

RESEARCH ARTICLE

Utilising traditional fermented foods as carriers of probiotics: the case of *Maheu* containing *Lactobacillus rhamnosus* yoba 2012

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ABSTRACT

Traditional fermented foods can be utilised as probiotic carriers. This study evaluated the potential of traditional *maheu* as a carrier for *Lactobacillus rhamnosus* yoba 2012, its nutritional importance, and acceptability as a sustainable probiotic food with health benefits. Acidity, pH, microbial counts, consumer acceptability, and shelf-life stability was determined. Nutrient content was (g / 100 g wb) protein 4.6, carbohydrates 7.1, fibre 2.8, moisture 82.3, ash 2.0, and total soluble solids 4.8. Energy provision was 278 Kcal / 100 g and significant ($p = 0.032$). Mineral content was significantly different ($p < 0.001$). Probiotic *maheu* had *Lb. rhamnosus* yoba count of 7 Log CFU / mL, pH (3.4), and titratable acid of 0.30 %. Consumer acceptability was 6.8 and significant ($p < 0.01$). Acidity, pH, and *Lb. rhamnosus* counts of probiotic *maheu* varied within 0.6 – 0.82 % ($p < 0.0001$), 3.4–3.3 ($p < 0.0001$), and 7–8.2 Log CFU / mL ($p < 0.0001$), respectively in storage. Coliforms, yeast and moulds were < 1 Log CFU / mL ($p < 0.001$). Probiotic *maheu* was estimated to meet over 20 % and 45 % of the recommended dietary allowance for protein and iron in children. This study showed that fermentation can enrich traditional foods with probiotics and makes them available and accessible to the population in sub-Saharan Africa.

Keywords: Fermented cereal beverage; Finger millet; *Lb. rhamnosus* yoba; *Maheu*; Probiotics

INTRODUCTION

The utilisation of probiotics and their products has a projected world market share of US \$15 billion (Bhadoria and Mahapatra, 2011) and make-up a great fraction of the global market for functional foods (Figueroa-González et al., 2011). Functional foods are foods that contain ingredients that are beneficial to health and examples include prebiotics, probiotics, vitamins, and minerals (Franz et al., 2014). International food research is now focused on probiotic bacteria and their health effects (Mpofu et al., 2017). The probiotics are being used in suitable applications in some developing nations in Africa and other developed countries in the world (Westerik et al., 2018). Probiotic is defined as a live microorganism that can move through the gastrointestinal tract passage, reaching the intestinal tract in its active form insufficient viable counts that would positively affect the health condition of the host (Franz et al., 2011). A probiotic bacterium is consumed as part

of the food to confer its health benefits (WHO, 2002; Mpofu et al., 2014). Existing probiotic supplements and products primarily contain strains of the bacterial genera *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Enterococcus*, and the yeast *Saccharomyces boulardii* (Kerry et al., 2018). These effective probiotics are limited and not affordable to the poor in Southern Africa (Kort et al., 2015).

The mechanism of action of probiotics on improving health have been found to include fighting pathogens, improvement of lactose intolerance symptoms, immune-modulation, anticarcinogenic, and antimutagenic processes, reducing blood cholesterol levels, and protection of intestinal mucosa conditions (Kerry et al., 2018, Kumari et al., 2011).

Diarrhea was reported to cause more than 800, 000 deaths in children every year (UNICEF/WHO, 2009). Diarrhea is also the principal cause of malnutrition in children who

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are under five years old in Sub-Saharan Africa (WHO, 2013, Lancet, 2015). In Sub-Saharan Africa, it accounts for approximately 37 % of deaths among children (Chawafambira et al., 2020a; Mpofu et al., 2015, Franz et al., 2014).

Meta-analysis study results on probiotics have concluded the potential benefits of specific bacteria strains on the treatment of diarrhea in children (Salari et al., 2012, WHO, 2013). There is, but, limited data on the use of probiotics in Africa. As of 2005, the only recognised market for probiotics products in Africa was in the form of fortified foods, supplements, and fermented dairy products with most strains being used in South Africa (Brink et al., 2005).

Maheu is a lactic acid non-alcoholic fermented maize-based beverage produced at homes (Pswarayi and Gänzle, 2019) and recently at the industrial level in southern African (Matsheka et al., 2013). It is naturally fermented at a temperature of 20–30 °C by dominant microorganisms belonging to *Lactococcus lactis* subsp. *Lactis* (Blandino et al., 2003) and contains very little or no alcohol with a pH between 2.74–3.5 (Mashau et al., 2019). Consumption of *maheu* by a black adult is estimated to be around 12–14 L per year (Steinkraus, 2004). In Zimbabwe, *maheu* is mostly consumed by all age groups together with other traditional fermented foods such as mutwiwa or ilambazi lokubilisa a fermented maize porridge, and mukaka wakakora / amasi or hodzeko a fermented milk product (Idowu et al., 2016). A study by Pswarayi and Gänzle, (2019) reported the presence of *Lactobacillus fermentum* FUA3588 and FUA3589 and *Lactobacillus plantarum* FUA3590 in *maheu* produced in Zimbabwe. Another study by Fadahunsi and Soremekun, (2017) observed that microorganisms associated with the fermentation process were *L. bravis* (54%), *L. casei* (23%), *L. plantarum* (12%), *L. lactis* (8%), and *S. cerevisiae* (70%) in Nigeria. Many studies have been conducted on maheu. More so, a study by Olusanya et al. (2020) reported an increase in calcium content by 350, 700, and 950% in *maheu* supplemented with 2, 4, and 6% Moringa oleifera leaf powder in South Africa respectively. No study has been conducted on maheu as a carrier of *L. rhamnosus* yoba 2012.

The utilisation of traditional fermented foods and the incorporation of probiotics may potentially act as a promising way of improving the health of children in Africa (Franz et al., 2014). FAO (2001) reported the benefits of lactic acid bacteria strains on food safety. This was supported by Mpofu et al. (2015) who observed the suppression effect of *Lb. rhamnosus* yoba 2012 on five food pathogens in probiotic mutandabota in Zimbabwe. *Lactobacillus rhamnosus* GG is a potential strain to be used in Africa. *Lactobacillus rhamnosus* GG (LGG) is the most widely studied probiotics that have proven treatment of

gastrointestinal diseases (Kerry et al., 2018). Recently, LGG, in the form of *Lactobacillus rhamnosus* yoba 2012, a generic probiotic is being applied as practical solutions to be used to access probiotics in food processing by many rural folks in Africa (Kort and Sybesma, 2012). The probiotic, *L. rhamnosus* yoba has some related genomic properties with *L. rhamnosus* GG (Petrova et al., 2018), and it has the possibility to inactivate other pathogenic bacteria and fungi present in the urogenital tract (Reid, et al., 2018). Franz et al. (2014) reported the use of *Lb. rhamnosus* yoba 2012 in traditional food products because of their availability and affordability by most people. Traditional fermented foods are diverse in Africa and *Lb. rhamnosus* yoba 2012 has been used in traditional products like Mutandabota, Obushera, and Uji (Mpofu et al., 2014; Kort et al., 2015; Wacoo et al., 2019). Cereal based fermented food products offer good prospects for use as probiotic carriers since they are widely consumed in Africa. The objectives of this study were to determine the potential of traditional *maheu* as a carrier of *Lb. rhamnosus* yoba 2012 and analyse the functional, nutritional, and sensory properties of the prepared probiotic *maheu*.

METHODS AND MATERIALS

Sample collection

White dried maize and sorghum grains were obtained from the rural area of Bikita (dry area located 20. 5 S° 31.37° E in Zimbabwe) were cleaned of extraneous matter. The sorghum grains were soaked in water for 1 hour and then dried under the sun for 12 hours. The dried sorghum grains and maize grains were both milled in a laboratory mill (Model RLA, 201 – 80014, Hammer mill, UK) and passed through a sieve with a size 800 µm (Model. HL 20, Gallenkamp and Co Ltd) to obtain fine maize and sorghum meal. The milled grains were then stored in an airtight polythene plastic (0.77 mm thickness) at 25 °C.

Maheu preparation

Traditional *maheu* samples were prepared according to a traditional method by Chelule et al. (2010) with modifications. Finger millet malt (Fig. 2) was used as a substitute for wheat flour in the original method. Maheu was prepared by mixing 480g of maize flour and water (4L). The mixture was cooked with constant stirring up to 95 °C for 15 minutes until a smooth gel was produced. The gel was cooled to room temperatures (25 °C). Thereafter, 65 g of finger millet malt flour was added to the gel before inoculation (Fig. 3 a). The slurry was then inoculated with *Lb. rhamnosus* yoba starter to a concentration of 5.8 log CFU/mL. Fermentation was carried out at 37°C for 36 hours (h) (Fig. 3 b). Sugar (100 g) was then added before consumption and the *maheu* was stored at 4 °C

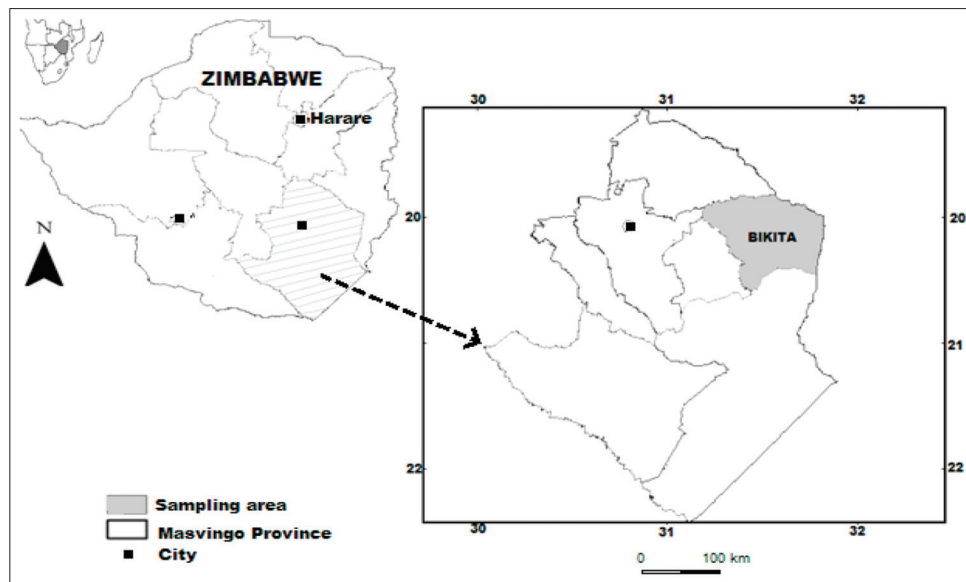


Fig 1. Finger millet and maize grain sampling area in Bikita, Zimbabwe



Fig 2. White maize meal and Malt

(Fig. 3 c). Total titratable acid, pH, total soluble solids were determined at $t = 0, 6, 12, 18, 24, 30$, and 36 h and microbial counts at $t = 0, 12, 24$, and 36 h.

Preparation of Inoculum

An isolate, *Lb. rhamnosus* yoba 2012 was obtained from a commercial product containing *L. rhamnosus* GG and was identified, and confirmed using 16S rRNA sequencing (Kort and Sybesma, 2012). Pure strains of *Lb. rhamnosus* yoba was purchased from the Yoba for Life Foundation, Amsterdam, Netherlands, and kept at -80°C . The pure strains of *Lb. rhamnosus* yoba was reactivated by sub-culturing anaerobically in De Man, Rogosa, and Sharpe agar (MRS) broth at 37°C for 18 h. A pre-mix of *mabeu* was mixed with sugar, boiled, and subsequently cooled to room temperature (25°C). One gram of *Lb. rhamnosus*

yoba pure strain was then precultured in the medium (1000 mL) and incubated at 37°C for 36 h. The growth of the bacterium was monitored until the number of live cells was >5 Log CFU / mL.

Inoculation of *mabeu*

Traditional *mabeu* was opened under aseptic conditions and inoculated with a (1% v/v) fresh pre-cultured *mabeu* (backslopping) (Fig. 4). *Lb. rhamnosus* yoba cell suspensions of the culture were gradually mixed with the *mabeu*. In the control experiment, the traditional *mabeu* sample was inoculated with distilled water.

Determination of growth rate of *Lb. rhamnosus* yoba

The growth rate of *Lb. rhamnosus* yoba in the traditional *mabeu* was determined at $t = 0, 12, 24$, and 36 h. One millilitre of the sample was aseptically taken from the *mabeu* at a 12 hourly interval. Thereafter, serial decimal dilutions were carried out in peptone physiological salt solution (pH 7 , 8.5 g / L NaCl, and 1 g / L neutralized bacteriological peptone from Oxoid). Diluents of $100\ \mu\text{L}$ were plated onto de Man, Rogosa, and MRS agar (1.2% agar, bacteriological peptone from Oxoid, added to de Man, Rogosa, and Sharpe broth, Merck) in triplicate. MRS agar plates were incubated at 37°C under anaerobic conditions in Gas Pack anaerobic jars (Becton Dickinson Microbiology Systems, Baltimore, Maryland, USA). Colonies on MRS agar were counted and expressed as colony-forming units per milliliter (CFU / mL) of *Lb. rhamnosus* yoba.

Nutritional and Functional analysis

Proximate analysis on moisture content using (AOAC method 925.45), ash content using dry ashing (AOAC method 938.08), crude fibre using the enzymatic gravimetric method (AOAC



Fig 3. Prepared *maheu* gel (a), Inoculated and incubated *maheu*, (b) Fermented *maheu* (c)

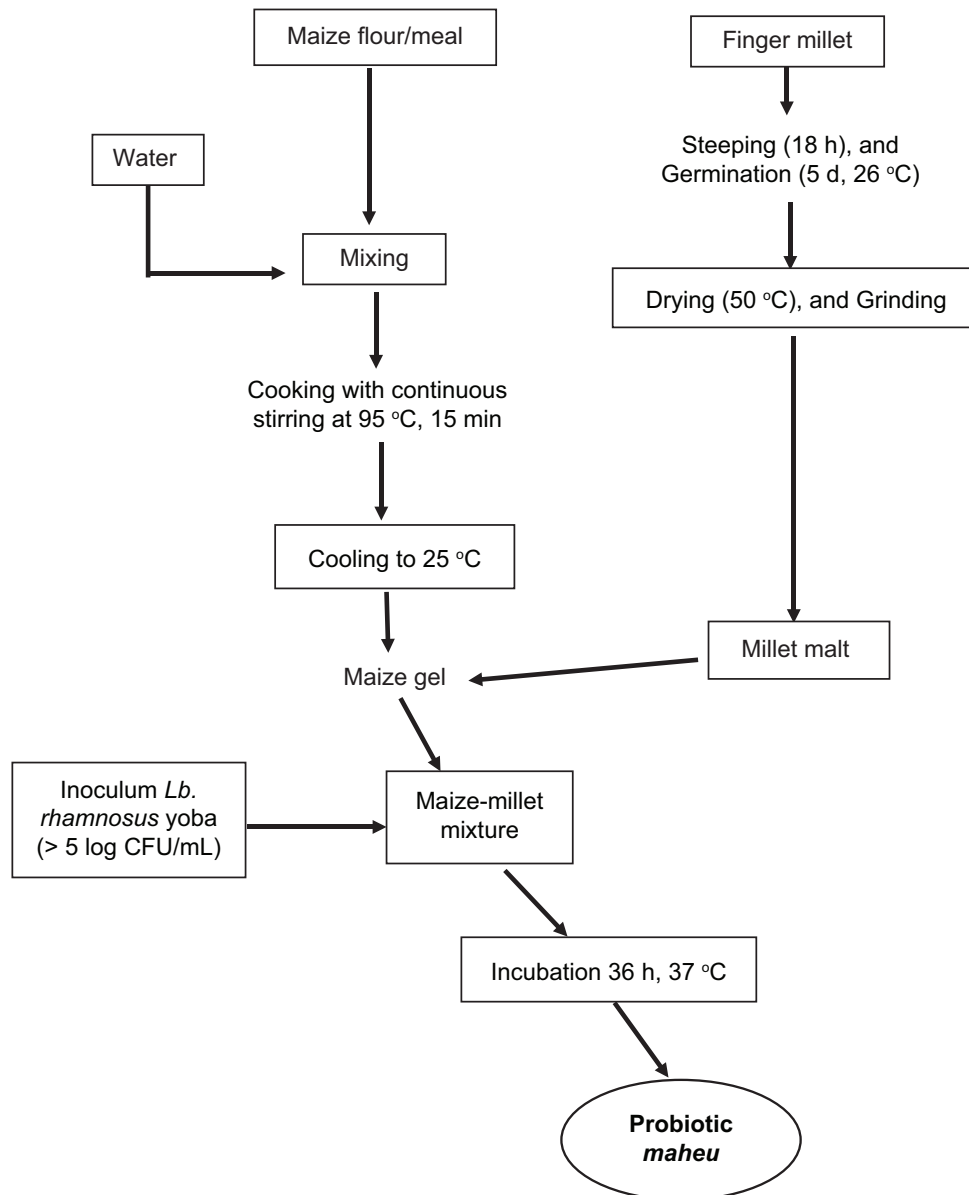


Fig 4. Flow chart for the Probiotic *maheu* production

method 985.29), crude fat using Soxhlet method (AOAC 989.05), and crude protein using Kjeldahl (AOAC method 991.20) were determined according to standard methods by the Association of Official Analytical Chemists (AOAC,

2005). Total carbohydrate was determined by the difference method. The probiotic *maheu* was estimated on its % contribution in the diet on the recommended daily allowances of nutrients per 100 g consumption of different age groups.

pH, total soluble solids, Total titratable acidity

The pH, total soluble solids, and total titratable acidity (TTA) were determined according to a standard method adopted from AOAC (2005). The pH and TTA measurements were taken at $t = 0, 6, 12, 18, 24, 30$, and 36 h and in storage ($d = 0, 5, 10, 15$, and 20).

Mineral content analysis

Mineral analysis of the *mabeu* samples was determined using an Inductively Coupled Plasma–Optical Emission Spectrometer (ICP-OES) (Agilent 5100, Agilent Technologies, Santa Clara, California, USA) (AOAC, 2005). The samples were digested using concentrated solutions of nitric acid (HNO_3) and sulphuric acid (H_2SO_4), followed by the addition of ultrapure hydrogen peroxide (H_2O_2) to complete digestion. The sample was then fed into the ICP-OES and mineral results were recorded.

Estimation of shelf-life and microbiological analysis

Fermented *mabeu* samples were stored at refrigeration temperature for 20 days. Ten (10 ml) of each *mabeu* sample was taken after every five days up to 20 days for pH, acidity, and microbiological analyses. A 1 ml sample of fermenting *mabeu* samples was aseptically collected and suspended in sterile 9 ml distilled water tubes. The samples were then serially diluted. An aliquot of 0.1 ml volume of dilutions was inoculated on sterile disposable Petri dishes using the pour plate method. Potato dextrose agar (PDA, deMann Rogosa Sharpe (MRS) agar, and violet red bile agar (VRBA) were used for yeast and mould, lactic acid bacteria (LAB), and coliforms respectively. The inoculated plates with MRS agar, PDA, and VRBA were incubated at 30°C in an anaerobic jar for 72 h, 25°C for 120 h, and 37°C for 24 h respectively. Microbiological counts for each media was then carried out.

Sensory analysis

An untrained panel ($n = 50$) consisting of students, staff members, and families in the rural Bikita area was used to assess the acceptability of the probiotic *mabeu* after fermentation. Each untrained panelist was served with 50 mL of probiotic *mabeu* sample. The panellists determined the product's overall acceptance and scored their responses on a 9 point hedonic scale (1 = dislike extremely to 9 = like extremely). Product attributes that were analysed were taste, colour, sourness, and appearance. The samples were stored at 4°C for 20 days.

Statistical analysis

The results were expressed as the mean \pm standard deviation (SD), and all experiments were conducted in triplicates. The least significant differences (LSD) test was used to determine a significant difference in the means at $p < 0.05$. Results on customer acceptance were analysed

with a one-way Analysis of variance (ANOVA) for the F-test. Changes in titratable acidity and pH in *mabeu* samples were analysed using a student *t*-test. All the analysis were done using SPSS package version 18.0 (Coakes and Ong, John Wiley & Sons, Queensland, Australia).

RESULTS AND DISCUSSION

Chemical composition

The nutrient content of the probiotic *mabeu* was carbohydrate 7.10 ± 1.05 g / 100 g, fat 1.08 ± 0.01 g / 100 g, crude protein 4.62 ± 0.02 g / 100 g, ash 2.06 ± 0.01 g / 100 g, crude fiber 2.80 ± 0.05 g / 100 g (Table 1). The nutrient contents were significantly different at $p < 0.05$. Fadahunsi and Soremekun (2017) reported a decrease in protein, fat, ash, fibre and carbohydrate contents from 11.00%, 4.83%, 1.55%, 1.10% and 66.72% to 9.21%, 2.02%, 1.03%, 0.83%, and 63.01% respectively in *mabeu*. The probiotic *mabeu* could act as a good source of dietary carbohydrate, protein, fibre, and water in the human body. The nutrient content results are attributed to the ingredients used in making *mabeu*. Abiose and Ikujenlola (2014), reported a nutrient content (g / 100 g) of carbohydrate 73.83 ± 0.04 , fibre 2.60 ± 0.02 , protein 9.80 ± 0.01 , and moisture 7.65 ± 0.10 in maize flour. Furthermore, Ramasha et al, (2018) reported a carbohydrate (75 - 83.3 g / 100 g), protein (7.7 g / 100 g), fat (1.8 g / 100 g), fibre (15-22 g / 100 g), and moisture (7.15 – 13.1 g / 100 g) content in finger millet. The fibre and fat content of the probiotic *mabeu* might have been improved by the malting process on millet grains before milling. This was supported by Ramashia et al. (2019) who reported an improvement in crude fibre, crude fat, vitamin B, C, mineral content, and a decrease in antinutritional compounds.

Table 1: Nutrient content of probiotic and control *mabeu*

Nutrient (g / 100 g)	Probiotic <i>mabeu</i>	Control	p-value
Carbohydrates (g / 100 g)	7.10 ± 1.05^a	6.87 ± 0.01^b	0.037
Crude Protein (g / 100 g)	4.62 ± 0.02^a	3.20 ± 0.05^b	0.012
Fat (g / 100 g)	1.08 ± 0.01^a	0.72 ± 0.02^a	0.142
Crude fiber (g / 100 g)	2.80 ± 0.05^a	2.61 ± 0.03^a	0.161
Ash (g / 100 g)	2.06 ± 0.01^a	1.86 ± 0.05^a	0.115
Moisture (g / 100 g)	82.3 ± 0.02^a	84.7 ± 0.01^a	0.712
Total soluble solids (g / 100 g)	4.8 ± 0.01^a	4.6 ± 0.03^a	0.820
Energy (Kcal / 100 g)	278 ± 0.05^a	265 ± 0.05^a	0.032

Mean \pm standard deviations are reported. Means in a row with different superscripts (a,b) are significantly different at $p < 0.05$

Nutrient deficiency is widespread in many rural and urban areas of sub-Saharan Africa, especially protein-energy malnutrition. The use of finger millet in probiotic *mabeu* would benefit in the consumption of quality protein. Finger millet grains were observed to contain 44.7 % of essential amino acids (Singh and Raghuvanshi, 2012) comprising methionine, tryptophan, and cysteine (Manjula et al., 2015; Ramashia et al., 2018), lysine (Mamatha and Begum, 2013); leucine, phenylalanine, isoleucine (Sood et al., 2017), and threonine which is beneficial in lowering cholesterol levels and reducing the risk of obesity and cancer in the human body (Thapliyal and Singh, 2015). The low fat and relatively high dietary fiber content make the probiotic *mabeu* a good beverage for health-conscious people. More so, Vanithasri et al. (2012) and Banusha and Vasantharuba, (2013) were in agreement and reported that low fat, relatively high dietary fibre, and presence of some non-starchy polysaccharides help in physiological benefits such as hypoglycemic and hypocholesterolaemic effects and providing nutrition. Dietary fibre content could be attributed to the presence of pectin, lignin, cellulose, β -glucan, and arabinoxylan (Prashantha and Muralikrishna, 2014).

The mineral content of *mabeu* samples is illustrated in Table 2. Probiotic *mabeu* had high phosphorus (112.3 ± 6.24 mg / 100 g wb), potassium (87.2 ± 4.13 mg / 100 g wb), calcium (88.2 ± 3.21 mg / 100 g wb) and magnesium (96.2 ± 3.12 mg / 100 g wb) contents. A study by Fadahunsi and Soremekun (2017) reported a decrease in sodium, potassium, calcium, iron, zinc and manganese contents from 0.058, 0.10, 0.06, 2.5mg/kg, 0.10 and 0.70mg/kg to 0.05, 0.82, 0.55, 1.96, 0.91, and 0.52mg/kg in the *mabeu* samples. The high mineral content might be attributed to the addition of finger millet as an ingredient. Ramashia et al. (2019) reported a mineral content (mg / 100 g) for calcium 398, phosphorus 130-250, potassium 430-490, sodium 49, iron 3.3-14 and zinc 2.3 in millet. Phosphorus helps in energy metabolism and the development of the body (Ramashia et al., 2018). This is supported by Chawafambira et al. (2020a) and Hina et al. (2018) who reported the importance of phosphorus in the synthesis of biomolecules and the formation of energy-rich molecules such as guanosine triphosphate (GTP), cytidine triphosphate (CTP), adenosine triphosphate (ATP), uridine triphosphate (UTP), cytidine triphosphate (CTP), phosphoenolpyruvate (PEP) and carbohydrate metabolism (Razaq et al., 2017). Probiotic *mabeu* also contain a significant amount of essential minerals such as iron zinc and magnesium. Chawafambira et al. (2020b) also noted an increase in iron and zinc bioaccessibility in probiotic jam containing *Lb. rhamnosus* yoba. The mineral content results for both probiotic and control *mabeu* were significantly different at $p < 0.05$ using a student *t*-test.

Table 2: The mineral content of probiotic and control *mabeu* per 100 g wet basis

Mineral (mg)	Probiotic <i>mabeu</i>	Control	p-value
Iron	3.10 \pm 0.02 ^a	1.85 \pm 0.03 ^b	<0.001
Magnesium	96.2 \pm 3.12 ^a	87.2 \pm 1.23 ^b	0.015
Calcium	88.2 \pm 3.21 ^a	72.8 \pm 2.40 ^b	<0.001
Zinc	1.85 \pm 0.08 ^a	1.12 \pm 0.05 ^a	0.09
Potassium	87.2 \pm 4.13 ^a	78.6 \pm 2.80 ^b	0.021
Phosphorus	112.3 \pm 6.24 ^a	101.2 \pm 4.60 ^b	<0.001
Copper	0.20 \pm 0.01 ^a	0.10 \pm 0.01 ^a	0.621
Sodium	3.22 \pm 0.03 ^a	2.86 \pm 0.01 ^b	0.022

Mean \pm standard deviations are reported. Means in a row with different superscripts (^{a, b}) are significantly different at $p < 0.05$

Table 3: Viability of *Lb. rhamnosus* yoba in probiotic *mabeu*

Time (hours)	Viable cells (Log CFU / mL)
Inoculation: t = 0	5.8 \pm 0.1 ^a
12	6.4 \pm 0.2 ^b
24	6.6 \pm 0.1 ^b
End of incubation t = 36	7 \pm 0.1 ^c
p- value	<0.0001

Values are mean \pm standard deviations. Means with different superscripts (^{a, b, c}) in a column are significantly different at $p < 0.05$

Enumeration of *Lb. rhamnosus* yoba in the probiotic jam

Lb. rhamnosus yoba was able to grow in, and fermented, *mabeu* with its cell counts increasing from 5.8 log Log CFU / mL to 7 log Log CFU / mL in 36 h ($p < 0.0001$). Mpofu et al. (2014), reported *Lb. rhamnosus* yoba level of 5.8 ± 0.1 Log CFU / mL in the inoculum used to produce probiotic *mutandabota*. Chawafambira et al. (2020b) observed a *Lb. rhamnosus* yoba viable count of 6.2 Log CFU / mL in probiotic jam prepared for *U. kirkiana* fruits. Mukisa et al. (2019) noted an increase in cell counts of *Lb. rhamnosus* yoba 2012 from 7.2 to 8.8 log CFU/ml in a sorghum fermented, Obushera food after 24 h. The results could explain that the *mabeu* food matrix was able to support the growth of *Lb. rhamnosus* yoba. Moreover, Wood and Holzapfel (1995) reported that *Lb. rhamnosus* yoba grows at a wide temperature range of 15 – 40 °C and is a mesophile bacteria. The simple sugars produced from the breakdown of starch and other complex sugars could have acted as sources of carbon needed to support the growth of *Lb. rhamnosus* yoba. This is supported by a study by Mpofu et al. (2014). Maize flour acted as the main substrate and source of sugars. Ahmed and Mital (1990) reported the simulated growth of *L. acidophilus* spp caused by the presence of glucose, fructose, manganese, and magnesium which act as growth promoters of the bacterium. Mukisa et al. (2017) reported *Lb. rhamnosus* yoba growth of 7– 9 log CFU / g in probiotic dairy yoghurt containing cooked banana/ *matooke* puree after 24 h of fermentation. Also, Mukisa et al. (2019) noted an increase in *Lb. rhamnosus* yoba count from 7.2 to 8.8 log CFU / ml in 24 h ($p < 0.0001$) in probiotic Obushera.

pH and acidity

Table 6 shows the changes in the pH and acidity of probiotic *mabeu* samples during the incubation period of 36 h. The results showed a decrease in pH values during the fermentation period and ranged between pH 6.85 and pH 3.4 ($p < 0.001$). The decrease in pH values in probiotic *mabeu* samples could be ascribed to *Lb. rhamnosus* yoba bacteria that was able to compete with other natural microorganisms that were available in the fermented *mabeu* sample and dominated the other microorganisms by natural selection and succession process. This was supported by Ramaite, (2004). Furthermore, *Lb. rhamnosus* yoba and other natural microorganisms were capable of producing high amounts of organic acids and surviving the acidic environment (Fowoyo and Ogunbanwo, 2010). The low pH observed during the fermentation process is beneficial in reducing the growth of most bacteria, including the pathogenic microorganisms, and this makes the *mabeu* microbiologically safe, as well as extending its shelf life (Halm et al., 1993). Mashau et al. (2019) reported a pH content of 3.9 which significantly decreased ($p < 0.001$) during storage in *mabeu* inoculated with wheat flour. Mukisa et al. (2019) noted a decrease in pH from 4.9 to 3.9 ($p < 0.0001$) and increase in titratable acidity from 0.31 % to 0.74 % ($p < 0.0001$) after 24 h in *Obushera*. Other authors have reported that pH 6 is important to support the growth of most microorganisms in food products (Parente et al., 1994; Akerberg et al., 1998).

Titratable acidity (TA) increased significantly ($p < 0.05$) during the fermentation period from 0.3 and 0.6 % lactic acid (v/v). The TA results showed a general increase in probiotic *mabeu* at 18 h (0.4), and it continued to increase up to the end of incubation 36 h (0.63). The observed increase in TA suggests the action of lactic acid bacteria, *Lb. rhamnosus* yoba and other natural fermentative microorganisms in *mabeu*. Adesokan et al. (2011) reported that lactic acid bacteria breakdown sugars to produce lactic acid as well as other secondary fermentation products, hence, the sour taste of *mabeu*. More so, Sanni, 1993 explained that other secondary products of lactic acid fermentation such as acetic, butyric, and propionic acids that are produced can result in the reduction of the pH to 3 and a TA of almost 0.6 %. The noted increase in TA would be significant in limiting the growth of harmful bacteria that result in bad fermentation in *mabeu*. The obtained TA results on the control *mabeu* (without probiotic bacteria) sample were in agreement with other previous studies by other researchers on *mabeu* (Adesokan et al., 2008; Gotcheva et al., 2001). Mashau et al. (2019) noted a TA of 0.2 % in *mabeu* inoculated with wheat flour.

There was a significant difference ($p < 0.05$) in the TSS content in the probiotic *mabeu* (4.8 °Brix). The observed

decrease in the TSS could be attributed to the high bacterial count of *Lb. rhamnosus* yoba, which suggests the increase in the utilisation of available solids (Kutyauripo et al., 2009). Mashau et al. (2019) reported a TSS of 4.7 °Brix in the *mabeu* sample produced using wheat flour as an inoculum. As the microbial counts of *Lb. rhamnosus* yoba increased it resulted in the breakdown of accessible sugars. This is correlated with the increase in the lactic acid results observed in this study (Jay et al., 2005). The amount of sugar utilisation was higher in probiotic *mabeu* as compared to the control sample. Zvauya et al. (1997) reported the leveling off of solids caused by the fermentation process as it prevents metabolic activity. Mukisa et al. (2019) reported a decrease in TSS from 15.2 to 14.5 °Brix after 24 h of fermentation during the production of probiotic *Obushera* in Uganda.

Microbiological properties of *mabeu* samples

There was a relatively small increase in numbers of lactic acid bacteria (LAB), coliform bacteria, and yeast and moulds in both *mabeu* samples (Table 5). The increase in microbial counts was significant ($p < 0.05$) although the counts were very low and with acceptable limits in food. Results on coliforms indicated the level of basic hygiene during the processing of *mabeu*. Kutyauripo et al. (2009) reported the source of microorganisms as the ingredients used in the production of the *mabeu* such as maize flour, malt, utensils, and water. Minervini et al. (2015) reported that malt microbiota does not only serves as inoculum for the lactic acid fermentation but it provides endophytic microbiota since *mabeu* is consumed without further heating after fermentation. The highest LAB counts were observed after day 5 in the control sample. Also in probiotic *mabeu*, the *Lb. rhamnosus* yoba increased to 8.0 Log CFU / mL after day 4. The LAB counts increased from 3.0 Log CFU / mL (day 0) to 3.3 Log CFU / mL in day 10 ($p < 0.05$). This suggests that during the fermentation process, the LAB increased which caused quick fermentation of *mabeu* samples. This explanation supports the observed increase in *Lb. rhamnosus* yoba in probiotic *mabeu* in storage. More so, the low pH of *mabeu* due to the increase in lactic acid might have allowed the growth of *Lb. rhamnosus* yoba and inhibited most competing microorganisms. Kalui et al. (2009) and Reid, (2008) reported that LAB produces antibiotic substances that could inhibit the growth of other microorganisms during fermentation. Furthermore, the increase in LAB counts in the control *mabeu* sample during storage might be attributed to the ability of the LAB isolates to dominate and conquer the *mabeu* solution thereby suppressing the growth of other unwanted microorganisms. Oyeyiola (1990) noted similar results on *mabeu*.

There was a significant decrease ($p < 0.05$) in mould growth in *mabeu* samples on day 10 in storage. Probiotic *mabeu* had

Table 4: pH and acidity of the probiotic *maheu* during fermentation period of 36 h

Time (hours)	pH		p-value	Acidity (% lactic acid)		p-value
	Probiotic <i>maheu</i>	Control		Probiotic <i>maheu</i>	Control	
Inoculation: t = 0	6.85±0.12 ^{ax}	6.85± 0.12 ^{az}	0.10	0.30±0.01 ^{ax}	0.31±0.02 ^{ax}	1.08
6	6.0±0.10 ^{ax}	6.2± 0.10 ^{bx}	0.06	0.36±0.04 ^{ax}	0.34±0.02 ^{ax}	0.09
12	5.5±0.10 ^{ax}	5.6± 0.10 ^{ay}	0.055	0.44±0.07 ^{ay}	0.40±0.01 ^{bx}	0.04
18	4.9±0.11 ^{ay}	5.0± 0.12 ^{ay}	0.10	0.49±0.05 ^{ay}	0.46±0.02 ^{ay}	0.08
24	4.5±0.10 ^{ay}	4.4± 0.10 ^{ax}	0.1	0.55±0.01 ^{by}	0.49±0.02 ^{ay}	0.02
30	3.8±0.02 ^{az}	3.9± 0.12 ^{bx}	<0.01	0.58±0.01 ^{az}	0.52±0.01 ^{by}	0.02
End of incubation t = 36	3.4±0.11 ^{az}	3.6± 0.01 ^{bx}	<0.001	0.63±0.05 ^{az}	0.55±0.03 ^{bx}	0.01
p-value	<0.0001	<0.0001		0.01	0.03	

Mean±standard deviations are reported. Means in a row with different superscripts (^{a, b}) are significantly different at $p < 0.05$. Values in the same column with different superscripts (^{x, y, z}) are significantly different ($p < 0.05$)

Table 5: Physiochemical and Microbiological properties of *maheu* in storage

Day	Sample	pH	TA	LAB	Yeast	Moulds	Coliforms
0	Probiotic <i>maheu</i>	3.4	0.63	7	ND	ND	ND
	Control	3.6	0.55	3.0	<1	2	ND
5	Probiotic <i>maheu</i>	3.3	0.68	7.5	ND	ND	ND
	Control	3.5	0.64	3.0	1	1	ND
10	Probiotic <i>maheu</i>	3.2	0.75	7.8	ND	ND	<1
	Control	3.4	0.72	3.1	1	1.2	<1
15	Probiotic <i>maheu</i>	3.1	0.78	8.0	>1	>1.1	<1
	Control	3.3	0.81	3.2	2	1.5	<1
20	Probiotic <i>maheu</i>	3.0	0.82	8.2	1	1.2	1
	Control	3.2	0.93	3.2	1	1.3	<1
	p-value	0.023	0.03	< 0.0001	<0.0001	0.000	0.02

Mean bacterial counts Log CFU / mL; ND = Not Detected; LAB = Lactic acid bacteria

Table 6: Mean scores for sensory acceptability of *maheu* samples

Sample	Appearance	Taste	Colour	Sourness	Overall acceptability
Day 0					
Probiotic <i>maheu</i>	8.0±1.1 ^a	7.8±1.1 ^c	7.2±1.5 ^c	6.7±1.4 ^c	6.8±1.2 ^a
Control	7.3±1.4 ^b	7.2±1.6 ^b	6.0±1.6 ^a	6.1±1.5 ^b	5.5±2.1 ^b
Day 20					
Probiotic <i>maheu</i>	7.6±1.3 ^b	7.7±1.1 ^c	6.8±1.5 ^b	5.8±1.8 ^a	5.4±1.1 ^b
Control	7.0±1.1 ^c	6.8±1.2 ^a	5.7±1.3 ^a	5.6±1.5 ^a	5.3±1.0 ^b
p-value	0.037	0.026	<0.001	0.022	0.032

Mean±SD. Mean values in the same column with different superscripts (^{a, b, c}) are significantly different from each other ($p < 0.05$)

a decrease to <1 Log CFU / mL and control *maheu* sample decrease from 2 to 1 Log CFU / mL. The observed results could be ascribed to the effect of a decrease in pH during fermentation (Table 5). Mashau et al. (2020) and El-Gendy and Marth, (1980) indicated that acids produced by bacteria were shown to inhibit the growth of moulds. Yeast counts increased during storage ($p < 0.0001$). The increase in the yeast counts might be attributed to the decrease in the pH which was found to favour the growth of yeast (Serna-Saldivar and Rooney, 1995).

Sensory properties of *maheu* samples

Probiotic *maheu* was significantly different from control in terms of appearance, taste, colour, sourness, and overall acceptability on days 0 and 20 (Table 6). Probiotic *maheu* had a high acceptance score and this suggests that the addition of *Lb. rhamnosus* yoba affected the organoleptic

characteristics. Salmeron et al. (2015) and Nyanzi et al. (2010) reported the possibility of changes in flavour profile, sensorial properties, and acceptability of fermented products due to the action of probiotic starters. Therefore, it became important to assess the effect of adding *Lb. rhamnosus* yoba on the acceptability of traditional fermented *maheu* in this study. More so, Nyanzi et al. (2010) suggested the importance of evaluating the market potential of novel probiotic products as compared with other existing related traditional food products. This study revealed that *Lb. rhamnosus* yoba starter had a significant effect ($p < 0.001$) on the acceptability of probiotic *maheu*. Mukisa et al. (2019) noted no significant effect of *Lb. rhamnosus* yoba on the acceptability of Obushera, a traditionally fermented sorghum-based beverage in Uganda. Wacoo et al. (2019) reported similar results on the effect of *Lb. rhamnosus* on

Kwete, is a fermented maize product from Uganda. Panelists showed their willingness to buy the probiotic *mabeu* and thus increases the accessibility of probiotics in Zimbabwe.

CONCLUSION

Traditional *mabeu* is part of the food cultural heritage of most rural and urban communities in Zimbabwe. The good fibre, protein, TSS, and mineral content as noted in this study, makes probiotic *mabeu* a good source of nutrition, especially where malnutrition needs important attention in Sub-Saharan Africa. Traditional *mabeu* was able to support the grow *Lb. rhamnosus* yoba and proved that locally available traditional foods have the potential to be carriers for probiotics. The *Lb. rhamnosus* yoba starter was able to grow and ferment traditional *mabeu*. *Lb. rhamnosus* yoba counts were 7 Log CFU / mL with a food with a low pH (3.4) and TA (0.63) after 36 h at 37 °C. The probiotic *mabeu* was acceptable by people and this forms the basis for its consumption as a source of nourishment to the nutritionally risk groups of people in society such as children, pregnant and lactating women in rural areas. Probiotic *mabeu* remained stable in storage under refrigeration conditions and low level of contamination. Consequently, traditional fermented foods can be adapted to produce probiotic foods that become acceptable and accessible to the local population in Zimbabwe. Moreover, there is a need to carry out further studies on the effect of yoba *mabeu* on the inactivation of pathogenic microorganisms in fermented *mabeu* as a way of bringing assurance on the safety of the product as compared to home-made traditional *mabeu*. Furthermore, nutritional research on the bioavailability/bioaccessibility of essential minerals is necessary.

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Authors' contributions

Chawafambira Armistice conceived the study design, data acquisition, and performed the experiments together with Tafadzwa Mkungunugwa. Mkungunugwa Tafadzwa did the data analysis and Chawafambira Armistice wrote the draft manuscript. Mkungunugwa Tafadzwa proofread the manuscript.

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