 Log CFU / mL, making it a potentially probiotic food [42], although the bacterial counts were lower than those noted by Mpofu *et al.* [17] in the probiotic *mutandabota* (8.8 ± 0.5 Log CFU / mL). Stadlymayr *et al.* [43] reported the proximate composition of *U. kirkiana* fruit pulp as crude protein 0.3 g / 100 g, fibre 2.1 g / 100 g, fat 0.4 g / 100 g, ash 0.8 g / 100 g, and carbohydrates 28.7 g / 100 g. The growth of the *L. rhamnosus* yoba was promoted by the presence of sugars to supply carbon. The jam is a potentially probiotic food because it contained over 6 Log CFU / mL viable *L. rhamnosus* yoba cell on jam consumption. Furthermore, the probiotic jam has a potential to improve the health and wealth of most poor rural folks in many ways such as good source of energy, reduction in bacterial spoilage due to the fermentation process (low pH), increased nutritional properties of the jam on the release of more iron and a high TSS, potential prevention of diarrhea resulting from the intake of a *L. rhamnosus* yoba, and improvement of livelihoods when the jam is processed at household level and sold. Kort *et al.* [8] reported the ability of *L. rhamnosus* yoba in producing vitamins, detoxifying carcinogens, and increased antimicrobial activity.

CONCLUSION

This study revealed that an underutilised fruit, *U. kirkiana* can successfully be used to culture and grow a probiotic bacterium. A functional food that contained *Lactobacillus rhamnosus* yoba, an isolate of *L. rhamnosus* GG, with a viability of 6.2 ± 0.2 Log CFU / mL was developed. A probiotic jam was produced that had live *L. rhamnosus* yoba cells at the recommended intake level of > 6 Log CFU / mL in the jam. The probiotic jam is a good source of sugars and energy for the rural population. *L. rhamnosus* yoba was able to ferment glucose. The use *L. rhamnosus* yoba in fermentation potentially improved the iron content in the jam and making it a good source of dietary iron. The low pH due to lactic acid production during fermentation makes the jam microbiologically safe. The TSS and sugar content were significant, thereby improving the nutritional and sensorial properties of the probiotic jam. This study has provided more insights on the need to use more probiotic strains in processing other indigenous fruits and enhance access to beneficial probiotics for rural populations who need them in Africa. Research to determine the mineral (iron and zinc) bioaccessibility and sensorial qualities of the probiotic jam is recommended.

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Table 1: Biochemical and functional properties of the probiotic jam and composite pulp

Biochemical and functional properties	*Jam with *L. rhamnosus* yoba	*Control jam sample	**Composite fruit pulp
Vitamin C (mg/100 g)	0.34 ± 0.02^{a}	0.28 ± 0.03^{b}	17.4 ± 0.13^{c}
Total Titratable Acidity (g/L)	2.2 ± 0.11^{a}	2.1 ± 0.10^{a}	0.4 ± 0.02^{b}
pH	3.5 ± 0.12^{a}	3.3 ± 0.10^{b}	4.3 ± 0.02^{c}
Total soluble solids (TSS) (%)	68.5 ± 0.2^{a}	68.0 ± 0.1^{a}	20.6 ± 0.12^{c}
Antioxidant activity (%)	3.7 ± 1.12^{a}	3.3 ± 1.0^{b}	35 ± 1.02^{c}
Moisture content (%)	32.8 ± 1.1^{a}	32.5 ± 1.2^{a}	72.2 ± 0.3^{b}
% Pectin	-	-	0.25 ± 0.05^{a}

Mean ± standard deviations are reported. Means with identical superscripts in a row are not significantly different at $p < 0.05$. *Values are based on the fresh weight (FW), ** Values are based on the edible portion (EP) of the fruit

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