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Development of a locally sustainable functional food based on mutandabota, a traditional food in southern Africa

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ABSTRACT

A probiotic dairy product was developed on the basis of a traditional dish called mutandabota to enable resource-poor populations in southern Africa to benefit from a functional food. Mutandabota is widely consumed in rural southern Africa, making it an ideal food matrix to carry probiotics. First, a process to produce probiotic mutandabota was designed. Raw cow milk was boiled and subsequently cooled to ambient temperature (25°C). Next, dry pulp from the fruit of the baobab tree (*Adansonia digitata* L.) was added to the milk at a concentration of 4% (wt/vol). This mixture was inoculated with the probiotic *Lactobacillus rhamnosus* yoba and left to ferment for 24 h, while the growth of the bacterial culture was monitored. Final ingredients were then added to produce probiotic mutandabota that had 14% (wt/vol) baobab fruit pulp and 7% (wt/vol) sugar in cow milk. The pH of probiotic mutandabota was pH 3.5, which ensures that the product is microbiologically safe. The viable plate count of *L. rhamnosus* yoba increased from 5.8 ± 0.3 log cfu/mL at the point of inoculation to 8.8 ± 0.4 log cfu/mL at the moment of consumption, thereby meeting the criterion to have a viable count of the probiotic bacterium in excess of 6 log cfu/mL of a product. Baobab fruit pulp at 4% promoted growth of *L. rhamnosus* yoba with a maximal specific growth rate (μ_{\max}) of 0.6 ± 0.2 /h at 30°C. The developed technology, though specific for this particular product, has potential to be applied for the delivery of probiotics through a variety of indigenous foods in different regions of the world. Upon consumption, probiotic mutandabota is expected to improve the population's intestinal health, which is

especially relevant for vulnerable target groups such as children and elderly people.

Key words: *Lactobacillus rhamnosus*, milk, baobab fruit, probiotic mutandabota

INTRODUCTION

Ingestion of probiotics is associated with health benefits (FAO/WHO, 2001; Lacroix and Mollet, 2007). To date, enhancing health and nutrition by provision of probiotics to less-affluent communities in a locally sustainable way is still a major challenge. The use of indigenous foods as potential vehicles for transmission of probiotics has been given little attention even though it is an option with great potential in developing countries. Indigenous foods are made from locally available resources. These traditional foods not only have a long history of safe use, they can also be afforded by the local communities. Mutandabota is one such indigenous food product known in southern Africa (Ministry of Agriculture, 2001). It is particularly popular in Zimbabwe and has potential for use as a probiotic carrier. It is made at a household level by mixing raw milk from cows or goats and dry baobab (*Adansonia digitata* L.) fruit pulp (Ministry of Agriculture, 2001; Mpofu et al., 2014). Mutandabota is considered a source of proteins and other micronutrients in southern Africa, where it is consumed as breakfast or lunch on a daily basis. The ingredients of mutandabota are 79% (wt/wt) milk, 14% (wt/wt) baobab fruit pulp, and 7% (wt/wt) crystalline sucrose (Mpofu et al., 2014).

The Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO, 2001) defines probiotics as live microorganisms that, when consumed in adequate amounts as part of food, confer a health benefit to the host. This definition emphasizes that first, probiotics must be consumed in a food matrix that allows them to survive passage through the stomach and exposure to bile, and second,

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a product should contain a certain number of viable probiotic cells that has been shown to deliver a health benefit. Although no cell-count level is recognized to guarantee a health effect (Reid, 2008), a minimum level of 6 log cfu/g of product is needed for a product to be considered probiotic (Shah, 2000; Adikhari et al., 2003). Studies on probiotic bacteria with higher (Gionchetti et al., 2007) or lower (Whorwell et al., 2006) viable cell counts have been published. The viable cell count of the probiotic bacteria is critical in the evaluation of the quality and functionality of a probiotic food product. Guandalini et al. (2000) demonstrated the beneficial effect of *Lactobacillus rhamnosus* GG on children suffering acute, watery diarrhea. In this study, children 1 mo to 3 yr of age, presenting recent onset of acute diarrhea, were enrolled in a double-blinded and placebo-controlled intervention trial. They concluded that the administration of *L. rhamnosus* GG in the oral rehydration solution to children with diarrhea was safe and resulted in marked and significant shorter duration of diarrhea, less chance of a protracted course, and faster discharge from the hospital.

Lactobacillus rhamnosus GG is one of the most thoroughly studied probiotics (Kankainen et al., 2009; von Ossowski et al., 2010; Kort and Sybesma, 2012). It is a lactic acid bacterium that meets the United Nations standard for probiotics and the requirements for clinical trial documentation (FAO/WHO, 2001). Evidence exists of beneficial effects of *L. rhamnosus* GG for prevention and treatment of antibiotic-associated diarrhea (Ruszczynski et al., 2008), rotavirus diarrhea (Grandy et al., 2010), gastrointestinal and upper respiratory tract infections in children (Hojsak et al., 2010) and inhibiting growth and adhesion of enteropathogens (Mack et al., 1999; Gopal et al., 2001). *Lactobacillus rhamnosus* GG shows a high tolerance to the acidic conditions prevailing in the stomach (Tuomola et al., 2000; Corcoran et al., 2005), survives intestinal passage (Sandholm-Mattila et al., 1999), is able to adhere to human colonic mucosa (Alander et al., 1999; Rinkinen et al., 2003), and transiently colonizes the gastrointestinal tract after treatment (Sandholm-Mattila et al., 1999; Tuomola et al., 2000).

In southern Africa, probiotic foods are scarce and expensive. They are not consumed by the rural population, in which lack of hygiene, poor sanitation, malnutrition, and enteric infections frequently lead to diarrheal disorders (Olivieri et al., 2008; Food and Nutrition Council, 2010). This study was meant to facilitate access of the rural population to the benefits of probiotics. To produce probiotic mutandabota in a sustainable way, a strategy was developed based on 2 main considerations. First, mutandabota is a nonfermented dairy product, which takes less than half an hour to

prepare (Mpofu et al., 2014). It is consumed within an hour after preparation. The easiest way to provide probiotics through this food would be to add the appropriate quantity of the probiotic to mutandabota just before consumption. This option, however, would require large quantities of the probiotic and was thus considered costly, unsustainable, and beyond the reach of the targeted communities. A second option is to have one producer in the village who would propagate the probiotic to produce an inoculum to be distributed to other villagers, who would, in turn, use the inoculum to produce their own probiotic mutandabota. Because commercially produced media for propagating probiotics are beyond the reach of the target population, locally available resources such as full-fat milk from local cows and indigenous baobab fruit pulp were considered in this study as the propagation medium. This study describes the development of a probiotic variant of mutandabota as a sustainable, nutritious, and health-promoting food produced at the village level.

MATERIALS AND METHODS

Extraction of Baobab Fruit Pulp

Mature, dry baobab fruits were collected from the wild in Binga district, Zimbabwe. Baobab fruits ripen and dry out while they are still on trees. Harvesting is done by gathering the dropping, dry fruits from the ground. To extract pulp, fruits were cracked by hitting them against a hard surface such as a rock. The dry pulp together with seeds was separated from the pericarps. Pulp was then separated from seeds by sieving and collected in a winnowing basket for preparation of mutandabota.

Medium and Inoculum for Probiotic Mutandabota

A strain of *L. rhamnosus* GG under the name *L. rhamnosus* yoba (Kort and Sybesma, 2012) was used throughout this study. The strain was obtained from Yoba for Life Foundation, Amsterdam, the Netherlands. It was stored at -80°C before being freeze-dried for long-term storage at 4°C in 50-mL tubes (Greiner Bio-One BV, Alphen a/d Rijn, the Netherlands). *Lactobacillus rhamnosus* GG grows slowly in milk (Hekmat and Reid, 2007; Valík et al., 2008). Glucose or another appropriate fermentable sugar is usually added to stimulate growth (Jyoti et al., 2003; Gaudreau et al., 2005). In southern Africa, these substrates have to be imported in most cases, which implies extra costs to production. In this study, locally available baobab fruit pulp was added to full-fat cow milk that had been boiled and subsequently cooled to ambient temperature

(25°C) before using it to cultivate *L. rhamnosus* yoba. The appropriate quantity of baobab fruit pulp for the medium was determined by adding pulp to milk at different concentrations and observing the pH changes and the subsequent growth abilities of the bacterium. *Lactobacillus rhamnosus* yoba was precultured in this medium in a fermentation vessel and incubated at 37°C for 36 h to a level exceeding 9 log cfu/mL. This culture was used for producing probiotic mutandabota.

Determination of the Growth Rate of *L. rhamnosus* Yoba

The growth rate of *L. rhamnosus* yoba in heat-treated full-fat cow milk supplemented with 4% (wt/vol) baobab fruit pulp was evaluated at 20, 25, 30, and 37°C. Sampling was done at hourly intervals over a period of 24 h. Sequential 10-fold dilutions of the culture samples were made in peptone physiological saline solution (8.5 g/L of NaCl and 1 g/L of neutralized bacteriological peptone, LP0034, Oxoid Ltd., Basingstoke, UK) and subsequently plated in triplicate onto de Man, Rogosa, and Sharpe agar (MRS; 1.2% Agar bacteriological, Oxoid Ltd., LP0011 added to de Man, Rogosa, and Sharpe broth, VM986641, Merck KGaA, Darmstadt, Germany). de Man, Rogosa, and Sharpe agar plates were incubated at 37°C in GasPack anaerobic jars (Becton Dickinson Microbiology Systems, Baltimore, MD). Colonies on MRS agar were counted, and results were expressed as colony forming units per milliliter (cfu/mL) of *L. rhamnosus* yoba. The maximal specific growth rates (μ_{\max}) for the *L. rhamnosus* yoba cultures were determined using the plate-count data.

Preparation of Probiotic Mutandabota

Probiotic mutandabota was prepared in triplicate on separate days in Binga district, Zimbabwe (17°36'S, 27°32'E), using a modification of the traditional process (Ministry of Agriculture, 2001; Mpofu et al., 2014). Village women under our supervision prepared probiotic mutandabota using process steps illustrated in Figure 1. To prepare 2 L of probiotic mutandabota, 1,570 mL of cow milk was boiled for about 5 min and cooled to an ambient temperature of 25°C. *Lactobacillus rhamnosus* yoba inoculum (5 mL) that had been propagated in milk with 4% baobab fruit pulp (as explained earlier) was inoculated into the cooled milk. Next, 63 g of baobab fruit pulp was added with continuous stirring. This milk with 4% baobab fruit pulp was left to ferment for 24 h at 23 to 37°C, while the growth of the bacterium was monitored. After the fermentation step, 217 g of baobab fruit pulp and 140 g of crystalline sucrose were

added and mixed for 7 min or until a homogeneous mixture was obtained. Probiotic mutandabota was then ready for consumption. The composition of probiotic mutandabota was 14% (wt/vol) baobab fruit pulp, 7% (wt/vol) sugar, and 79% (wt/vol) cow milk with viable *L. rhamnosus* yoba cells. In the negative control experiment, all conditions and procedures were the same as in the production of probiotic mutandabota, except for the addition of 5 mL of autoclaved distilled water instead of the *L. rhamnosus* yoba inoculum.

Incubation Conditions for Probiotic Mutandabota

Inoculated milk with 4% baobab fruit pulp was incubated for 24 h. Because neither electricity nor incubation facilities were available in Binga district, a remote rural area, the vessel with mutandabota was put in the sunlight during the day to absorb maximum heat. At night it was left next to the fireplace in a traditional grass-thatched kitchen (Pinterest, 2013). The preparation temperature was measured using i-button temperature loggers (DS 1922T-FS#, HQTronics, Dokkum, the Netherlands). One i-button was placed in the fermentation vessel, a second i-button was placed in the open air, and a third i-button was placed in the traditional kitchen. The i-buttons logged temperature at 15-min intervals over a 24-h period.

***L. rhamnosus* Yoba in Probiotic Mutandabota and pH Measurement**

Lactobacillus rhamnosus yoba was enumerated in probiotic mutandabota and the control experiment (i.e., without added *L. rhamnosus* yoba). *Lactobacillus rhamnosus* yoba enumeration was done (i) in the inoculum just before inoculation, (ii) in milk with 4% baobab fruit pulp just before 24-h incubation ($t = 0$), (iii) at the end of 24-h incubation ($t = 24$), and (iv) just before consumption of probiotic mutandabota. Plating on MRS agar and incubation conditions were the same as explained earlier. Colonies were checked microscopically to confirm absence of yeasts.

The pH values during preparation of probiotic mutandabota were measured using a combined glass electrode pH meter (WTW, Weilheim, Germany). The pH meter was calibrated using standard buffer solutions (Merck, Darmstadt, Germany) at pH 4.0 and 7.0. A sample of probiotic mutandabota was taken from the fermentation vessel and pH was determined. The pH was measured (1) at the start of the incubation ($t = 0$), (2) at the end of 24-h incubation ($t = 24$), and (3) in probiotic mutandabota that was ready for consumption.

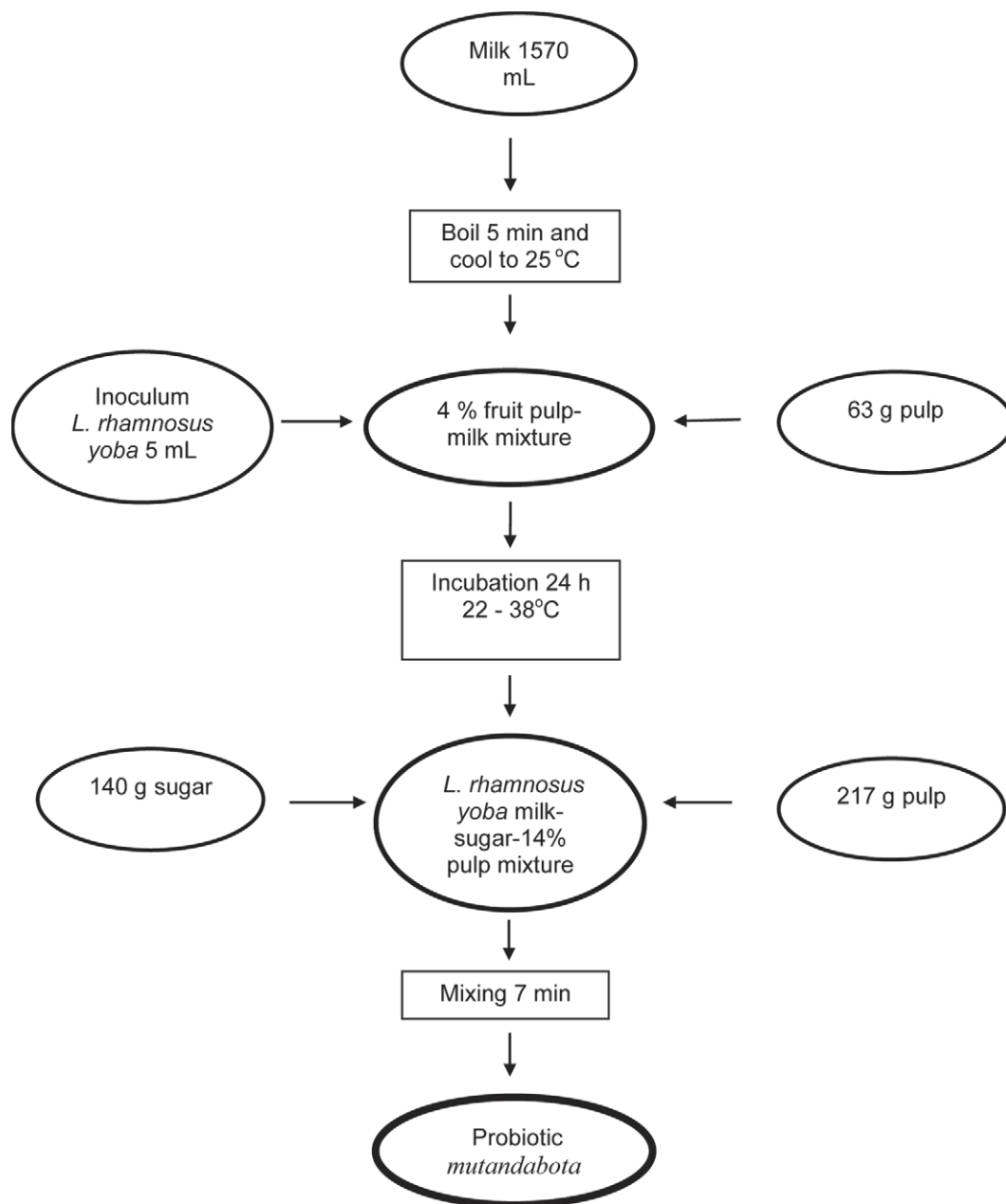


Figure 1. Flowchart for the production of probiotic mutandabota. *L. rhamnosus* = *Lactobacillus rhamnosus*.

Statistical Analysis

The mean values of ingredients and pH of all samples as well as average levels of *L. rhamnosus* yoba in experiments were compared using a one-way ANOVA. Statistical analysis was done using SPSS 13.0 for windows (Apache Software Foundation, Forest Hill, MD) and Excel (Microsoft Corp., Redmond, WA). Descriptive statistics such as means, percentages, frequencies, and variances were computed and used to describe the data.

RESULTS AND DISCUSSION

pH Changes During Production of Probiotic Mutandabota

It is imperative from a health-supporting perspective that probiotic mutandabota contains *L. rhamnosus* yoba in excess of 10^6 viable cells per milliliter at the point of consumption (Shah, 2000; Adikhari et al., 2003). Growth of *L. rhamnosus* GG and other probiotic lactic acid bacteria in dairy products has been stimulated by

addition of fruit juice or fruit pulp (Espirito-Santo et al., 2011). In this study baobab fruit pulp was added to milk to stimulate growth of *L. rhamnosus* yoba, and the pH was monitored to ensure that it remained within the pH range that allows growth of *L. rhamnosus* yoba. The relationship between final pH and amount of baobab fruit pulp is shown in Table 1. A fruit pulp content of 2% gave a pH of 5.4 ± 0.1 . This pH value allowed growth of *L. rhamnosus* yoba but was not enough to sustain growth of the bacterium over a 24-h period due to substrate limitation. Doubling the amount of fruit pulp to 4% resulted in a pH of 4.6 ± 0.1 . Increasing the fruit pulp concentration above 6% resulted in a further decrease in pH, which allowed survival of *L. rhamnosus* yoba, but not its growth. The low pH of the mixture could be attributed to the acidic nature of dry baobab fruit pulp. Airan and Desai (1954) first highlighted the presence of organic acids in baobab fruit pulp. Later reports by Nour et al. (1980) and Vertuani et al. (2002) confirmed the presence of citric, tartaric, malic, succinic, and ascorbic acids in baobab fruit pulp. According to Liew et al. (2005) the optimum pH value for growth of *L. rhamnosus* is in the range of pH 6.4 to 6.9. The lowest pH for growth is within the range of pH 4.4 to 3.4 (Helland et al., 2004). Therefore, milk fortified with 4% (wt/vol) baobab fruit pulp with a corresponding pH of 4.6 ± 0.1 was used for effective propagation of *L. rhamnosus* yoba.

In probiotic mutandabota preparation, the pH of the milk with 4% fruit pulp at the point of inoculation was pH 4.5 ± 0.1 . After 24 h of fermentation with *L. rhamnosus* yoba, the pH decreased to 3.9 ± 0.1 . Organic acids such as lactic acid produced by *L. rhamnosus* yoba during fermentation could have been responsible for the decrease in pH value. After all ingredients were added, the final probiotic mutandabota with 14% fruit pulp had a pH of 3.5. Such a low pH ensures microbiological safety of probiotic mutandabota. Most food pathogens do not survive or grow at such a low pH (International Commission on Microbiological Specifications for Foods, 2002). However, to secure safety of

probiotic mutandabota, it is necessary to evaluate the risk food pathogens pose to consumers at the point of consumption. The final pH in the control experiment was also 3.5.

Temperature Changes During Production of Probiotic Mutandabota

Temperature is a dominant factor determining the bacterial growth rate (μ_{\max}). The μ_{\max} for *L. rhamnosus* yoba in milk supplemented with 4% baobab fruit pulp at 20°C was 0.22/h, whereas at 37°C it was 0.96/h (Figure 2). An essential aspect of enabling the propagation of probiotics in a traditional setting, such as the one where mutandabota originates from, is to maintain the cultivation temperature in a physiologically acceptable range without the use of modern means such as electricity for controlled heating. In this study, the objective was to attain a growth rate around 0.6/h. Consequently, the vessel with mutandabota was placed next to a wall in direct sunlight during the day to ensure that the temperature inside the fermentation vessel was as close as possible to optimum growth temperatures of *L. rhamnosus* yoba. At night it was placed in a traditional Ndebele kitchen near the fireplace. Traditional Ndebele kitchens are built of bricks or mud with a grass thatching (Pinterest, 2013). This makes them well insulated from the hot daytime temperature, which can reach 44°C, and from the cold night temperature of about 10°C. In the kitchen, the vessel was placed near a fireplace, where residual heat from the fireplace kept the temperature in the vessel higher than the kitchen temperature.

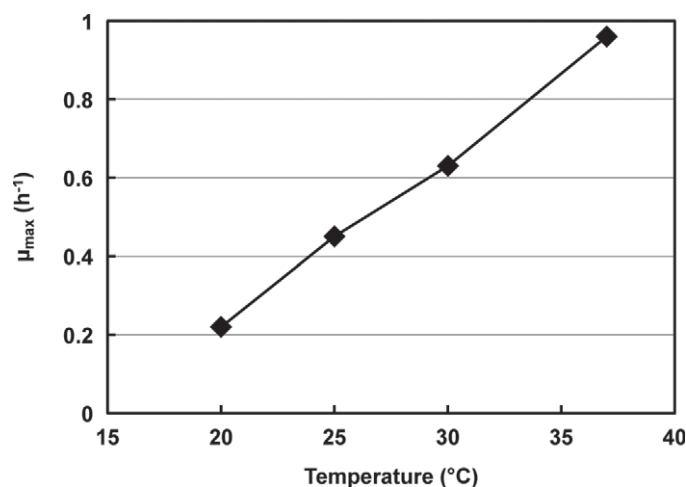


Figure 2. Specific growth rate of *Lactobacillus rhamnosus* yoba in milk supplemented with 4% baobab fruit pulp as a function of incubation temperature.

Table 1. Mean pH of milk mixed with baobab fruit pulp (n = 3)

Pulp (%)	Pulp (g)	Milk (g)	pH
0	0	25	6.6
2	0.5	24.5	5.4 ± 0.1
4	1	24	4.6 ± 0.1
6	1.5	23.5	4.2 ± 0.1
8	2	23	3.9 ± 0.1
10	2.5	22.5	3.7 ± 0.1
12	3	22	3.6
14	3.5	21.5	3.6 ± 0.1
16	4	21	3.5

The mean temperature of the environment and product during the production process of probiotic mutandabota was logged using i-buttons at 15-min intervals over a 24-h period (Figure 3). The incubation started in the morning at 1120 h. The initial temperature in the fermentation vessel was $29.8 \pm 3.8^\circ\text{C}$; this was also the ambient temperature because boiled milk was cooled to ambient temperature before inoculation. The temperature rose to reach a maximum of $36.5 \pm 6.7^\circ\text{C}$ after approximately 3.5 h, at 1430 h, usually the hottest period of the day in this part of the country. The temperature gradually decreased in the fermentation vessel to a minimum of $22.5 \pm 3.3^\circ\text{C}$ after approximately 21 h from the start. This occurred at around 0700 h. The vessel was removed from the warm kitchen environment at around 0600 h, hence the temperature equilibrated to the environmental temperature outside. The temperature started to increase again to $34.5 \pm 8.5^\circ\text{C}$ when the experiment ended after 24 h. Wood and Holzapfel (1995) noted that *L. rhamnosus*, as a mesophile, grows well at temperatures between 15 and 40°C .

The temperature in the fermentation vessel stayed above both kitchen and environmental temperature during the day and at night (Figure 2). Thus, putting the pot in an advantageous position during the day and at night enabled attainment of the temperature that favored growth of *L. rhamnosus* yoba. The initial temperature within the kitchen was $27.3 \pm 5.6^\circ\text{C}$, the maximum kitchen temperature recorded was $29.4 \pm 3.5^\circ\text{C}$ after 3.5 h, and the minimum kitchen temperature recorded was $19.5 \pm 3.3^\circ\text{C}$ after 19 h. The open environment temperature showed the highest variation

with a maximum of $32.2 \pm 5.3^\circ\text{C}$ after 2 h and the minimum of $16.0 \pm 1.8^\circ\text{C}$ after 19 h. Generally, monthly average high temperature in Binga district ranges from 21 to 31°C , and monthly average low temperature ranges from 15 to 22°C (Worldweatheronline, 2013). This implies that seasonal variations are confined in a narrow range, which allows production of probiotic mutandabota using this procedure throughout the year. However, if this technology was to be applied in other remote, cold regions without electricity, keeping the fermentation vessel close to the fireplace for 24 h would ensure physiologically acceptable growth temperature for *L. rhamnosus* yoba.

Enumeration of *L. rhamnosus* Yoba in Probiotic Mutandabota

Viable counts of *L. rhamnosus* yoba in probiotic mutandabota were determined at different stages of the preparation process (Table 2). The milk supplemented with 4% baobab fruit pulp was inoculated with *L. rhamnosus* yoba at a level of $5.8 \pm 0.3 \log \text{cfu/mL}$. After 24 h of incubation at a temperature regimen of 23 to 37°C (Figure 3), the viable plate count of *L. rhamnosus* yoba increased to a level of $8.8 \pm 0.3 \log \text{cfu/mL}$. This indicates that 4% baobab fruit pulp was sufficient to support an increase in cell numbers of *L. rhamnosus* yoba by 3 orders of magnitude within 24 h. Arnold et al. (1985) and Chadare et al. (2009) reported proximate composition of baobab fruit pulp on a dry-weight basis as (g/100 g) CP 5.3, fiber 13.7, fat 3.6, ash 4.9, and carbohydrates 74.9. The carbon sources in baobab fruit pulp and full-fat cow milk were apparently enough to support growth of *L. rhamnosus* yoba. Murray et al. (2001) reported 35.6 g/100 g (dry-weight basis) simple sugars in baobab fruit pulp. Babu and coworkers (1992) and Kailasapathy and Supriadi (1996) noted that tomato juice and papaya pulp stimulated growth of a related lactic acid bacterium *L. acidophilus*. In the latter case, the growth stimulation was explained by an increased availability of simple sugars, mainly glucose and fructose, and the minerals magnesium and manganese, which are growth promoters for *L. acidophilus* (Ahmed and Mital, 1990). Minerals reported in baobab fruit pulp included (mg/100 g) iron 9.3, calcium 295, magnesium 90, manganese 0.7, zinc 1.8, sodium 2.8, and potassium 1,240 (Osman, 2004). In mutandabota, growth of *L. rhamnosus* yoba was also supported by milk constituents other than lactose. Moreover, boiling of milk releases some free amino acids that promote bacterial growth (Hekmat and McMahon, 1992). The chosen process with preheated milk provided a practically sterile environment for the growth of *L. rhamnosus* yoba, and therefore, no competition existed for nu-

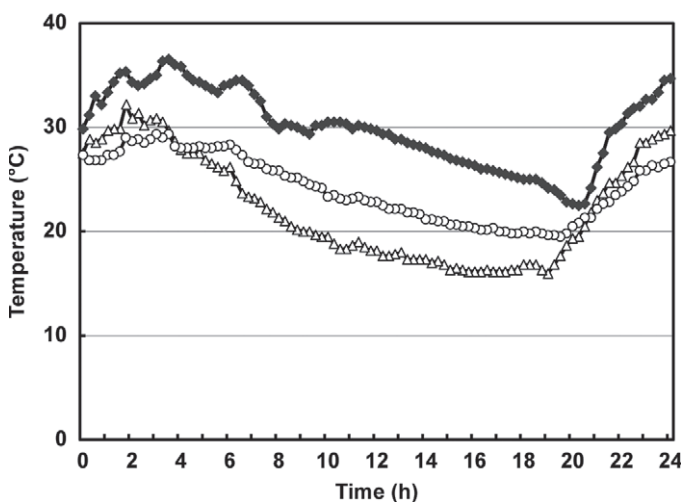


Figure 3. Mean temperatures changes during the production of probiotic mutandabota ($n = 3$). The temperature inside the product mutandabota (closed diamonds), the ambient temperature outside (open triangles), and the temperature in the kitchen (closed circles) were monitored during a period of 24 h.

Table 2. Probiotic mutandabota composition and numbers of *Lactobacillus rhamnosus* yoba at different stages of preparation (n = 3)

Ingredient	Stage			
	Inoculum	At inoculation <i>t</i> = 0	End of incubation <i>t</i> = 24	Probiotic mutandabota on consumption
Pulp (% wt/wt)	4	4	4	14
Milk (% wt/wt)	96	96	96	79
Sugar (% wt/wt)	0	0	0	7
<i>L. rhamnosus</i> yoba (log cfu/mL)	9.3 ± 0.2	5.8 ± 0.3	8.8 ± 0.3	8.8 ± 0.4

trients with other microorganisms associated with raw milk (Mutukumira et al., 1996; Mpofu et al., 2014). A pH value of 4.5, even though suboptimal for growth of *L. rhamnosus* yoba, allowed significant growth to occur. Sheehan et al. (2007) observed that among lactobacilli and bifidobacteria, *L. casei* DN-114 001, *L. rhamnosus* GG, and *L. paracasei* NFBC43338 displayed the highest robustness surviving at levels above 10⁷ cfu/mL in orange juice (pH 3.65) and above 10⁶ cfu/mL in pineapple juice (pH 3.40) for at least 12 wk.

To finish the preparation of probiotic mutandabota, crystalline sucrose and more baobab fruit pulp were added and mixed for 7 min. The general practice is that mutandabota is consumed within the first 12 h after preparation. As shown in Table 2, the viable plate count of *L. rhamnosus* yoba at the moment of consumption was 8.8 ± 0.4 log cfu/mL. Mutandabota is consumed once or twice a day, with servings ranging from 250 to 450 mL depending on the age of the consumer. As such, a meal of mutandabota delivered the probiotic bacterium in numbers well above the recommended beneficial threshold value (Tamime et al., 1995; Kijander et al., 2008). Recovered *L. rhamnosus* yoba cells from probiotic mutandabota just before consumption had typical white, round, and convex colonies. Cell morphology was confirmed by microscope. No colony forming units existed on MRS agar that was spread plated with material from the control sample. No yeast cells were detected in probiotic mutandabota and in the control experiment.

CONCLUSIONS

Probiotic mutandabota was produced at the village level. The amounts of the ingredients in probiotic mutandabota were similar to those in traditional mutandabota, namely 14% (wt/vol) baobab fruit pulp and 7% (wt/vol) sugar in full-fat cow milk. Additionally, probiotic mutandabota had 8.8 ± 0.4 log cfu/mL viable *L. rhamnosus* yoba cells at the moment of consumption. These results show that the criterion for a probiotic food, namely to have a viable cell concentration in excess of 6 log cfu/mL of product, was met. The pH of

probiotic mutandabota was pH 3.5, which ensured its microbiological safety. Baobab fruit pulp at 4% concentration in milk promoted growth of *L. rhamnosus* yoba by 3 orders of magnitude within 24 h. Unlike the current trend where exotic fruits are used in probiotic dairy food products, in this study an indigenous fruit was successfully used to grow a probiotic strain, thus opening an avenue to exploit indigenous fruits as ingredients in the formulation of locally produced probiotic products. Although this work focused on growth of *L. rhamnosus* yoba in mutandabota, the potential exists to apply this approach on other traditional foods as well, thereby enhancing access to probiotics for communities who might need them most. In conclusion, we have developed a means of generating access to *L. rhamnosus* yoba, a probiotic strain, to the rural population of southern Africa, using mutandabota, a traditional food, with viable *L. rhamnosus* yoba in excess of recommended daily intake levels.

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