Development of probiotic *mutandabota*, a locally sustainable functional food incorporating *Lactobacillus rhamnosus*

AUGUSTINE MPOFU

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Contents

Abstract

Mutandabota or *umlondo* is an indigenous food that is consumed in Southern Africa on a daily basis. The product is made by mixing raw cow's or goat's milk with 14 % (wt/vol) dry pulp of the baobab fruit (*Adansonia digitata* L.) and 7 % sugar. *Mutandabota* has a high protein content, and is rich in vitamin C and minerals. It also provides fibre to the diet, which evidently has potential health benefits in preventing diabetes, cardiovascular diseases, some cancers and constipation. Predominant microorganisms were isolated from *mutandabota* and identified. This indicated that different species of bacteria and yeast survive the acidity and low pH of 3.4±0.1 in *mutandabota*. While no pathogens were isolated, the identified microorganisms are capable of spoiling the product. Preparation of *mutandabota* is a gendered activity dominated by women.

A probiotic dairy product was then developed at village level on the basis of *mutandabota* to enable resource-poor populations in Southern Africa to accrue health benefits from a functional food. Raw cow's milk was pasteurised and dry baobab fruit pulp was added to the milk at a concentration of 4 % (wt/vol). This mixture was inoculated with the probiotic *Lactobacillus rhamnosus* yoba, an isolate of *Lactobacillus rhamnosus* GG, and left to ferment for 24 h. Baobab fruit pulp at 4% promoted growth of *L. rhamnosus* yoba. More pulp and sugar were then added to produce probiotic *mutandabota* with 14 % (wt/vol) baobab fruit pulp and 7 % sugar. The final pH of probiotic *mutandabota* was pH 3.5, which ensured the microbiological safety of the product. Viable plate count of *L. rhamnosus* yoba was 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 in the product.

There was no significant difference ($p=0.31$) in consumers' preference between traditional and probiotic *mutandabota*, despite a significant difference ($p<0.001$) in sensorial properties of the two products. Challenge tests to evaluate the impact of *L. rhamnosus* yoba on competing pathogens in *mutandabota* were done. In traditional *mutandabota* (pH 3.4±0.1) some food-borne pathogens survived and withstood the acids and low pH of the product. However, probiotic *mutandabota* (pH 3.4±0.1) inactivated all tested food-borne bacterial pathogens during the 24 h potential consumption time. This demonstrated that probiotic *mutandabota* can be safer stored than traditional *mutandabota*. The *L. rhamnosus* yoba showed robustness and grew from 5.5 log cfu/mL to 9.0 log cfu/mL within 24 h in the presence of pathogens in probiotic *mutandabota*.

The outcome of this work was a safe, healthy, optimum-quality product of relevant nutritional value. Although this work focused on growth of *L. rhamnosus* yoba in *mutandabota*, the potential exists to apply this approach to other traditional foods worldwide as a low-cost method to improve dietary quality and gastro-intestinal health of consumers. Probiotic *mutandabota* processing and trading may ameliorate the well-being of rural households through improvements in health status and livelihoods.

Chapter 1

Introduction

1.1 Background

Research and development of underutilised foods in Sub Sahara Africa provides a viable option to combat increasing hunger and malnutrition. According to FAO/WHO (1991) and Stevens et al. (2012), the Sub-Saharan population of Africa, notably women and children, suffer from insufficient intake of protein and energy, and a lack of micronutrients. The Food and Nutrition Council of Zimbabwe (2010) reported that 33.8 % of children under five years of age had chronic malnutrition. These malnourished children are more susceptible to disease, may suffer cognitive impairment, have poorer educational outcomes and are likely to experience reduced productivity in their endeavours. Improvement in nutrition in Zimbabwe and the Southern Africa region is needed to reverse this trend. An option with great potential is to improve nutrition at community level through the use of indigenous foods. Indigenous foods are made from locally available resources. These traditional foods not only have a long history of safe use, local communities can also afford them. *Mutandabota* or *umlondo* (Fig. 1.1) is such an underutilized food product found in drier areas of Southern Africa (Kadzere et al., 2004). *Mutandabota* is consumed as a major source of proteins and micronutrients, and it is sometimes used as a supplementary food for infants (Ministry of Agriculture, 2001). The product is made by mixing raw cow's or goat's milk with 14 % (wt/vol) dry pulp of the baobab fruit (*Adansonia digitata* L.) and 7 % sugar (Mpofu et al., 2014).

Producing a probiotic variant of *mutandabota* at village level in a locally sustainable way would ensure access to probiotics by resource-poor communities in Southern Africa. Ingestion of probiotics is associated with health benefits (FAO/WHO, 2001a; Lacroix and Mollet, 2007). The Food and Agriculture Organization and the United Nations/World Health Organization (FAO/WHO, 2001a) define probiotics as live microorganisms that, when consumed in adequate amounts as part of food, confer a health benefit to the host. One of the most thoroughly studied probiotics is *Lactobacillus rhamnosus* GG (Gorbach

et al., 1987; Kankainen et al., 2009; Ossowski et al., 2010). It is a lactic acid bacterium that meets the United Nations standard for probiotics and the requirements for clinical trial documentation (FAO/WHO, 2001a). Evidence exists of beneficial effects of *L. rhamnosus* GG derived from clinical trials with double-blind and placebo-controlled cross-over designs for prevention and treatment of diarrhoea, gastrointestinal and upper respiratory tract infections in children (Grandy et al., 2010; Guandalini et al., 2000; Hojsak et al., 2010), and inhibiting growth and adhesion of enteropathogens (Gopal et al., 2001; Mack et al., 1999). Incorporation of *L. rhamnosus* GG in *mutandabota* would produce a probiotic variant of *mutandabota* that will improve the population's intestinal health or would restore it when it is transiently affected, in addition to the nutritional benefits of the product.

Fig. 1.1: Freshly prepared *mutandabota* being served

1.2 The baobab tree: Occurrence and distribution

The baobab (Figs 1.2 and 1.3) is a deciduous tropical fruit tree with a natural distribution in Sub-Sahara Africa, where it grows in hot dry areas at low altitudes (Sanchez et al., 2010; Wickens, 1982). The baobab belongs to the family *Bombacaceae* and the genus *Adansonia* (Baum, 1995; Palgrave, 1957). According to Wickens (1982), the genus *Adansonia* has 9 species. The predominant ones are *Adansonia gregorii* found in western Madagascar and western Australia and *Adansonia digitata* found in tropical Africa. The binomial *Adansonia digitata* was given by Linnaeus, the genus name honouring Michael Adanson, who had been to Senegal in the eighteenth century and described the baobab (Adanson, 1771). Since the baobab is not a cultivated tree nor properly domesticated, there are currently no baobab plantations or known varieties (Baum, 1995; Buchmann et al., 2010). However, the tree has been introduced in areas outside Africa and grown successfully (Sidibe and Williams, 2002). It has also been grafted in some parts of Africa (Southampton Centre for Underutilised Crops, 2006).

The baobab has a hugely swollen trunk of up to 28 m in circumference and reaches a height of 18 – 25 m (Wickens, 1982). Wood is pulpy, bark is smooth, reddish brown to grey, soft and fibrous (Baum, 1995). Spreading branches twist and taper sharply and are leafless for much of the year. Fruits continue to hang from long stalks after the leaves have fallen. Leaves alternate and stalks are made up of 5- 9 leaflets, the central one being the largest (Tredgold, 1986). Flowers grow singly from the leaf axils, they are large, snow white and beautiful. The tree produces an extensive shallow lateral root system with roots ending in tubers (Wickens, 1982).

The baobab tree tolerates an annual rainfall of between 300 - 600 mm, a rainy season of 3-5 months, described as arid to semi-arid (FAO, 1988). Its ideal typical mean annual temperature is 20 – 30 ^{o}C, but it can tolerate very high temperatures with a mean

maximum of 40 – 42 ºC (Baum, 1995). The tree sheds its leaves early and the trunk contracts in the dry season and expands in the wet season (Owen, 1974). The baobab can grow on a wide variety of soils such as stony, non-agricultural soils (Astle et al., 1969; Jenic and Hall, 1976). In Zimbabwe it is found on poorly drained soils (Wickens, 1982).

Fig. 1.2: A baobab tree in Zimbabwe

1.3 Baobab fruit and its composition

Baobab fruits (Figs 1.4 and 1.5) are very variable, globose to ovoid but sometimes oblong, cylindrical and often irregular in shape. They can be 20 cm in length (Baum, 1995; Tredgold, 1986). The apex is pointed or obtuse, covered by velvety greenish to yellowish hairs. Pericarp is woody, enclosing a dry mealy pulp (Wickens, 1982). Seeds are kidney shaped and embedded in a powdery white pith that separates into floury pulp blocks, each containing a single seed (Baum, 1995). Harvested baobab fruits from

Zimbabwe show an average pulp content of 24.3 % (wt/wt, dry base) (Kadzere et al., 2004). Chadare et al. (2009) reviewed the composition and nutritional value of baobab fruit pulp and reported an average (wt/wt, dry base) moisture content of 11.6 %, crude protein 5 %, carbohydrates 74 %, crude lipids 3 %, fiber 13 %, ash 5 % and energy 1247 kJ/100g. Amino acids in baobab fruit pulp include aspartic acid, glutamic acid, serine, glycine, histidine, arginine, threonine, tyrosine, valine, methionine, isoleucine, phenylalanine, cysteic acid, lysine, tryptophan, alanine, leucine and proline.

Fig. 1.3: Fruiting baobab tree during the wet season in Zimbabwe

Vitamins reported were B1 thiamine, B2 riboflavin, B3 niacin, vitamin A and vitamin C. The vitamin C in fruit pulp was on average 283 mg/100g, the maximum was 500 mg /100g (Arnold et al., 1985; Becker, 1983; Nour et al., 1980). Such high vitamin C content gives fruit pulp a particularly high antioxidant capability (Vertuani et al., 2002).

Antioxidants protect the cells of organisms from damage by free radicals (Frei, 1991). A review by Padayatty (2003) notes that a deficiency of vitamin C weakens the immune system, promotes susceptibility to disease and can result in scurvy. Consumption of 40 g baobab fruit pulp covers 84 to more than 100 % of the Recommended Daily Intake (RDI) of vitamin C for a pregnant woman of 19-30 years (FAO/WHO, 2001b).

Fig. 1.4: Baobab fruits

Baobab fruit pulp is acidic due to the presence of organic acids including citric, tartaric, malic, succinic and ascorbic acid (Airan and Desai, 1954). Mineral content of baobab fruits included calcium, copper, iron, potassium, magnesium, manganese, sodium, phosphorus and zinc (Nordeide et al., 1994; Nour et al., 1980). The recorded amounts of iron and calcium were 302 mg/100g and 4.3 mg/100g respectively (FAO/WHO, 2001b), which is more than double the amounts in milk. The above nutritional values suggest that with proper exploitation, fruit pulp is a good source of nourishment. The number of baobab trees in Zimbabwe has been estimated at 5 million (Bio-innovation Zimbabwe, 2014). The baobab trees are densely clustered in drier areas of the country with a sub-population of 2.8 million people.

1.4 Processing and utilisation

The baobab is a very long-lived tree with multiple uses. In Southern Africa, fruits are edible in the months of April to October (Tredgold, 1986). However, when properly stored, dry fruits can be eaten throughout the year. Ibiyemi and co-workers (1988), reported that storage of pulp of the dry baobab fruits is improved by the use of sodium metabisulphite as a preservative. Pulp can also be frozen if ground to a powder (Obizoba and Amaechi, 1993). Fruit pulp has been mixed with water and is consumed in Mali (Nordeide et al., 1994). The cattle-owning Fulani and Hausa of northern Nigeria use the fruit pulp emulsion to mix with milk to be taken as a drink (Nicol, 1957). In Southern Africa dry fruit pulp (Fig. 1.5) is mixed with milk to make *mutandabota* (Ministry of Agriculture, 2001). The pulp is also used as a baking powder substitute (Baum, 1995).

Seeds are roasted and eaten as snacks, used as a substitute for coffee or used as a source of cooking oil after extraction by pounding (Palmer and Pitman, 1972). Seedcake is fed to animal stock in Tanzania (Nkana and Iddi, 1991). Fruit shells are used in the manufacture of pots (Dovie, 2003), and as fuel in Tanzania (Nkana and Iddi, 1991). Leaves are used in

Southern Africa, they are cooked to make a sauce (Nordeide et al., 1994; Williamson, 1975). In Zimbabwe they provide a substitute for fresh vegetables (Dovie, 2003). Leaves are routinely browsed by animals (Toure et al., 1998). Large caterpillars found feeding on the leaves are prepared as relish (Dovie, 2003). Roots of young trees are eaten raw and bulbs that form at the root ends are dried, ground and used to make porridge (Dovie, 2003). Fibre from inner bark is strong and widely used for making rope, basket nests, snares, fishing lines and for weaving. The green bark is also used as a dye and for decoration (Toure et al., 1998). Moreover, the baobab is used widely in traditional medicine throughout its distribution (Dweck, 1997; Jayaweera, 1981; Ramadan et al., 1996). Finally, the hollow trunks have been put to a variety of uses as houses and stores, as well as dens for wildlife (Dovie, 2003).

Recently, the European Commission authorized the placing on the market of dry baobab fruit pulp as a novel food ingredient (Vassiliou, 2008). The first known commercial processor of baobab was the Baobab Fruit Company, formed in 2001 in Verona, Italy. The company manufactures dietary supplements and cosmeceuticals from the fruit pulp. It imports the raw material from Senegal. Another Italian company that uses the baobab fruit pulp is Specchiasol which manufactures a synbiotic health product; synbiotics are products that combine the two biotic claims; pro- and prebiotics, the living bacteria and specific substrates for specific intestinal microbiota. The dried fruit pulp was approved in the USA in 2009 as a food ingredient in blended fruit drinks and cereal bars (Addy, 2009). The Southern African Natural Products Trade Association is promoting baobab seed oil production and fruit beverages production using standardised processing (Phytotrade, 2014).

Fig. 1.5: Farmers processing baobab fruits

1.5 Milk and its constituents

Mutandabota contains 79 % milk. Milk is essentially an emulsion of fat in a watery solution of sugar and mineral salts, with proteins in a colloidal suspension. According to Eckles et al., (2000), millennia ago, perhaps as early as 6000-8000 BC, ancient man learned to domesticate species of animals for the provision of milk. These included cows, buffaloes, sheep, goats, yak and camels, all of which are still used in various parts of the world for the production of milk for human consumption. There are many factors that affect milk composition; in case of cattle, these include breed variations, cow to cow variations, herd to herd variations, including management and feed considerations, seasonal and

geographic variations (Haug, 2007). With such variations, only an approximate composition of milk can be given. According to Eckles et al. (2000) the main constituents of milk are water 87.2 % and dry matter 12.8 % (fat 3.8 %, protein 3.5 %, sugar 4.8 % and ash 0.7 %). Proteins of milk contain all essential amino acids (Haug, 2007; Swaisgood, 1995). In addition, the amounts of these amino acids in milk protein exceed the amounts in proteins from other sources such as eggs, the common meat cuts and vegetables. Milk is an excellent source of vitamins that are essential to health and physiological processes, including the fat soluble vitamins A, D, E, and K, and the dietary water soluble vitamins: thiamine, riboflavin, pyridoxine, cyanocobalamin, niacin and pantothenic acid (Jenkins, 2006). There is also a small amount of vitamin C present in raw milk but it is very heat-labile and easily destroyed by pasteurization. A sufficient supply of minerals in the human diet is a matter of great importance. All 22 minerals considered essential to the human diet are present in milk (Eckles et al., 2000). Southern African diets are based on cereal products (Food and Nutrition Council, 2010). Such diets are especially lacking in calcium. Milk is one of the best sources of this important element. Milk and baobab fruit pulp therefore contribute to the nutritional diversity of *mutandabota* as their constituents complement each other.

1.6 *Lactobacillus rhamnosus* GG

Several studies have shown the benefits derived from ingestion of *L. rhamnosus* GG. Evidence exists of beneficial effects of *L. rhamnosus* GG for prevention and treatment of antibiotic-associated diarrhoea (Ruszczynski et al., 2008), rotavirus diarrhoea (Grandy et al., 2010), gastrointestinal and upper respiratory tract infections in children (Hojsak et al., 2010) and inhibiting growth and adhesion of enteropathogens (Gopal et al., 2001; Mack et al., 1999). Guandalini and co-workers (2000) demonstrated the beneficial effect of *L. rhamnosus* GG on children suffering acute, watery diarrhoea. In their study, children 1 month to 3 years of age, presenting recent onset of acute diarrhoea, were enrolled in a

double-blinded and placebo-controlled intervention trial. It was concluded that the administration of *L. rhamnosus* GG in the oral rehydration solution to children with diarrhoea was safe and resulted in a marked and significant shorter duration of diarrhoea, less chance of a protracted course, and faster discharge from the hospital.

As a probiotic, *L. rhamnosus* GG shows robustness in stress adaptation and survival along its entire life cycle. In upstream processing, the bacterium survives dehydration and successive storage conditions. Having been incorporated into a fermented food product, it remains viable to the end of the product's shelf-life. Once consumed with the product, it persists in the gastrointestinal tract conditions before it reaches the proposed site of action. As such it shows tolerance to high temperature, survival at low pH and adaptation to osmotic shocks (Sunny-Roberts, 2008; Ananta, 2004; Prasad, 2003). Attributes of *L. rhamnosus* GG that enhance its functionality are its high tolerance to the acidic conditions prevailing in the stomach (Corcoran et al., 2005; Tuomola et al., 2000), survival during intestinal passage (Mattila-Sandholm et al., 1999), and its ability to adhere to human colonic mucosa (Alander et al., 1999; Rinkinen et al., 2003), thereby transiently colonizing the gastrointestinal tract after treatment (Mattila-Sandholm et al., 1999; Tuomola et al., 2000).

Recently the concept of “generic probiotics” was introduced, as a practical solution to create access to probiotics for people in the developing world (Kort and Sybesma, 2012). *Lactobacillus* was isolated from a commercially available product, containing *L. rhamnosus* GG. The identity of the isolate was confirmed by 16S rRNA sequencing and the isolate was deposited at the Belgian Co-ordinated Collections of microorganisms/Laboratorium voor Microbiologie Gent (BCCM/LMG) culture collection under the name of *L. rhamnosus* yoba (Kort and Sybesma, 2012). *L. rhamnosus* yoba is the isolate that was used in experiments reported in this thesis.

1.7 Rationale of the thesis

Mutandabota is part of the food cultural heritage of Southern Africa and Zimbabwe in particular. The high protein content of *mutandabota*, in addition to micronutrients, vitamins, and minerals, gives it potential usefulness as a food protein source in Southern Africa where child malnutrition needs to be combated and eradicated. It is estimated that 12,000 preventable child deaths per year are attributable to under nutrition in Zimbabwe (Food and Nutrition Council, 2010). *Mutandabota* complements well an otherwise nutrient-poor, staple cereal–based diet in rural communities, however, its consumption is limited to localized areas where the baobab tree occurs. *Mutandabota* is acceptable to the people and this forms a firm basis for promoting its consumption beyond the current scope and makes it an affordable and readily available source of nourishment for all groups considered to be nutritionally at risk, such as children, pregnant women, lactating mothers, rural communities, and urban poor. Rural communities might not recognise the health benefits of *mutandabota*, but many people in urban areas are now aware of its benefits leading to increased demand for the baobab fruit to make an imitation of *mutandabota* called *icelolo*, which is made by sprinkling sugar on dry baobab fruit pulp, mixing with water or milk and freezing it, to be consumed as a frozen block. Even though consumption of *mutandabota* is increasing and its benefits are apparent, *mutandabota* is still poorly researched and documented. There is a need to fill this research gap and set the research and development agenda of this underutilised product. In formulating the research agenda for developing practices to improve the quality and safety of *mutandabota* under local conditions, it was necessary to investigate the technology used to process milk and baobab fruit pulp into *mutandabota*, and to ascertain its socio-economic significance. Considering that: first, *mutandabota* is a food made from raw unpasteurised milk, and second: it is used as a weaning food for infants, it became imperative that its chemical and microbiological composition be determined as a way of evaluating food quality and safety.

Benefits of *mutandabota* could be greatly enhanced by incorporation of a probiotic into the product. Upon consumption, probiotic *mutandabota* would be expected to improve the population's intestinal health and nutrition, which is, especially relevant for vulnerable target groups such as children and the elderly. In Southern Africa, because of scarcity and price, probiotic foods are not consumed by the rural population, in which lack of hygiene, poor sanitation, malnutrition, and enteric infections frequently lead to diarrhoeal disorders (Food and Nutrition Council, 2010; Olivieri et al., 2008). The use of indigenous foods as potential vehicles for transmission of probiotics is an option with great potential. In producing probiotic *mutandabota*, a new process would have to be designed based on the traditional *mutandabota* production process. This could significantly alter its organoleptic properties and thus its acceptability by consumers. Majchrzak et al. (2010) noted that the difference concerning health benefits of probiotic and conventional non probiotic food is not always clear to consumers. Preference for a product ultimately depends on many factors of which the most important is sensorial quality. It was thus imperative to, first, evaluate if a detectable sensorial difference existed between probiotic and traditional *mutandabota*, and second, to determine consumer preference for probiotic *mutandabota*. This would guide in successfully introducing probiotic *mutandabota* to targeted communities.

While the beneficial effects of probiotic strains and their mechanism of action have been demonstrated quite well (Guandalini et al., 2000; Kankainen et al., 2009; Ossowski et al., 2010), little information is available on the survival and growth of pathogens in probiotic foods. Good survival of the probiotic bacteria in food products during their specified shelf-life is of importance, as well as the antimicrobial action that the probiotic product may have against contaminating pathogens during the production process and subsequent storage. If *mutandabota* is contaminated by pathogens that are later consumed with it, they might cause microbial infection amongst its consumers. It was thus, important to investigate the inactivation of bacterial pathogens in traditional and probiotic

mutandabota. The ultimate goal was to develop a safe probiotic variant of *mutandabota* as a sustainable, nutritious, and health promoting food to be produced at the village level.

1.8 Objectives of the study

Broad objective: The broad objective of the study was to improve quality, safety and functionality of *mutandabota*, produced under local conditions.

In order to achieve the overall objective, the following specific objectives were formulated:

1. To document qualitatively and quantitatively the process of *mutandabota* production,
2. To determine the chemical and microbiological composition, and the socio-economic significance of *mutandabota*,
3. To isolate and identify predominant microbes in *mutandabota* during preparation,
4. To incorporate *Lactobacillus rhamnosus* yoba in *mutandabota* and produce a locally sustainable probiotic food based on *mutandabota*,
5. To compare sensory properties and consumer preference of probiotic *mutandabota*, and
6. To investigate inactivation of bacterial pathogens in probiotic *mutandabota*.

1.9 Outline of thesis

Chapter 1 formulates the justification and relevance of the research, its objectives and outline. It provides general information on the two major raw materials for *mutandabota* production, the baobab fruit and milk. Furthermore, a summary of the relevant literature on the probiotic bacterium *Lactobacillus rhamnosus* GG is provided. **Chapter 2** documents the current practice of processing milk and baobab fruit pulp into *mutandabota.* Moreover, the chapter describes an evaluation of the chemical and microbiological composition of the finished product as well as the socio-economic significance of the product. This was

meant to enable formulation of a research agenda for developing practices to improve the quality and safety of *mutandabota* under local conditions, and ultimately increase the contribution of this food product in the fight against malnutrition. **Chapter 3** focuses on developing a probiotic variant of *mutandabota* as a sustainable, nutritious and health-promoting food produced at village level to enable resource-poor populations in Southern Africa to benefit from a probiotic food. **Chapter 4** evaluates whether a detectable sensorial difference exists between probiotic and traditional *mutandabota*, and secondly, determines the consumer acceptance of probiotic *mutandabota*. The rationale of the latter activity is to determine if the probiotic product is likely to be successful upon introduction in the targeted communities. **Chapter 5** documents challenge tests, first, to evaluate the impact of *L. rhamnosus* yoba on competing pathogens. Second, to investigate the survival and decline of bacterial pathogens in traditional and probiotic *mutandabota*. Inactivation of pathogens in *mutandabota* is of public health significance because food-borne pathogens endanger public health upon consumption of contaminated food, especially in Southern Africa, where there are many vulnerable consumers of *mutandabota* such as children, the elderly and immuno-compromised people living with HIV/AIDS. **Chapter 6** presents the general discussion on the obtained results together with concluding remarks on how far this thesis has realised its objectives. Furthermore recommendations are given and an outline of work currently being done to produce the probiotic *L. rhamnosus* yoba in local industries to make production of the probiotic *mutandabota* sustainable.

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Chapter 2

Mutandabota, a food product from Zimbabwe: Processing, composition and socioeconomic aspects

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Abstract

We evaluated processing technology, composition, and socioeconomic significance of *mutandabota*, a food product made by mixing cow's or goat's milk with dry baobab fruit pulp. *Mutandabota* production is a gendered activity dominated by women. Nutrient content was (g 100 g^{-1} w.b) protein 4.8 ± 1, fat 2.8 ± 0.9, fiber 1.1 ± 0.4, ash 0.9 ± 0.2, carbohydrates 20 ± 1.7, moisture 70.4 ± 3.7, and vitamin C 80 ± 25 mg/100g. Microbiological load (log cfu mL^{-1}) was high, 4.7 ± 1.2 mesophilic bacteria, 5.3 ± 2.1 lactic acid bacteria, and 5.0 ± 1.3 yeasts and moulds. The pH of milk was 6.7 and the final pH of *mutandabota* was 3.5 ± 0.1. *Mutandabota* is a major source of proteins and vitamin C. Its microbiological quality needs evaluation.

Keywords: baobab fruit, chemical composition, microbial load, milk, *mutandabota*

1. Introduction

Research and development of underutilized foods in Sub-Saharan Africa provides a viable option to combat increasing hunger and malnutrition. According to FAO/WHO (1991) and Stevens et al. (2012), the Sub-Saharan population of Africa, notably women and children, suffer from insufficient intake of protein and energy, and a lack of micronutrients. The Food and Nutrition Council of Zimbabwe (2010) reported that 34 % of children under five years of age in the country suffer from chronic malnutrition. The Council recommended scaling up nutrition, mainly by improving the nutritional quality of indigenous food products that are available to the community. *Mutandabota* or *umlondo* is one underutilized food product found in semi-arid regions of Zimbabwe (Ministry of Agriculture, 2001). It is made by mixing cow's or goat's milk and dry baobab (*Adansonia digitata* L.) fruit pulp. *Mutandabota* is consumed as a major protein source by both young and old, and is sometimes used as a supplementary food for infants. For generations, *mutandabota* has significantly contributed to people's livelihoods through subsistence use and as a safety net in drought periods. However, its consumption is limited to localized areas where the baobab tree occurs. The baobab tree is widely distributed throughout drier regions of Southern Africa (Sanchez et al., 2010).

Milk and baobab fruit pulp are the major raw materials in *mutandabota* production. Quality of milk and proximate composition of pulp have a direct bearing on the nutritional status of *mutandabota*. Milk has been considered the most nearly perfect food known, endowed with nutritional and immunological components that positively influence human health (Wong et al., 2006; Millsa et al., 2011). Raw cow's milk from Zimbabwe contains approximately (g 100 g^{-1}): total solids 12, crude protein 3.2, fat 3.5, and non-fat solids 8.3 (Mutukumira et al., 1996). Proteins of milk contain all essential amino acids. All 22 minerals and vitamins considered essential to the human diet are also present in milk (Eckles et al., 2000).

Baobab fruits from Zimbabwe showed a pulp content of 24 g 100 g^{-1}, range 10 - 57 (Ministry of Agriculture, 2001). Moisture content of fruit pulp was 11.6 g 100 g^{-1} on product dry weight basis. Proximate composition was found to be (g 100 g^{-1} d.w.): crude protein 5.3, fiber 13.7, fat 3.6, carbohydrates 74.9, and ash 4.9 (Arnold et al. 1985; Chadare et al., 2009). Fruit pulp is acidic due to the presence of organic acids, including citric, tartaric, malic, succinic, and ascorbic acids. Vitamin C content in fruit pulp was reported as 160 - 337 mg 100 g^{-1} (Ighodalo et al., 1991; Ministry of Agriculture, 2001). Such high vitamin C content gives fruit pulp a particularly high antioxidant capability (Vertuani et al., 2002). Minerals in fruit pulp included (mg 100 g^{-1}): iron 9.3, calcium 295, magnesium 90, zinc 1.8, sodium 2.8, and potassium 1240 (Osman, 2004).

The objectives of the present study were to monitor the technology used to process milk and baobab fruit pulp into *mutandabota*, to evaluate the chemical and microbiological composition, and to assess the socio-economic significance of *mutandabota*. This was meant to enable formulation of a research agenda for developing practices to improve the quality and safety of *mutandabota* under local conditions, and to ultimately increase the contribution of this food product in the fight against malnutrition.

2.0 Materials and methods

2.1 Study area

The study was conducted in Siyanzyundu (17^{o} 36' S, 27^{o} 32' E), Binga district, Zimbabwe, a communal farming area with a rich *mutandabota* tradition. The abundance of baobab trees and the widespread consumption of *mutandabota* in Binga (Ministry of Agriculture, 2001) determined the choice of this community as the study site. *Mutandabota* is believed to have originated here and is considered a food cultural heritage in this community.

2.2 Field data collection

The field research was conducted from August 2010 to July 2011. Permission to carry out the research was obtained from the headmen and councilor of the area. Informed consent was obtained from all participants and respondents. Data were collected through focus group discussions (FGD), formal interviews, practical observation, and demonstrations.

2.3 Focus group discussions and interviews

Siyanzyundu comprises eight villages. The names of villages and their population size is shown in Table 2.1. Seven of the villages are relatively close to each other. One village, Chilisegela, is relatively isolated from others and bigger, with a population of 280 inhabitants. From each of the eight villages, at least 5 % of the inhabitants were randomly selected to make a sample size of 40. Boyd and colleagues (1981) recommend a sample size of at least 5 % of the total population. Based on this, the number of participants picked from each village, as shown in Table 2.1, was considered to be representative.

Discussions and interviews were conducted in the local Tonga language that was best understood by all informants. Semi-structured interviews (Bernard, 2006) were used for FGDs. FGDs were held with two groups. The age of participants ranged from 24 to 65 years (mean = 45). The first group consisted of participants from seven villages. The number of participants from each village and their gender is shown in Table 2.1. The second group consisted of 15 participants from one village, Chalisegela. Four of the participants were men and 11 were women. The age range was 23 - 70 years (mean = 43). The headmen, as community leaders, were excluded from FGD to enable spontaneous and free flow of information.

Table 2.1: Number and gender of participants in focus group discussions in Siyanzyundu, Zimbabwe.

Village name	No. participants	Women	Men
Siabwande	5	5	0
Siantebele	8	8	0
Siamudele	2	2	0
Ndwani	1	1	0
Ngandu	2	2	0
Siamugwandu	5	3	2
Siabukoko	2	1	1
Chilisegela	15	11	4
Total	**40**	**33**	**7**

Formal interviews were held individually using semi-structured questionnaires (Bernard, 2006) to understand people's perceptions on preferences, availability, and consumption of indigenous tree-food products. This was important for comparison purposes and in judging whether the baobab tree was more or less important as a food resource than other indigenous fruit trees. The following people were interviewed: (i) the two headmen in Siyanzyundu (headmen are traditional leaders in Zimbabwe and are considered as the custodians of traditional knowledge and culture (Mupunga and Dube, 1992), (ii) the councilor for the area, and (iii) two elderly women recommended to us for their knowledge of *mutandabota*. None of these interviewees participated in the previous FGDs.

Data collected in FGDs and interviews included socio-demographic characteristics (e.g. age, gender), farming activities, cattle head and goat characteristics (e.g. numbers, composition, and uses), cattle and goat feeding management (e.g. sources of feed and grazing management), cattle and goat health management, and milking practices. The importance of the baobab as compared to other fruit trees as a food resource was

evaluated by asking participants to rank fruit trees in their area from the most important (ranked 1) to the least important (ranked 5). Data was collected on preparation of *mutandabota*, challenges encountered, fruit and pulp storage conditions, as well as *mutandabota*'s socio-economic and nutritional significance.

2.4 Milking and preparation of *mutandabota*

The milking of cattle and goats was observed. Three households prepared *mutandabota* in duplicate at their homesteads on separate days. The households prepared *mutandabota* using raw cow's milk according to their traditional technical know-how. Household members were observed while carrying out the operations. Quantitative data was collected on inputs (i.e. raw materials, labor, utensils), time, temperature of preparation and outputs (yields and by-products). Samples were collected during preparation for biochemical and microbiological analysis.

2.5 Biochemical and microbiological analysis

2.5.1 Biochemical analysis

The pH of milk and *mutandabota* were determined immediately on sampling in a 5 mL subsample using a combined glass electrode pH meter. Moisture, crude protein, fat, ash, and crude fiber content of *mutandabota* were determined according to AOAC (2005) methods. Total carbohydrate content of *mutandabota* was estimated by difference. Temperature of *mutandabota* was determined on sampling by inserting a thermometer into a 100 mL sample.

2.5.2 Microbiological analysis

Microbial load was evaluated and the type of bacteria was determined in cow's milk and *mutandabota*. From milk, a subsample (1 mL) was aseptically drawn from the preparation dish just before addition of pulp, and made into 10^{-1} dilutions using peptone physiological saline (PPS) solution (8.5 g NaCl and 1 g neutralized bacteriological peptone (Oxoid, LP0034) in 1 L demineralized water). At the end of *mutandabota* preparation, a subsample (1 mL) of *mutandabota* was aseptically drawn from the preparation dish and made into 10^{-1} dilutions using peptone physiological saline (PPS) solution. Immediately, in each case, from PPS serial dilutions, 100 µL of the appropriate diluent were inoculated onto selective media in duplicate. Lactic acid bacteria (LAB) were enumerated on de Man, Rogosa, and Sharpe Agar (MRSA: 1.5 % Technological Agar Oxoid, LP0013 added to de Man, Rogosa and Sharpe broth (Merck, VM986641), mixed and sterilized), supplemented with 100 mg 100 g^{-1} natamycin (2 g Delvocid (50 % natamycin, DSM). MRSA plates were stored in air-tight jars and incubated under a modified atmosphere (80 % N_2, 10 % CO_2, and 10 % H_2) at 30 ^oC for 48 ± 4 h. Colonies were checked microscopically to confirm presence/absence of yeasts. Presumptive LAB were confirmed by oxidase and catalase tests, and confirmed counts were reported as LAB. Aerobic mesophilic bacteria were enumerated on plate count agar (PCA, Oxoid CM0325). PCA plates were incubated at 25 ^oC for 48 ± 4 h, and reported as total viable count. Yeasts and moulds were enumerated on oxytetracycline glucose yeast extract agar (OGYEA, Oxoid CM0545) supplemented with oxytetracycline). OGYEA plates were incubated under aerobic conditions at 25 ^oC for 3 d. All colonies on respective plates were counted and results expressed as colony forming units (cfu) per mL of milk or *mutandabota*, taking into account the dilution factors.

2.6 Statistical analysis

Statistical analysis was done using SPSS 13.0 for Windows (Apache Software Foundation, USA) and Microsoft Excel. Descriptive statistics such as means, percentages, frequencies, and variances were computed and used to describe the data.

3.0 Results

3.1 Siyanzyundu: The study area

Siyanzyundu, in Binga district, Zimbabwe, is a communal farming area in a very arid and marginal agro-ecological zone in the country. The baobab tree does very well in the area due to its adaptation to hot, dry areas (Arnold et al., 1985). Table 2.2 shows the importance of the baobab tree as a food resource for the community in Siyanzyundu in relation to other fruit bearing trees. The baobab tree was ranked first, mainly because of its use in *mutandabota* preparation, and also because different parts of the tree are processed into different foods. Fruits are sucked and chewed. Leaves are used as a sauce ingredient. Fruits are sold to generate income and can also be used in barter trade in exchange for the staple millet grains (*Pennisetum glaucum*). Second ranked was the *nyii* tree (*Berchemia discolor*), followed by *mateme* (*Strychnos cocculoides*) then *musika* tree (*Tamarindus indica*). The remaining trees (Table 2.2) were the least ranked as food resources.

Table 2.2: Ranking of trees as food resources by local farmers in Siyanzyundu, Zimbabwe

Rank	Local name of tree	English Name	Botanical name
1	*Muuyu*	Baobab	*Adansonia digitata*
2	*Nyii*	Mountain date	*Berchemia discolor*
3	*Mateme/Mkemeswane*	Corky monkey-orange	*Strychnos cocculoides*
4	*Musika*	Tamarind	*Tamarindus indica*
5	*Mutsubvu/Mutswankela*	Chocolate berry	*Vitex payos*
5	*Mkambo*	Monkey orange	*Strychnos innocua*
5	*Mbubu/Umviyo*	Velvet wild medlar	*Vangueria infausta*
5	*Zakalanda/moringa*	Horse-radish tree	*Moringa oleifera*

The average head of cattle per homestead in Siyanzyundu was 9 (range 2 - 30). The predominant breed in Siyanzyundu was the small, hardy Sanga type with a small proportion of cross-bred Jersey, Friesian, and Brahman. The goat breed was the indigenous Mashona type. Of the 40 farmers, 26 % reared cattle only, 39 % had cattle and goats, while 17 % had goats only. A few farmers (18 %) had neither goats nor cattle. Of those who had goats, the average number of goats per farmer was 19. Farmers stated that their cattle and goats were healthy. Veterinary extension services provided treatment and vaccinations against important diseases, among them anthrax, and foot-and-mouth disease. The Ministry of Agriculture considers extension and veterinary services in these areas satisfactory (Agricultural Extension Services, 2005). All farmers (n = 40) reported that their cattle and goats grazed on communal rangelands throughout the year, with goats additionally browsing bushes. The principal grasses in the area are *Aristida, Eragrostis*, and *Hyparrhenia* species. While cattle and goats in this community, like anywhere else in the country, are kept for a number of purposes (Feresu and Muzondo, 1989), the provision of meat and production of milk are the most important benefits, thereby providing the family with a protein source.

3.2 Milking in Siyanzyundu

When milking, cow hind legs along with the tail were tied and the udder region was washed with clean water using bare hands. Holding the teats diagonally, milking was done quickly, silently, and completely with dry hands (Fig. 2.1). Foam formed on milking in the milking jar was used to lubricate the teats. It was observed that farmers' milking practices were deficient in basic sanitary techniques like disinfection of the milker's hands before milking, or teat dipping. The effects of teat dipping, according to Dodd and Phipps (1994), are to lower the level of microorganisms in milk as well as to help the healing of teat skin injuries.

Milk yield per cow was on average 2 liters per day, depending on farm-management practices and season. Higher milk yields were recorded during the rainy season, when the pasture is plentiful. In the dry season, yields are much lower. Most farmers (70 %) said they milked 1 or 2 cows per year, 30 % milked 3 or more cows per year. According to Empson (1992), generally levels of milk production from the communal farming sector are low. The figures in Siyanzyundu compare well with the country average in the communal sector. These are 1–3 liters per day (150 lactation days) for indigenous breeds; 4–10 liters per day (average 240 lactation days) for crossbreeds; and more than 10 liters per day (300 lactation days) for purebred exotic cows (Mupunga and Dube, 1992).

Milk yield per 9 goats was 2.5 liters (on average 278 mL per goat) per day. Goats were milked with one person holding the hind legs, a second holding the head, and a third person extracting the milk (Fig. 2.2). As with cow milking, the udder region was washed with clean water using bare hands. Holding the teats diagonally, milking was done quickly, silently, and completely with dry hands. Foam formed on milking in the milking jar was used to lubricate the teats. Milk produced per homestead is pooled together before use, keeping goat's and cow's milk separate.

Fig. 2.1: Cow milking in Siyanzyundu, Zimbabwe

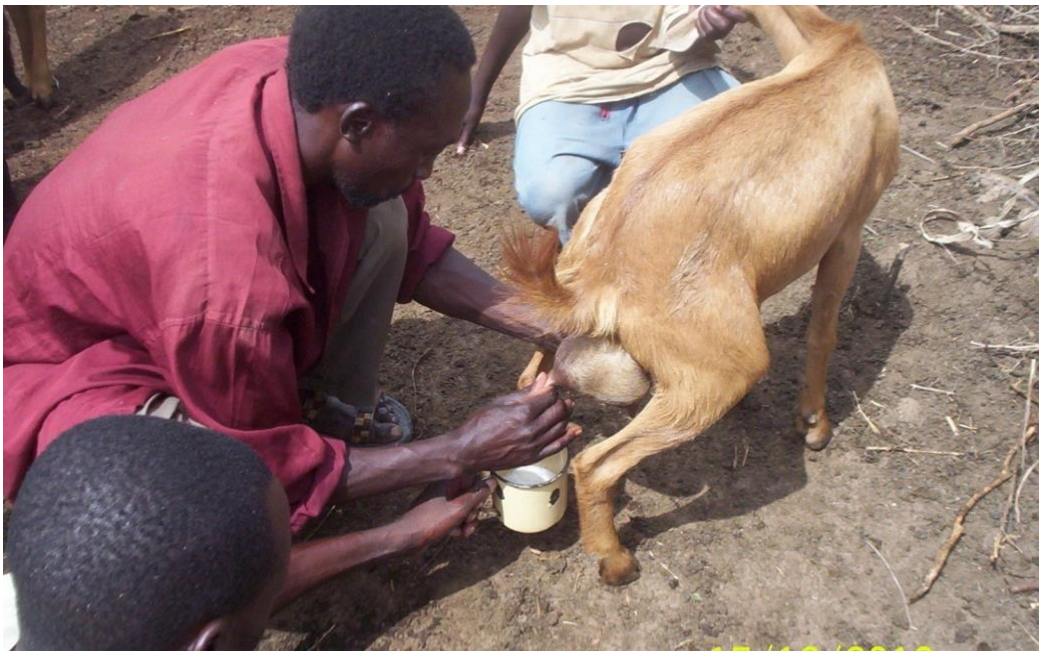

Fig. 2.2: Goat milking in Siyanzyundu, Zimbabwe

Milk was filtered using a clean (non-sterile) cloth to remove any physical contaminants such as animal fur, manure, and pieces of grass, before consumption and/or processing. There are no refrigeration or other cooling facilities in Siyanzyundu. Therefore, milk is consumed immediately after milking or processed into *mutandabota* for immediate consumption. Some of the farmers (60%) sell some of their cow's milk within local communities to raise some income.

3.3 Post-harvest storage of baobab fruits

Villagers store baobab fruits in cribs (huts specially designed to ensure a dry, non humid internal environment). Fruits can be stored for six months or more without loss of quality in such cribs. If exposed to a humid environment, fruits are said to absorb moisture and this affects the quality of pulp. Farmers reported the problem of fruits being weevil or insect damaged. Damage is detected by the presence of holes, a floury dust, or tiny pinkish crystal-like structures in the pulp, a criterion to which villagers pay special attention when choosing fruits for processing. Pulp from such fruits is considered unsuitable for processing. Chadare et al. (2008) also observed pulp-storage problems in Benin, such as insect larvae infestation and color changes.

3.4 Extraction of fruit pulp and *mutandabota* preparation

In the method of preparing *mutandabota* employed in Siyanzyundu, as recorded in this study, mature dry baobab fruits were collected from the wild. Baobab fruits ripen and dry while they are still on trees. Large quantities (two to five sacks, each sack containing on average 120 fruits) were brought to the homestead. Farmers mentioned that they preferred less-bitter fruits from good trees identified over the years. Such fruits are said to produce better *mutandabota*. Fruits were graded according to size (small, medium and large). Fruits of the same size were processed together, which was a way of ensuring

homogeneity in terms of color, flavor, and aroma of the final product. The illustration of process stages used for the preparation of *mutandabota* is shown in Fig. 2.3. In this method fruits were cracked by hitting them against a rock or log. Naturally dehydrated pulp together with seeds was separated from the pericarps. Pericarps were stored for later use. After removal of the pericarp, pulp and seeds were separated by sieving, pulp was collected in a winnowing basket. The seeds were pounded in a mortar with a pestle to completely separate the pulp adhering to the seeds. A second sieving was done and the collected pulp was added to the main portion of the pulp in the winnowing basket. This was repeated three times or until the seeds were free of pulp. Seeds and some fibrous matter were put aside for later use. Pulp was gradually added to milk, while continuously stirring, in an 8-liter plastic bowl. Crystalline sugar was also added to the mixture. Stirring was continued for approximately 7 minutes or until a homogeneous mixture was achieved. The preparation temperature was 30 ^{o}C.

Table 2.3 shows a summary of the raw materials and their quantities used in preparing *mutandabota* and the resultant product yield. Average yield was 2,180 g of *mutandabota* from 334 g of pulp, 151 g of sugar, and 1,814 g of milk. The average percentage yield was 94 % based on total ingredient weight. *Mutandabota*, shown in Fig. 2.4, had an appetizing cream colour with a thick yogurt-like consistency.

Table 2.3: Ingredients used in producing a batch of *mutandabota* (*n=6*) in Siyanzyundu, Zimbabwe

Ingredients	**Minimum**	**Maximum**	**Mean (*n*=6)**	**SD**
Pulp (g)	280	394	334	57.4
Cow's milk (g)	1,691 (1,742 mL)	1,973 (2,032 mL)	1,814 (1,868 mL)	141
Sugar (g)	112	189	151	38.5
Mutandabota (g)	1,943	2,380	2,180	220.8
Yield (%)	91	96	94	2.5

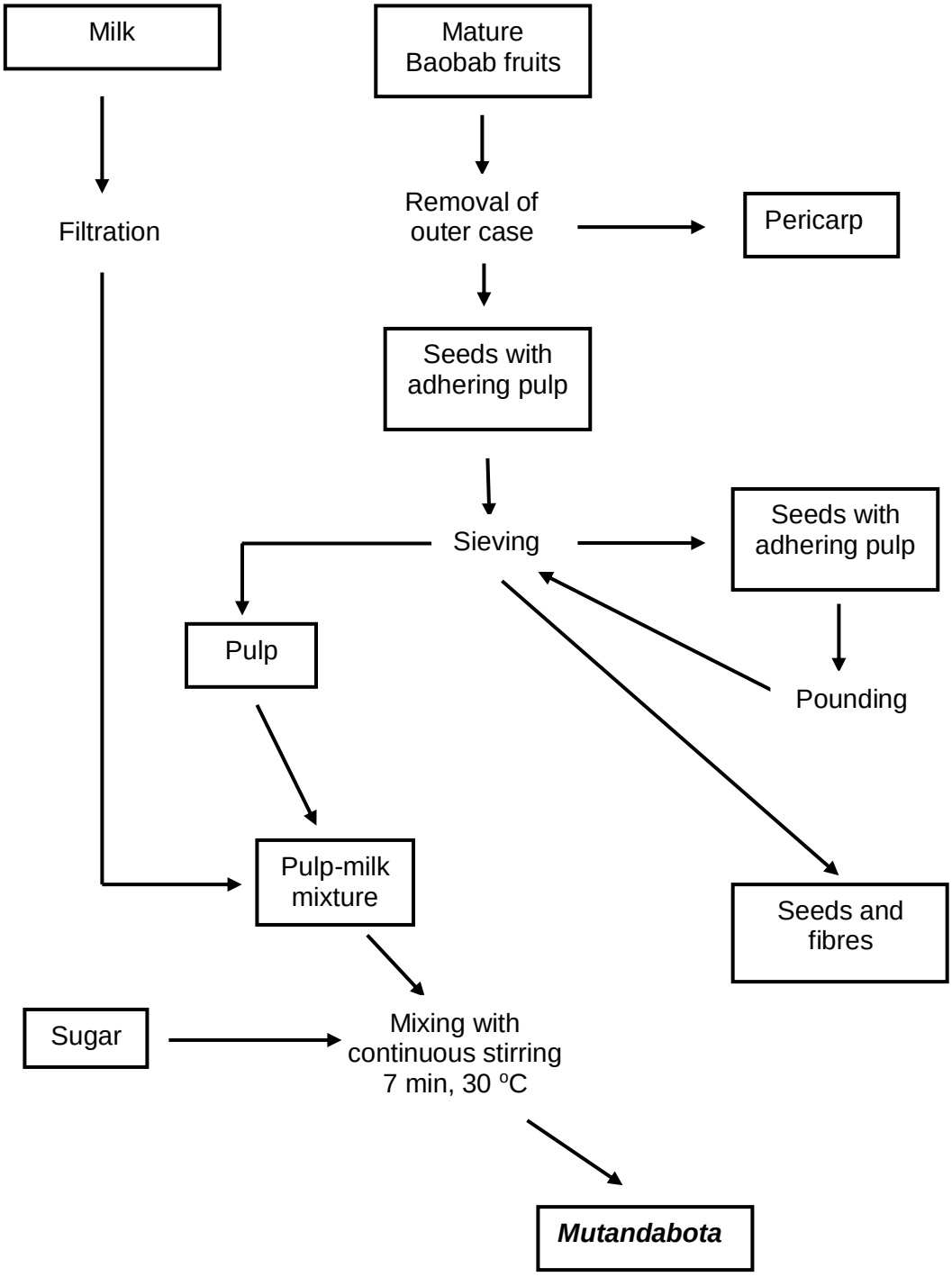

Fig. 2.3: Flow chart of *mutandabota* preparation in Siyanzyundu, Zimbabwe.

3.5 Chemical composition

The nutrient content of *mutandabota* was (g 100 g^{-1} fresh weight) crude protein 4.8 ± 1, fat 2.8 ± 0.9, crude fibre 1.1 ± 0.4, ash 0.9 ± 0.2, carbohydrates 20 ± 1.7, moisture 70.4 ± 3.7 and vitamin C 80 ± 25 mg 100 g^{-1}. *Mutandabota* is a major source of protein in the Siyanzyundu community, which depends, as is the case with most rural Sub-Saharan communities, on millet, sorghum and maize grains for food (Oniang'o et al., 2003). The content of vitamin C (80 ± 25 mg 100 g^{-1}) makes *mutandabota* an attractive natural source of vitamin C for pregnant and lactating women as well as for children and the elderly.

Fig. 2.4: *Mutandabota* being served for lunch in Siyanzyundu, Zimbabwe

3.6 Microbiological and biochemical properties

Microbiological load of raw cow's milk on processing to produce *mutandabota* was high. On average, mesophilic bacterial counts were 5.8 log cfu mL^{-1}, lactic acid bacterial counts were 5.1 log cfu mL^{-1} and yeasts and mould counts were 3.6 log cfu mL^{-1}. Identification of 131 single-colony isolates from the raw milk and *mutandabota* showed the absence of potential pathogenic microorganisms (unpublished results). Mesophilic bacterial counts exceeded the regulatory limit of 5.7 log cfu mL^{-1} for milk intended for processing in Zimbabwe (Ministry of Agriculture, 1992). Possible causes for such high bacterial counts could be contamination by the milker, hands of the milker, environment at the milking site, milk filter cloth, and/or the milking vessels that may harbour microorganisms. Moreover, the environmental temperatures of 30 ± 1.2 ^{o}C during processing and a time lapse of approximately 2 hours before processing to *mutandabota* could also have contributed to bacterial growth. The International Dairy Federation (1990) and Walstra and Jenness (1984) reported that the high temperatures in raw milk in tropical conditions favour mesophilic bacterial growth. While there are antimicrobial substances in milk, such as lysozyme, lactoperoxidase, lactoferrins, and immunoglobulins (Harding, 1999; Silanikove et al., 2006), these substances usually inhibit bacterial growth only for some minutes after milking (Fonteh et al., 2002; Zhang et al., 2008).

On preparing *mutandabota*, the pH of cow's milk was 6.7 ± 0.4, a pH value within the biological variation described by Walstra et al. (2006). Milk pH is affected by a number of factors that include farm-management practices, type of feed, and stage of lactation. The pH of the resulting *mutandabota* was 3.5 ± 0.1, at a temperature of 30 ^{o}C. The low pH of *mutandabota* 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 and colleagues (1980) and Vertuani and colleagues (2002)

confirmed the presence of citric, tartaric, malic, succinic, and ascorbic acids in baobab fruit pulp.

In *mutandabota*, mesophilic bacterial counts were, on average 4.7 ± 1.2 log cfu mL^{-1}, lactic acid bacteria were 5.3 ± 2.1 log cfu mL^{-1}, yeasts and moulds were 5.0 ± 1.3 log cfu mL^{-1}. The drop in bacterial load, when compared to microbial load in milk on processing (5.8 log cfu mL^{-1} mesophilic bacteria, 5.1 log cfu mL^{-1} lactic acid bacteria, and 3.6 log cfu mL^{-1} yeasts and moulds), was probably due to the acidic nature of baobab pulp and the resulting pH of *mutandabota*, which is not very conducive to microbial proliferation and probably inactivated or killed many microorganisms. The lactic acid bacteria seem to have been affected less by the acidic nature of *mutandabota* because of their inherent tolerance of acidic environments (Hyronimus et al., 2000). Yeast and moulds increased in numbers, probably because, in addition to yeasts and moulds from milk, some yeasts and moulds came from dry baobab pulp. Yeasts and moulds have been isolated from baobab fruit pulp (Saka et al., 2007).

3.7 Socio-economic significance of *mutandabota*

Mutandabota is of social, economic and nutritional significance in the livelihoods of people living in Siyanzyundu and many other Zimbabwean communities. This is the case with other fruit based products in Zimbabwe (Nyanga et al., 2008). The whole family is involved in production of *mutandabota* from fruit harvesting, milking and mixing of ingredients. This brings the family together, fostering family and community cohesion, especially if *mutandabota* is shared with neighbours. The dominance of women in focus groups (87.5 % in the first group and 73.3 % in the second group) indicates that *mutandabota* production is a gendered activity dominated by women. Women are responsible for fruit harvesting and *mutandabota* preparation. *Mutandabota* is consumed by all members of the family and is a major component of the diet. On average an adult

consumes a serving of 400 g *mutandabota* per day. This makes economic sense in that *mutandabota* is made from locally available resources and can be consumed as either breakfast or lunch.

4.0 Discussion

The climatic conditions in the study area Siyanzyundu, in Binga, Zimbabwe, could have influenced the inhabitants to exploit the baobab fruit as their main forest-food resource. Siyanzyundu is a very arid and marginal agroecological zone in the country. Rainfall is too low (<600 mm/annum) and erratic for the reliable production of even drought-resistant fodder and grain crops (Ndebele et al., 2007). Vincent and Thomas (1960) noted that extensive cattle or game ranching is the only sound farming system for this region. Farming in Siyanzyundu is based on minimal dry-land cropping and grazing natural pasture. All farmers in Siyanzyundu grow sorghum (*Sorghum bicolor*) and millet (*Pennisetum glaucum*) as their staple food crops, providing most of the carbohydrates to the diet. Processing milk and baobab fruit to produce *mutandabota* ensured a constant supply of proteins, vitamin C and minerals to the diet throughout the year, since the raw materials, milk is always available and the baobab fruit pulp can be stored for use throughout the year. However pulp preservation needs improvement. Pulp storage can be improved by the use of sodium metabisulphite as a preservative against moulds (Ibiyemi et al., 1988). *Mutandabota* provides additional fibre to the diet, which evidently has potential health benefits, in particular for preventing diabetes, cardiovascular diseases, various cancers and functional constipation in children (Smith, 2010).

The practice of milking both cattle and goats in the cattle kraal and goat pens, respectively, in an open environment, raised a hygiene concern for the reason that, while milk is immediately poured into a container and closed, there is a high contamination risk during milk extraction from the teats, because at this time the container is not closed and

foreign materials like manure blown by the wind can contaminate the milk (Figs 2.1 and 2.2). Milk has many times been identified as a source of food-borne diseases (Adesiyun et al., 1995; Arslan and Ozdemir, 2008). Control of bacterial content in raw milk is very important for public health, because consumption of dairy products processed from milk with high microbial counts increases the risk of ingestion of toxins or infective organisms. However, microbiological quality is not only dependent on bacterial count, but on the specific bacterial types. A high count of lactobacilli, for example, may actually be beneficial. Lowering of milk pH from 6.7 to 3.5 in *mutandabota* within the 7 ± 2 minutes preparation time, through natural means, can be considered a major benefit to *mutandabota* in the enhancement of microbiological safety and shelf-life.

On average, an adult consumes a serving of 400 g *mutandabota* per day. According to the World Health Organization (2007), the recommended level of protein intake (RLPI) for a toddler (1 – 3 years old) is 0.83 g per kg of body weight. A serving of 300 g *mutandabota* would provide 60 % of the RLPI, if consumed twice a day, as is often the case, then 120 % of RLPI is covered. A 300 g serving of *mutandabota* will provide more than 100 % of the recommended vitamin C daily intake for a toddler. *Mutandabota* production is a gendered activity dominated by women. In recognition of the prominent role of women in the whole process, it is envisaged that any technological intervention has to prioritize the role of women, and the effect of the technology on their livelihoods. Pulp extraction and pulp preparation appear to be the most difficult processing operations. They are tedious manual operations mainly handled by women. A mechanical pulp extractor, as wished for by the local population, would significantly contribute to a sustainable solution in improving the livelihoods of processors. Finding solutions for such operations through research activities will result in a semi-mechanized process which will ultimately save time for women involved in this activity. The saved time can be invested in other remunerative activities, resulting in higher incomes that can be spent on child education and development. Pulp preparation also faces problems. It requires pounding in a mortar

with a pestle, and a sieving process for removal of fiber and seed. These two operations are made difficult by air currents that take away part of the product or introduce dust particles. It would be beneficial to investigate how the operations can best be facilitated for a rural population. *Mutandabota* is gaining interest and its production has expanded beyond its area of origin (Ministry of Agriculture, 2001). We can thus foresee further increase in consumption of *mutandabota*.

5.0 Conclusions

Mutandabota is part of the food cultural heritage of Zimbabwe and the community of Siyanzyundu in particular. The high protein content of *mutandabota*, in addition to nutrients, vitamins, and minerals, as recorded in this study, gives it potential usefulness as a food protein source in Sub-Saharan Africa where child malnutrition needs to be combated and eradicated. *Mutandabota* complements well an otherwise nutrient-poor, staple cereal–based diet in rural communities. *Mutandabota* is acceptable to the people and this forms a firm basis for promoting its consumption beyond the current scope and makes it an affordable and readily available source of nourishment for all groups considered to be nutritionally at risk, such as children, pregnant woman, lactating mothers, rural communities, and urban poor. Nutritional research on *mutandabota* focusing on digestibility and bioavailability of nutrients is necessary. It is beneficial to perform research on cumbersome processing operations and appropriately mechanize them to save time and improve safety considerations to enable wider distribution and contribution to reduce malnutrition. Storage conditions for the baobab fruit are indeed very poor and need to be studied and improved to increase the shelf-life of the fruit. Increasing the shelf-life of the fruit will add value to pulp for production of standardized *mutandabota*. The high microbial load of milk used for *mutandabota* processing and the correspondingly high microbial load in *mutandabota* pose a potential hygiene challenge. General hygienic practices should be investigated and improved, from milking through

all the processing stages. Lowering of milk pH from 6.7 to 3.5 in *mutandabota* within the seven minutes' preparation time, through natural means, can be considered a major benefit to the product in the enhancement of microbiological safety. Introduction of fermentation technology in *mutandabota* production could be beneficial, since it contributes to improvement of nutritional value, digestibility of food, and microbiological safety and is an inexpensive technology that can be applied successfully in rural situations. In addition, we recommend determining the feasibility of incorporating probiotics with suitable characteristics to improve the health benefits of *mutandabota*. The ultimate goal would be to end up with a safe, healthy, optimum-quality product of relevant nutritional value.

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Chapter 3

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's 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's milk. The pH of probiotic *mutandabota* was pH 3.5, which ensured that the product was 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 maximum 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*

1. 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 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 month to 3 years 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 diarrhoea 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 (Mattila-Sandholm 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 non-fermented 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 bacteria 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 bacteria 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.

2.0 Materials and methods

2.1 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*.

2.2 Medium and inoculum for probiotic *mutandabota*

An isolate 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; Valik 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's 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*.

2.3 Determination of the growth rate of *L. rhamnosus* yoba

The growth rate of *L. rhamnosus* yoba in heat-treated full-fat cow's 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, 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.

2.4 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 Fig. 3.1. 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 22 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's 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.

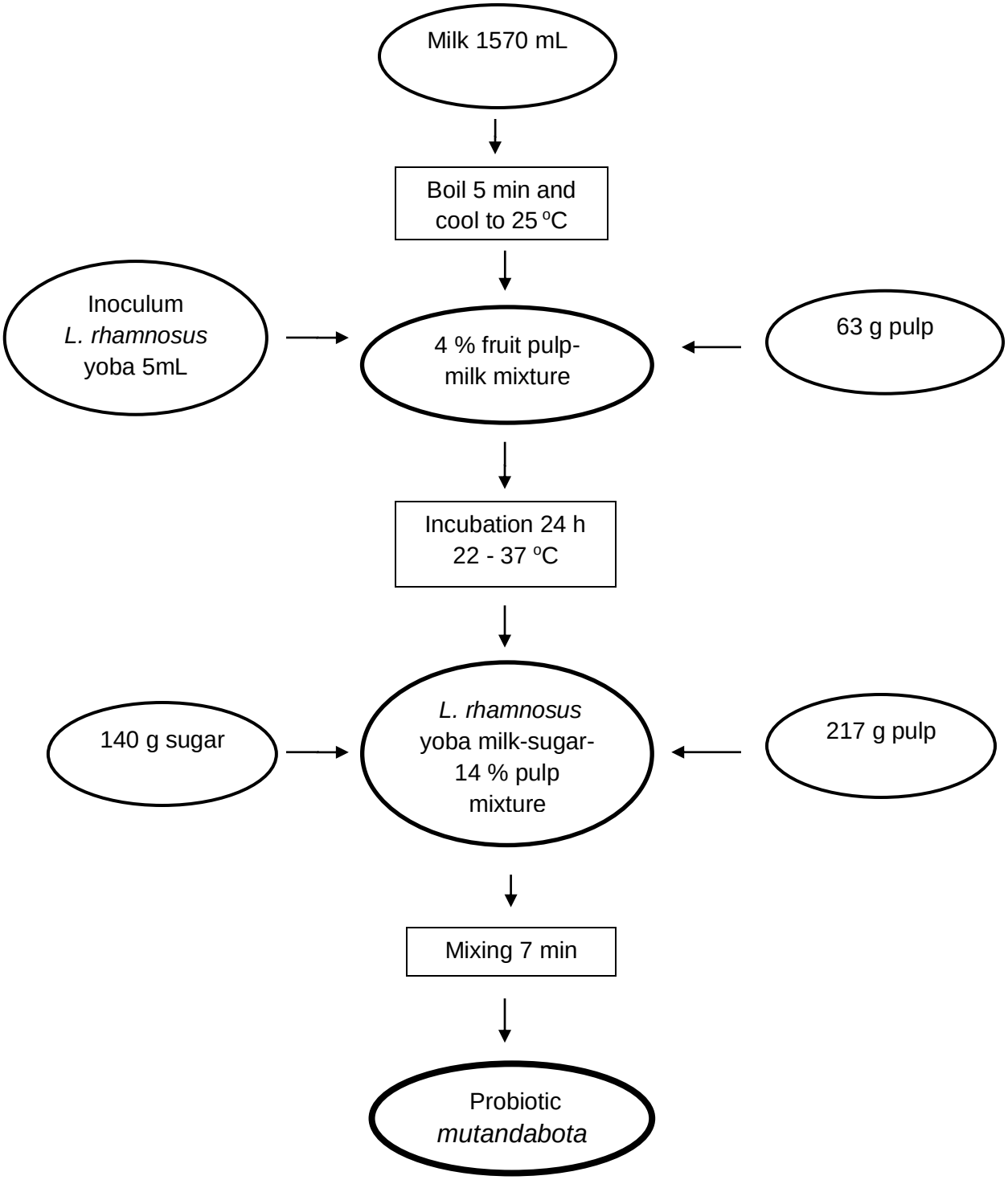

Fig. 3.1: Flowchart for the production of probiotic *mutandabota*

2.5 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.

2.6 *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.

2.7 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.

3.0 Results and discussion

3.1 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 (Espırito-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 3.1. 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.

Table 3.1: 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

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.

3.2 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 (Fig. 3.2). 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.

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 (Fig. 3.3). The incubation started in the morning at 11:20 h. The initial temperature in the fermentation vessel was 29.8 ± 3.8 °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 ± 6.7 °C after approximately 3.5 h, at 14:30 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 ± 3.3 °C after approximately 21 h from the start. This occurred at around 07:00 h. The vessel was removed from the warm kitchen environment at around 06:00 h, hence the temperature equilibrated to the

environmental temperature outside. The temperature started to increase again to 34.5 ± 8.5 °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 (Fig. 2.2).

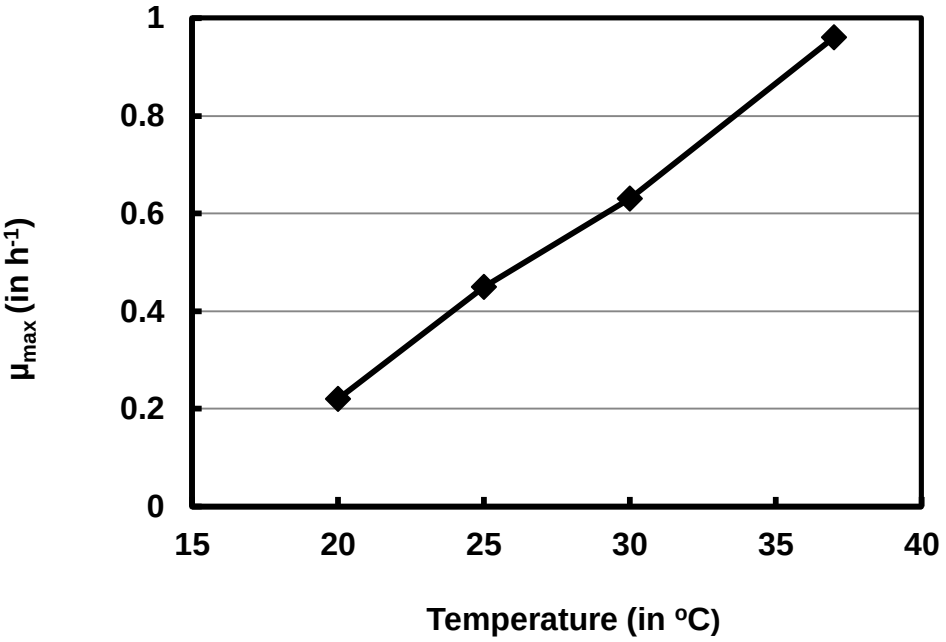

Fig. 3.2: Specific growth rate of *Lactobacillus rhamnosus* yoba in milk supplemented with 4 % baobab fruit pulp as a function of incubation temperature.

Thus, putting the pot in an advantageous position during the day and at night enabled attainment of the temperature that favoured growth of *L. rhamnosus* yoba. The initial temperature within the kitchen was 27.3 ± 5.6 °C, the maximum kitchen temperature recorded was 29.4 ± 3.5 °C after 3.5 h, and the minimum kitchen temperature recorded was 19.5 ± 3.3 °C after 19 h. The open environment temperature showed the highest variation with a maximum of 32.2 ± 5.3 °C after 2 h and the minimum of 16.0 ± 1.8 °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.

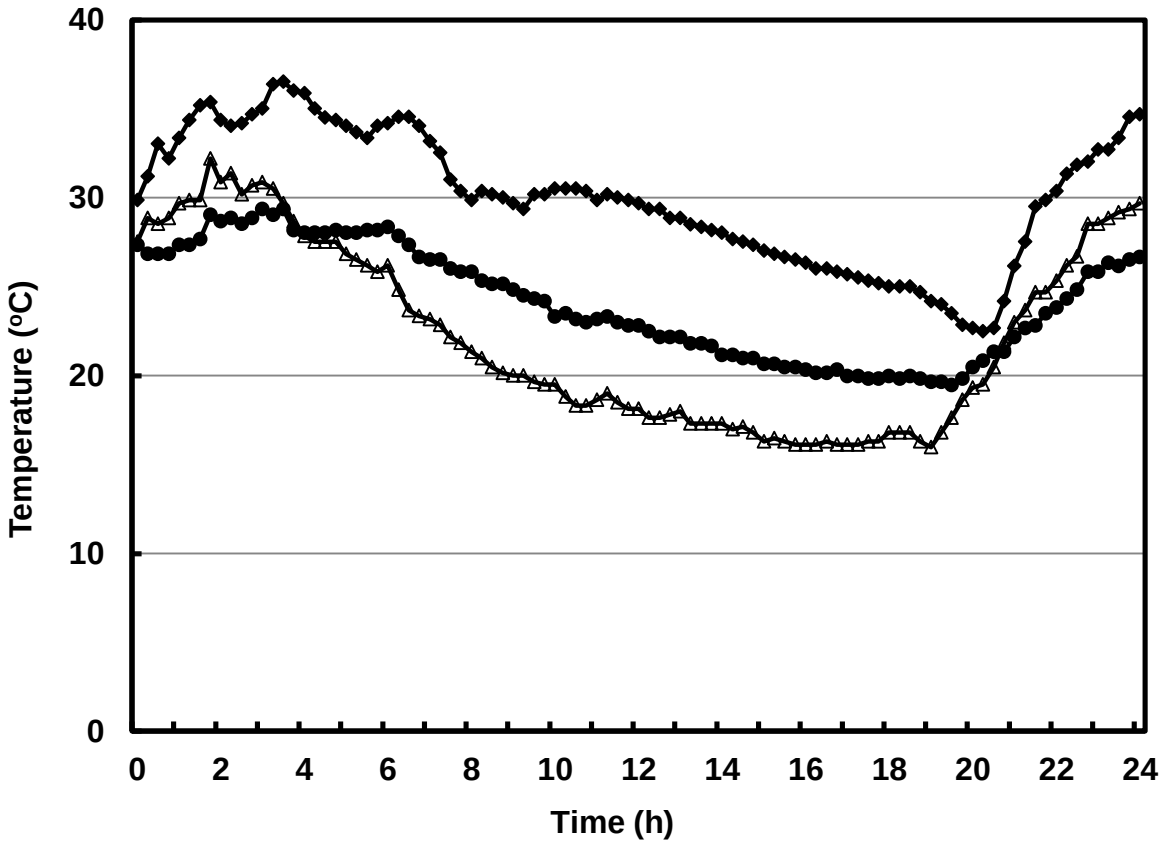

Fig. 3.3: Mean temperature changes during the production of probiotic *mutandabota* (n = 3). The temperature inside the product *mutandabota* (closed diamonds), the ambient temperature outside (open triangles), and the temperature in the kitchen (closed circles) were monitored during a period of 24 h.

3.3 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 2.2). The milk supplemented with 4 % baobab fruit pulp was inoculated with *L. rhamnosus* yoba at a level of 5.8 ± 0.3 log cfu/mL. After 24 h of incubation at a temperature regimen of 22 to 37 °C (Fig. 3.3), the viable plate count of *L. rhamnosus* yoba increased to a level of 8.8 ± 0.3 log 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) crude protein 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).

Table 3.2: Probiotic *mutandabota* composition and numbers of *Lactobacillus rhamnosus* yoba at different stages of preparation (n=3)

Ingredient	**Stage**			
	Inoculum	Inoculated t=0	End of incubation t=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
L. rhamnosus yoba (log cfu/mL)	9.3±0.2	5.8±0.3	8.8±0.3	8.8±0.4

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 nutrients 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 10^7 cfu/ mL in orange juice (pH 3.65) and above 10^6 cfu/mL in pineapple juice (pH 3.40) for at least 12 weeks.

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 2.2, 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; Kajander 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 microscopy. 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.

4.0 Conclusions

Probiotic *mutandabota* was produced at the village level. The amounts of 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 isolate, to the rural population of

southern Africa, using *mutandabota*, a traditional food, with viable *L. rhamnosus* yoba in excess of recommended daily intake levels.

5.0 Acknowledgments

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Chapter 4

Sensory properties and consumer preference of yoba *mutandabota*, a dairy product fermented with *Lactobacillus rhamnosus* yoba

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Abstract

Lactobacillus rhamnosus yoba, a probiotic bacterium, was cultured in *mutandabota* to enable resource-constrained communities to profit from ingestion of a probiotic food. *Mutandabota* is a dairy product and major protein source in rural Southern Africa. Sensory evaluation to determine the acceptability of yoba *mutandabota* was conducted by a panel (n=100) consisting of regular consumers of the product. Composition of traditional and yoba *mutandabota* was the same, namely 79 % (wt/wt) cow's milk, 14 % (wt/wt) baobab fruit pulp and 7 % (wt/wt) sugar. Additionally, yoba *mutandabota* had 8.8 log cfu/mL viable *L. rhamnosus* yoba cells. The sensory properties of traditional and yoba *mutandabota* differed significantly ($p<0.001$). Important product characteristics were milkiness, sweetness, sourness and creamy mouth feel. There was no significant difference ($p=0.31$) in consumers' preferences for traditional or yoba *mutandabota*. Yoba *mutandabota* was thus accepted by its regular consumers as a local, sustainable functional food with the recommended concentration of viable *L. rhamnosus* yoba cells that can deliver health benefits.

Key words: sensory evaluation, baobab, protein source, acceptability, consumers

1.0 Introduction

More than 827 dairy products containing probiotics were available in the year 2007 on the world market (Meyer, 2007). To date, not only has the number ballooned, but the variety of probiotic products has also increased to include milk-based desserts, powdered milk for new-born infants, ice-creams, butter, mayonnaise, cheese, products in the form of capsules and fermented foods of vegetable origin (Buriti et al., 2014; Ranadheera et al., 2010; Saad et al., 2013). Recently, yoba *mutandabota* was developed to enable resource-poor populations in Southern Africa to benefit from a functional food (Mpofu et al., 2014a). An isolate of *Lactobacillus rhamnosus* GG (LGG), under the name *Lactobacillus rhamnosus* yoba (Kort and Sybesma, 2012), was successfully cultured in *mutandabota*, a dairy product and major protein source in rural Southern Africa. Several studies have shown the benefits derived from ingestion of LGG. There is evidence of beneficial effects of LGG derived from clinical trials with double-blind and placebo-controlled cross-over designs for prevention and treatment of diarrhoea, gastrointestinal infections and upper respiratory tract infections in children (Grandy et al., 2010; Guandalini et al., 2000; Hojsak et al., 2010).

According to Mpofu et al. (2014b), *mutandabota* has a thick yogurt-like consistency, a sour taste and a pH of 3.5. It is made from raw unpasteurised cow's milk mixed with baobab (*Adansonia digitata* L.) fruit pulp. The nutrient content of *mutandabota* is (g 100 g^{-1} fresh weight) crude protein 4.8 ± 1, fat 2.8 ± 0.9, crude fibre 1.1 ± 0.4, ash 0.9 ± 0.2, carbohydrates 20 ± 1.7, moisture 70.4 ± 3.7 and vitamin C 80 ± 25 mg 100 g^{-1}. *Mutandabota* is a major source of protein in Siyanzyundu, a rural community in Zimbabwe, which depends, as is the case with most rural Sub-Saharan communities, on millet, sorghum and maize grains for food (Oniang'o, et al., 2003). The content of vitamin C (80 ± 25 mg 100 g^{-1}) makes *mutandabota* an attractive natural source of vitamin C for pregnant and lactating women as well as for children and the elderly.

Baobab fruit pulp, the raw material for *mutandabota* is highly nutritious, its protein has an excellent amino-acid profile, including essential ones such as lysine, methionine, cystine and tryptophan (Chadare et al., 2009; Sena et al., 1998).Vitamins present in baobab fruit pulp include B1 thiamine, B2 riboflavin, B3 niacin, vitamin A and vitamin C. Mineral contents of baobab fruit pulp include iron and calcium at 302 mg/100g and 4.3 mg/100g respectively, this is more than double the amount in milk.

In producing yoba *mutandabota*, a new process was designed based on the traditional *mutandabota* production process. It had two major additional steps, namely boiling of raw milk and fermentation. Both operations could significantly alter its organoleptic properties and thus its acceptability by consumers. Majchrzak et al. (2010) noted that the difference concerning health benefits of probiotic and conventional non probiotic food is not always clear to consumers. Preference for a product ultimately depends on many factors of which the most important is sensorial quality. Factors related to technological and sensory aspects of probiotic foods are of utmost importance since only by satisfying the demands of consumers can the food industry succeed in promoting the consumption of functional products (Mattila–Sandholm et al., 2002). Little work has been done on measuring the sensorial characteristics and acceptability of products fermented with LGG, even though it is one of the most studied bacteria (Gorbach and Goldin 1989; Kort and Sybesma 2012). It has been successfully incorporated into yogurts and fermented milks, both major vehicles for its distribution. The objective of this study was to determine the viable count of *L. rhamnosus* yoba in yoba *mutandabota* at the moment of consumption, to evaluate if a detectable sensorial difference existed between traditional *mutandabota* and yoba *mutandabota* and to determine consumer preference of yoba *mutandabota*. This was done to ascertain whether yoba *mutandabota* can be successfully introduced to targeted communities without further product development.

2.0 Materials and methods

2.1 Ingredients for *mutandabota* preparation

Mature, dry baobab fruits were collected from the wild in Binga district, Zimbabwe (17° 36' S, 27° 32' E). Baobab fruits ripen and dry out while they are still on the tree. Harvesting is by gathering the dropped, 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. Fruit pulp was then used by Binga women, under our supervision, to prepare both traditional and yoba *mutandabota*. Milk for *mutandabota* preparation was obtained from local cows, pooled together and used to produce both traditional and probiotic *mutandabota*.

2.2 Preparation of traditional *mutandabota*

Traditional *mutandabota* was prepared according to the common practice in Binga (Mpofu et al., 2014b). A total 2 kg of traditional *mutandabota* was prepared by gradually adding, while continuously stirring, 280 g of baobab fruit pulp to 1580 g of raw cow's milk in a preparation bowl. Crystalline sugar (140 g) was also added to the mixture. Stirring was continued for approximately 7 min or until a homogeneous mixture was achieved.

2.3 Inoculum and preparation of probiotic *mutandabota*

An isolate of *L. rhamnosus* GG under the name *L. rhamnosus* yoba (Kort and Sybesma, 2012) was used throughout this study. The isolate was obtained from Yoba for Life Foundation, Amsterdam, the Netherlands (www.yoba4life.com). 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* yoba was precultured in full-fat cow's milk that had been boiled and subsequently cooled to ambient temperature (25 °C), before addition of 4 % baobab fruit pulp (Mpofu et al., 2014a). The fermentation vessel was incubated at 37 °C for 36 h to give a concentration of 8 to 9 log cfu/mL viable *L. rhamnosus* yoba cells. This culture was sequentially diluted in milk and used for producing yoba *mutandabota*.

Yoba *mutandabota* was prepared according to Mpofu et al. (2014a). To prepare 2 kg of yoba *mutandabota*, 1570 g of cow's milk was boiled and cooled to an ambient temperature of 25 ^{o}C. *L. rhamnosus* yoba inoculum (10 g) was inoculated into the cooled milk, reaching a concentration of 5 log cfu/mL. Next, 63 g of baobab fruit pulp was added under continuous stirring. This mixture at 4 % baobab fruit pulp concentration was left to ferment for 24 h at approximately 37 ^{o}C, after which 217 g of baobab fruit pulp and 140 g crystalline sucrose were added and manually mixed for 7 min or until a homogeneous mixture of probiotic *mutandabota* was obtained. Yoba *mutandabota* was then ready for consumption. This procedure enabled attainment of 8 to 9 log cfu/mL viable *L. rhamnosus* yoba cells at the moment of consumption. Thus the final composition of both traditional and probiotic *mutandabota* was identical at 79 % (wt/wt) full-fat cow's milk, 14 % (wt/wt) baobab fruit pulp and 7 % (wt/wt) sugar. However, in addition, yoba *mutandabota* had 8.8 log cfu mL^{-1} viable *L. rhamnosus* yoba cells at the moment the sensory evaluation was undertaken.

2.4 Evaluation of *L. rhamnosus* yoba in yoba *mutandabota*

L. rhamnosus yoba was enumerated in yoba *mutandabota* just before the sensory evaluation. For that reason, a 1 mL subsample was aseptically taken from a portion that was about to be served to a consumer. Serial decimal dilutions were made in peptone physiological saline (PPS) (8.5 g/L NaCl and 1 g/L neutralized bacteriological peptone,

LP0034 Oxoid, Basingstoke Hampshire United Kingdom) solution. Immediately, in each case, from PPS serial dilutions, 100 µL of the appropriate diluent were spread plated in triplicate onto de Man, Rogosa and Sharpe Agar (MRSA) (1.2 % agar bacteriological, Oxoid, LP0011 added to de Man, Rogosa and Sharpe broth, VM986641, Merck, Darmstadt Germany). MRSA plates were incubated at 37 ^{o}C in GasPack anaerobic jars (Becton Dickinson Microbiology Systems, Baltimore, Maryland, USA). All colonies on MRSA were counted and results were expressed as colony forming units per millilitre (cfu/mL) of *L. rhamnosus* yoba, taking dilution factors into account.

2.5 Determination of pH

The pH values during preparation of both traditional and yoba *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 subsample of either traditional or yoba *mutandabota* was taken from the preparation vessel just before serving, and pH was determined.

2.6 Sensory evaluation

The sensory evaluation research was approved by the Senate Research Committee of the Chinhoyi University of Technology, Zimbabwe. Before the actual consumer taste panel, a pilot was conducted to determine how the taste panel should be run and how the samples should be prepared and presented. For the actual test panel, 100 regular consumers of *mutandabota* in Binga district were selected using a systematic random sampling procedure (Krebs, 1999). Demographic information about the panellists was collected from informed consent forms signed by panellists. Verbal instructions and questionnaires were translated into the local Shona language. No discussion by panellists was allowed during the evaluation process to ensure accurate data collection. Each sample weighed 28

g and was enough for retesting if the panellist so desired. Samples were served in ordinary dishes covered with aluminium foil. *Mutandabota* is normally served in ordinary dishes, additionally, these dishes do not impart any flavour or odour to products. Distilled water was used as palate cleanser before and between evaluations. A score sheet and pencil were provided along with the samples. The health benefits of yoba *mutandabota* were explained to the panellists after all evaluations were completed.

2.6.1 Triangular taste test

Both traditional and yoba *mutandabota* were subjected to a triangle taste test to determine whether addition of a probiotic bacterium *L. rhamnosus* yoba, and the accompanying modification of the production process resulted in a detectable difference between the two products. Each panellist received three samples coded with three random letters. Serving was at 11:00 a.m., the time that *mutandabota* is normally consumed in this community. The panellists were told that two of the three samples were the same and one was different, and that they had to identify the odd sample. The first 50 panellists tested two samples of traditional *mutandabota* coded RNJ and TQL and one of yoba *mutandabota* coded SPM. The other 50 panellists tested one sample of traditional *mutandabota* coded QSM and two samples of yoba *mutandabota* coded UKG and PNT. The order of the three samples was randomised for each panellist and the order in which each panellist should taste the samples was indicated by putting the sample code in the appropriate order on the score sheet. Analysis of the triangle test results was based on the probability that the difference is significantly different from being selected one third of the time.

2.6.2 Preference tests

The paired comparison test was used as an affective test to measure relative preference to yoba *mutandabota*, because, first, the differences in liking between the samples was expected to be small. Second, the literacy level of most panellists was very low. The same regular consumers of *mutandabota* who performed the triangular taste test, performed the paired comparison test. The panellists were presented with two coded samples, in a randomised order for each panellist. One sample (QSM) was traditional *mutandabota* and the other one (SMP) was yoba *mutandabota.* Panellists were asked which sample they preferred and to explain the reasons behind their choice. Fifty panellists tested a sample of traditional *mutandabota* first and the other 50 tasted a sample of yoba *mutandabota* first. Consumers were asked to provide a written opinion on their choice.

2.7 Statistical Analysis

Statistical analysis was done using SPSS 13.0 for Windows (Apache Software Foundation, USA) and Microsoft Excel. Descriptive statistics such as means, percentages, frequencies, and variance were computed and used to describe the data. Tables for probability in triangular taste tests prepared by Roessler et al. (1948) were used for analysis of triangular taste test data.

3.0 Results and discussion

3.1 The pH and concentration of *L. rhamnosus* yoba cells in probiotic *mutandabota*

In yoba *mutandabota* preparation, the pH of the milk with 4% fruit pulp at the point of inoculation was pH 4.5 ± 0.1. The low pH of the mixture could be attributed to organic acids in dry baobab fruit pulp. Nour et al., (1980) and Vertuani et al. (2002) reported the presence of citric, tartaric, malic, succinic, and ascorbic acids in baobab fruit pulp. 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 further decrease in pH value. After all ingredients were added, the final yoba c *mutandabota* with 14 % fruit pulp had a pH of 3.5. Yoba *mutandabota* had 8.8 log cfu mL^{-1} viable *L. rhamnosus* yoba cells at the moment the sensory evaluation was undertaken. This *L. rhamnosus* yoba count was clearly in excess of 1.0 x 10^6 cfu mL^{-1} of product, which is often quoted as the beneficial and/or therapeutic minimum (Alander et al., 1999; Hojsak et al., 2010). The pH of traditional *mutandabota* was 3.5. The low pH was due to organic acids in baobab fruit pulp. Since the pH of 3.5 is below the isoelectric point (4.5) of casein, the milk protein precipitates giving the product a viscous yogurt like structure. The low pH of both probiotic and traditional *mutandabota* is thought to enhance microbiological safety and reflects the sour taste of the two products.

3.2 Demographic information of panellists

The youngest panellist was 9 years old. From pre-test results, it was decided to exclude children below the age of 9 because they could not comprehend the sensory evaluation exercise. The oldest panellist was 69 years old. Life expectance in Zimbabwe is 60 years (WHO, 2013). *Mutandabota* is eaten by every member of the society, the age spread of the sensory panel is shown in Fig. 4.1. The dominant age groups were the 11 to 15 year

age group and the 16 to 20 year age group, with 17 people in each. Comparison with the population distribution in the area reveals that the population in Binga has more of these age groups compared to older age groups (WHO, 2013). The gender distribution of the panellists was 70 females and 30 males. Generally there are more females than males in rural Zimbabwe, because men have left the rural areas in search of employment in urban areas and neighbouring countries (Olivieri et al., 2008; Andersson, 2001).

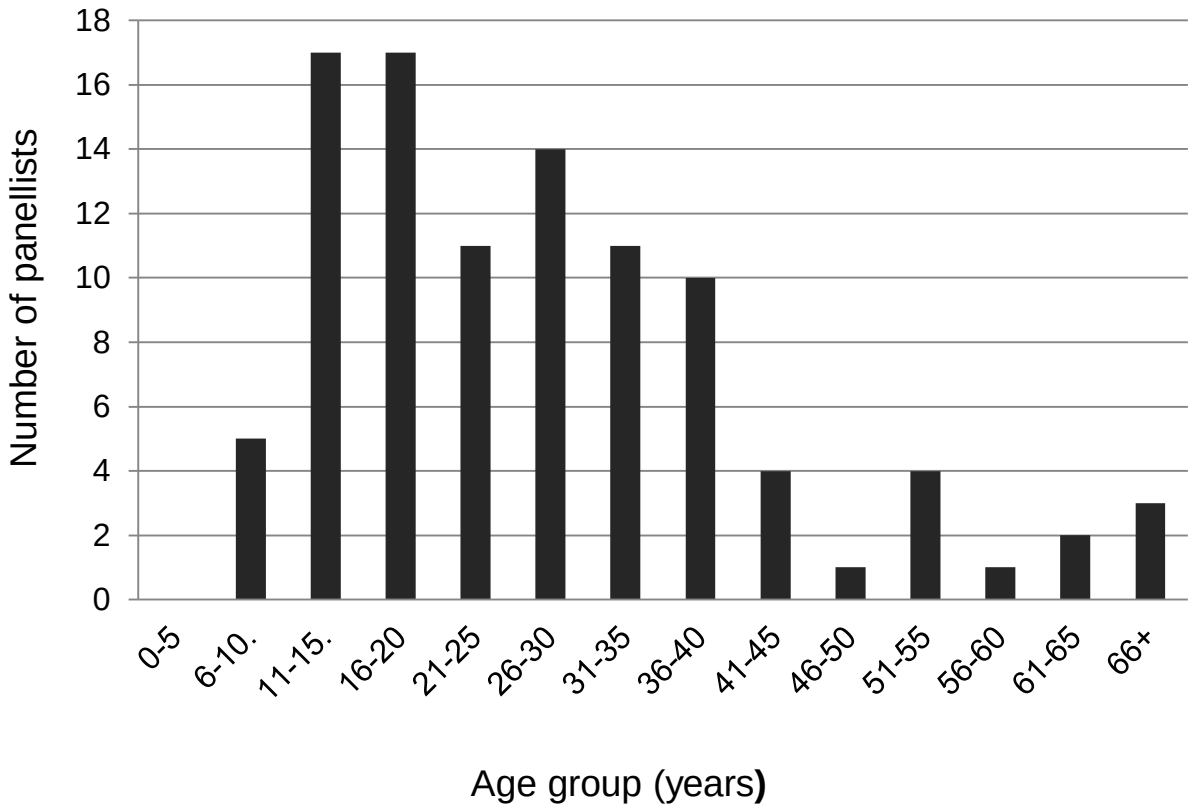

Fig. 4.1: Age distribution of the sensory panel

3.3 Triangular taste test

Of the first 50 panellists (testing two traditional products and one yoba product), 32 correctly stated sample SPM as the odd one, while 7 said RNJ was the odd one and 11 said TQL was the odd one. This meant that the difference between yoba and traditional *mutandabota* was statistically significant at $p<0.001$. From the second group (testing one traditional product and two yoba products), 30 correctly indicated sample QSM as the odd one, 9 said UKJ was the odd one and 11 said PNT was the odd one. Again the difference between the traditional and yoba *mutandabota* was statistically significant at $p<0.001$. The apparent detectable difference ($p<0.001$) between traditional and yoba *mutandabota* could be due to two main reasons. Firstly, milk used for producing yoba *mutandabota* was boiled, whilst for traditional *mutandabota* it was not boiled. Boiling milk denatures whey proteins and increases the water-holding capacity of milk proteins (Hekmat and McMahon, 1992). This eventually affects the structure, texture and aroma of the end product. Secondly, traditional *mutandabota* is a product made from fresh milk, whilst yoba *mutandabota* is a fermented product. By introducing *L. rhamnosus* yoba in *mutandabota* and allowing it to grow over a 24 h period, the fermentation process converts some simple sugars into lactic acid, and other flavouring compounds such as diacetyl and acetoin. This eventually affects the aroma and texture of yoba *mutandabota*. Diacetyl and acetoin are important flavour compounds produced by LGG (Jyoti et al., 2003). In a related study by Atunes et al. (2005), the probiotics *Lactobacillus acidophilus* and *Bifidobacterium longum* did not alter the sensory properties of fat-free yogurt. Hekmat and Reid (2006) observed that *L. rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 did not alter the sensory properties of yogurt. The main explanation for this difference with our results is that conventional and probiotic yogurts are both fermented products, while yoba *mutandabota* is fermented and traditional *mutandabota* is not a fermented product.

3.4 Preference test

From the 100 panellists, 53 preferred sample QSM, which was traditional *mutandabota*, while 47 preferred sample SPM, which was yoba *mutandabota*. Statistically there was no significant difference (p=0.31) in consumers' preferences for either yoba or traditional *mutandabota*. In work on yogurt, Hekmat and Reid (2006) showed that the appearance, flavour, texture, and overall quality of probiotic 1 % fat yogurt were comparable and similar to standard 1 % fat yogurt (control). In work done by Ranathunga et al. (2013), panellists preferred the colour of natural yogurt over probiotic yogurt containing *L. acidophilus* LA-5 and *Bifidobacterium lactis* BB 12. Further, they preferred the taste of natural yogurt in comparison to that of probiotic yogurt. Majchrzak (2010) observed that despite some differences in sensory properties, there were no significant differences in consumers' preferences between probiotic and conventional yogurt. Uysal-Pala, et al. (2006), observed that the intensities of flavors in products made by regular yogurt cultures were higher than those of drinkable yogurts with probiotic cultures. Our work with *mutandabota* was unique in that a non-fermented product had the same level of acceptance as that of a fermented product.

3.5 Consumer opinions on probiotic and traditional *mutandabota*

In food products, the associations that first come to the respondents' mind are regarded as the ones that are the most relevant for consumers' choice and their decisions related to product purchase (Roininen et al., 2006). Analysis of comments and descriptive words written by consumers to explain their choice revealed a trend in sensory attributes of what consumers who participated in this study value most in acceptance and preference of either traditional or yoba *mutandabota* (Table 4.1). This is important because it reveals areas that need to be worked on in the future to produce a product most preferred by consumers. According to Østlie et al. (2003) to succeed in promoting the consumption of functional probiotic products, the food industry has to satisfy the demands of the

consumer. At the same time all probiotic foods should be safe and have good sensory properties (Saarela et al., 2000).

Generally the results from both products were similar, minor non-significant differences were observed (Table 4.1). The main sensory descriptors used by panellists in their evaluations were “a sweet taste” and “a milky taste”. Several participants mentioned sweet *mutandabota*. This is important because children tend to prefer a sweet taste, and yoba *mutandabota* is primarily meant to benefit children in improving nutrition and gastrointestinal health. Only one panellist mentioned the baobab taste, which probably indicates that the baobab taste is generally masked by the sugar and milk taste. These results indicate that the addition of *L. rhamnosus* yoba did not significantly affect the texture, flavour and taste of yoba *mutandabota.* In our case acceptance means regular consumption of yoba *mutandabota* and thus improved nutrition and enhanced health

Table 4.1: Descriptive terms used for yoba and traditional *mutandabota*

Comment	Probiotic *mutandabota*	Traditional *Mutandabota*	Total
More sugar	21	20	41
More milk	18	15	33
Yogurt taste	1	0	1
Thicker	1	1	2
Long lasting taste	2	0	2
Cooked milk	2	0	2
Less sour	3	2	5
Fresh	0	3	3
Smooth	2	1	3
Baobab taste	1	0	1
Odour	1	0	1
Cream taste	3	0	3
Well mixed	1	2	3
Good flavour	1	0	1
Nice	4	3	7
Good	4	1	5
Appetising	1	0	1
Delicious	1	0	1

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4.0 Conclusions

The findings of the paired difference test indicated that there was no significant difference ($p=0.31$) in consumers' preferences between traditional and yoba *mutandabota* containing *L. rhamnosus* yoba, despite a significant difference ($p<0.001$) in sensorial properties of the two products. The main sensory descriptors used by assessors in their evaluations were "a sweet taste" and "a milky taste". Several participants mentioned sweet *mutandabota*. This is important because children tend to prefer a sweet taste, and yoba *mutandabota* is primarily meant to benefit children in improving nutrition and gastrointestinal health. A pH of 3.5 makes *mutandabota* a relatively low risk food

product for pathogenic contamination, which increases its appeal in regions where the cold chain is not always available. It is recommended that production of the probiotic bacteria be localised in Southern Africa. This will lead to establishment of microenterprises with the added benefit of capacity building, knowledge transfer, financial security, and promotes a sense of community empowerment. This project is an excellent show-case for a successful and sustainable nutrition and gastro-intestinal health intervention.

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Chapter 5

Inactivation of bacterial pathogens in yoba *mutandabota*, a dairy product fermented with the probiotic *Lactobacillus rhamnosus* yoba

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Abstract

Mutandabota is a dairy product consumed as a major source of proteins and micronutrients in Southern Africa. In this study the microbial safety of traditional and a probiotic variant of *mutandabota* was investigated by challenging the products with five important food pathogens: *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter jejuni*, *Escherichia coli* O157:H7 and *Bacillus cereus*. Pasteurised full-fat cow's milk was used for producing traditional and probiotic *mutandabota*, and was inoculated with a cocktail of strains of the pathogens at an inoculum level of 5.5 log cfu/mL. Survival of the pathogens was monitored over a potential consumption time of 24 h for traditional *mutandabota*, and over 24 h of fermentation followed by 24 h of potential consumption time for yoba *mutandabota*. In traditional *mutandabota* (pH 3.4±0.1) no viable cells of *B. cereus* and *C. jejuni* were detected 3 h after inoculation, whilst *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella* spp. significantly declined ($P<0.05$), but could still be detected (<3.5 log inactivation) at the end of the potential consumption time. In yoba *mutandabota*, the pH dropped from 4.2±0.1 to 3.3±0.1 after 24 h of fermentation, mainly due to organic acids produced during fermentation. Only *Salmonella* spp. was able to grow in yoba *mutandabota* during the first 9 h of fermentation, but then decreased in viable plate count. None of the tested pathogens were detected (>3.5 log inactivation) after 3 h into potential consumption time of yoba *mutandabota*. The probiotic *Lactobacillus rhamnosus* yoba grew from 5.5±0.1 log cfu/mL to 9.1±0.4 log cfu/mL in the presence of pathogens in yoba *mutandabota*. Inactivation of pathogens in *mutandabota* is of public health significance because food-borne pathogens endanger public health upon consumption of contaminated food, especially in Southern Africa where there are many vulnerable consumers of *mutandabota* such as children, elderly and immuno-compromised people with HIV/AIDS. The findings of this study demonstrate that yoba *mutandabota* fermented with *L. rhamnosus* yoba has antimicrobial properties against the tested pathogens and it is safer compared to the traditional *mutandabota*.

Keywords: food-borne infection, challenge test, fermentation, survival, baobab fruit, LGG

1. Introduction

Probiotic bacteria and their health effects are a focus of international food research. Incorporation of selected strains of the genera *Bifidobacterium* and *Lactobacillus* in milk products and lately in non-dairy products has been studied in detail (McMaster et al., 2005; Østlie et al., 2003; Van Tienen et al., 2011). The beneficial effects of probiotic strains on the host and their mechanism of action have also been demonstrated quite well (Guandalini et al., 2000; Kankainen et al., 2009; von Ossowski et al., 2010). However, little information is available on the survival and growth of pathogens in probiotic dairy foods. Not only good survival of the probiotic bacteria in food products during their specified shelf-life is essential, but also the potential antimicrobial action of the probiotic bacteria against contaminating pathogens during the production process and shelf-life is relevant.

Mutandabota is a non-fermented, milk-based food consumed daily as a major source of proteins and micronutrients, and it is also sometimes used as a supplementary food for infants in Southern Africa (Zimbabwe Ministry of Agriculture, 2001). The product is made by mixing raw cow's or goat's milk 79% (wt/wt), dry baobab (*Adansonia digitata* L.) fruit pulp 14% (wt/wt) and sugar 7% (wt/wt) (Mpofu et al., 2014a). *Mutandabota* has a thick, yogurt-like consistency, a sour taste and a pH of 3.4±0.1. Generally, low pH products are regarded as microbiologically stable and safe to eat (ICMSF, 2002). However, observations on preparation of traditional *mutandabota* evoked questions about its potential role as a vehicle for food-borne microbial infections. The traditional method utilises raw milk, which raises a food safety concern as such milk may contain pathogenic bacteria like *Salmonella* spp., *Listeria monocytogenes* and *Campylobacter jejuni*, which can cause illness in humans (Kumbhar et al., 2009; Nanu et al., 2007). Coliforms and enterotoxigenic *Escherichia coli* have been isolated in raw milk in Zimbabwe and South Africa (Gran et al., 2002; Ibtisam et al., 2008; Mhone et al., 2011).

Preparation of *mutandabota* is carried out at household level in a shaded open space and does not use aseptic techniques. When *mutandabota* is contaminated by pathogens and then consumed, it might cause microbial infection amongst its consumers.

On the basis of *mutandabota*, a probiotic product was developed to enable resource-poor populations in Southern Africa to benefit from a functional food (Mpofu et al., 2014b). *Lactobacillus rhamnosus* yoba, an isolate of *Lactobacillus rhamnosus* GG (LGG) (Kort and Sybesma, 2012) was used to produce yoba *mutandabota*. LGG, originally cultured from a healthy human intestinal source, has been thoroughly studied and used safely as a probiotic strain in a variety of probiotic foods for more than 20 years (Bernardeau et al., 2006; Hatakka, et al., 2001; Kalliomaki, et al., 2001). There is evidence of beneficial effects of LGG based on clinical trials with double-blind and placebo-controlled cross-over designs for prevention and treatment of diarrhoea and gastrointestinal and upper respiratory tract infections in children (Grandy et al., 2010; Hojsak et al., 2010; Guandalini et al., 2000). In producing yoba *mutandabota*, a new production process was designed based on traditional *mutandabota* preparation procedures. Two major steps were incorporated into the traditional procedure, namely the boiling of raw milk and fermentation with *Lactobacillus rhamnosus* yoba. Contamination of the product with pathogenic bacteria may occur after the heat treatment; bacterial pathogens have been isolated from pasteurised milk and products from pasteurised milk (Beukes et al., 2001; Gran et al., 2002; Nyatoti et al., 1997). The fact that food handlers play an important role as vectors for microbial infections has long been demonstrated (Chironna et al., 2004; Olsen et al., 2001). Producing yoba *mutandabota* through fermentation might enhance its microbiological safety. It is thought that bacterial pathogens cannot survive for long in fermented foods due to the presence of organic acids and secondary fermentation products produced during fermentation (Gadaga et al., 2004; Nout and Motarjemi, 1997; Saito et al., 1997). Whether contamination will lead to microbial growth and turn the wholesome food into a health hazard, depends on the nature of the contamination, the

intrinsic factors of traditional or yoba *mutandabota*, the processing and storage conditions. Therefore, this study was performed to investigate the survival of bacterial pathogens in traditional and yoba *mutandabota*.

2. Materials and Methods

2.1 Selection of bacterial pathogens

Five bacterial pathogens were selected to evaluate the food safety risk of traditional and yoba *mutandabota.* The pathogen selection was based on expert advice and scientific literature on pathogens identified as causing food-borne microbial infections in Southern Africa, particularly Zimbabwe. The selected pathogens were five strains each of *Campylobacter jejuni*, *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Bacillus cereus*. Then three strains of *Salmonella* subspecies Enteritidis, a strain each of *Salmonella* subspecies Paratyphi B and *Salmonella* subspecies Typhimurium. All pathogenic strains except four, were obtained from the Laboratory of Food Microbiology culture collection, Wageningen University, Wageningen, the Netherlands. Four of the *E. coli* O157:H7 strains were obtained from the Netherlands National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands.

2.2 Preparing cocktails of bacterial pathogens

All strains were stored in cryovials in a freezer at -80 ^{o}C and were revived by plating. *L. monocytogenes*, *Salmonella* spp., *E. coli* O157:H7 and *B. cereus* strains were plated individually on Brain Heart Infusion (BHI) agar [37 g BHI broth (Oxoid, Basingstoke, UK) and 12 g agar bacteriological (Oxoid), in 1 litre of distilled water]. *C. jejuni* strains were plated individually on blood agar [40 g blood agar base (Oxoid) and 7 % sterile blood in 1 litre of distilled water]. BHI agar plates with either *E. coli*, *L. monocytogenes* or *Salmonella* spp. were incubated aerobically at 37 ^{o}C for 24 h. Plates with *B. cereus*

were incubated aerobically at 30 ºC for 24 h. Plates with *C. jejuni* were incubated microaerobically at 41.5 ºC for 48 h. To prepare the inoculum, a colony of each strain was picked from a respective plate and inoculated into BHI broth, with heart infusion (HI) broth (Oxoid) being used for each *C. jejuni* strain. Incubation was done in stationary incubators for 24 h, reaching a bacterial broth culture concentration of approximately 9 log cfu/mL. A cocktail of five strains of each species in suspension was produced by mixing equal portions (2 mL) of each strain in a sterile test tube and mixing. The cocktail was then ready for inoculation into *mutandabota*.

2.3 Preparation of LGG inoculum

An isolate of the probiotic bacterium *Lactobacillus rhamnosus* GG, under the name *Lactobacillus rhamnosus* yoba (Kort and Sybesma, 2012), was used in this study. The bacterium was obtained from Yoba for Life Foundation (http://www.yoba4life.com), Amsterdam, the Netherlands. It was stored at -80 ºC, before being freeze-dried for long-term storage at 4 ºC in 50 mL tubes (Greiner Bio-One, BV, Alphen a/d Rijn, the Netherlands). To prepare the inoculum, baobab fruit pulp was added to UHT full-fat cow's milk to a concentration of 4 % (wt/wt). *L. rhamnosus* yoba, 5 log cfu/mL was pre-cultured in this medium in a fermentation vessel and incubated at 37 ºC for 36 h. This gave a concentration of approximately 9 log cfu/mL *L. rhamnosus* yoba. This culture was sequentially diluted in peptone physiological saline (PPS) with the final dilution 10^{-3} done in UHT full-fat cow's milk before use in producing yoba *mutandabota*. The inoculum for producing yoba *mutandabota* was approximately 5 log cfu/mL *L. rhamnosus* yoba.

2.4 Preparation of traditional and yoba *mutandabota*

Traditional *mutandabota* (100 g) was prepared based on the local practice in Binga district, Zimbabwe (17° 36' S, 27° 32' E) (Mpofu et al., 2014a). This was done by gradually adding, while continuously shaking, 14 g of baobab fruit pulp and 7 g of crystalline sucrose to 79 g of UHT full-fat cow's milk in a 250 mL sterile bottle. Manual stirring was continued for 7 min or until a homogeneous mixture was achieved. Yoba *mutandabota* was prepared as illustrated in Fig. 5.1 (Mpofu et al., 2014b). To prepare 100 g of yoba *mutandabota*, *L. rhamnosus* yoba inoculum (prepared earlier) was added to 78 g of UHT full-fat cow's milk to a concentration of 5.5 log cfu/mL. Dry baobab fruit pulp was then added to give a concentration of 4 % (wt/wt) and vigorously mixed. This mixture was left to ferment for 24 h at 37 ^{o}C in a stationary incubator. After the fermentation step, an additional 10 g of baobab fruit pulp and 7 g crystalline sucrose were added and mixed for 7 min to obtain a homogeneous mixture of yoba *mutandabota*. The product was now ready for consumption. This procedure enabled attainment of approximately 9 log cfu/mL *L. rhamnosus* yoba in the final product at consumption.

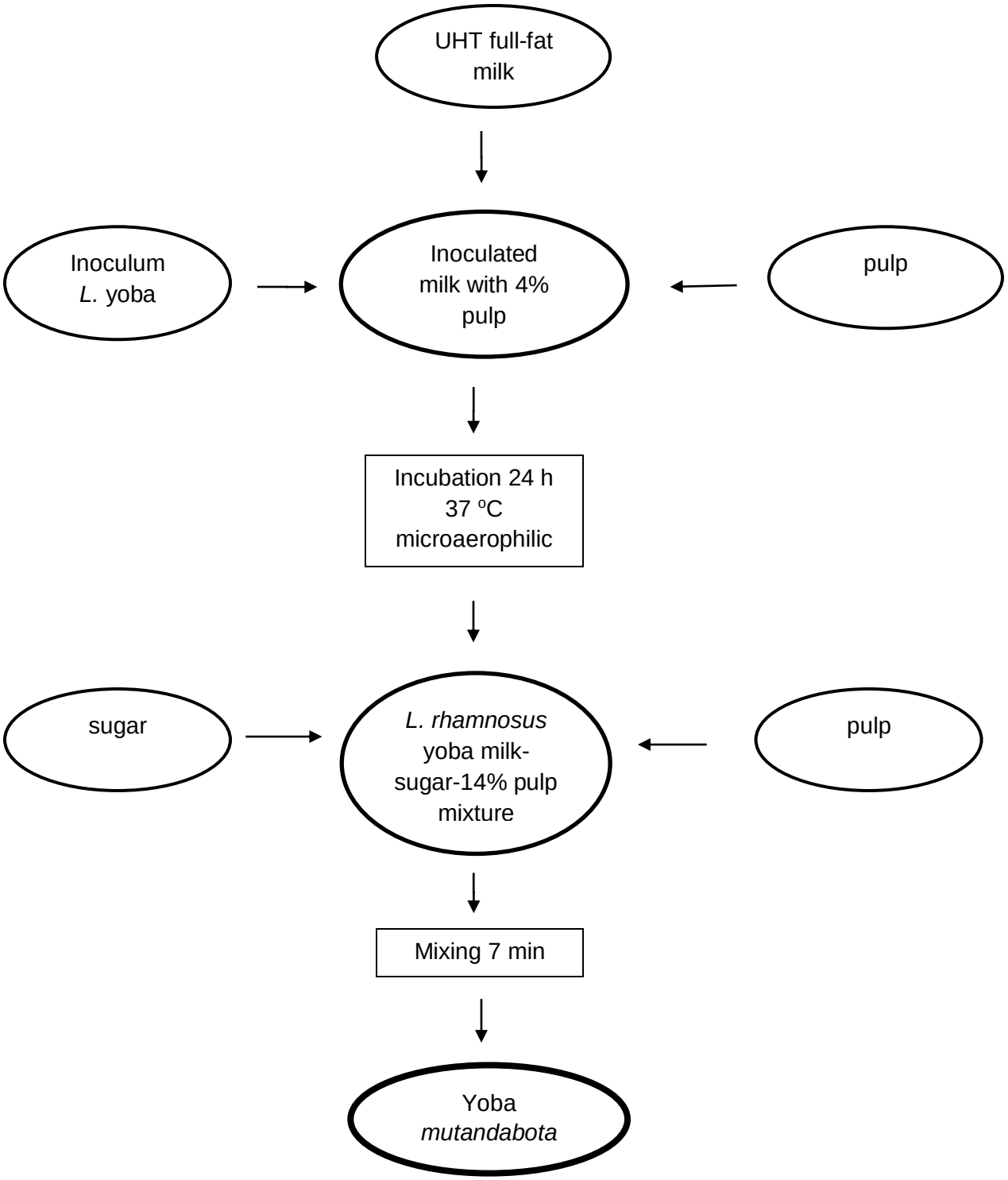

Fig. 5.1: Flow chart for the production of yoba *mutandabota*

2.5 Artificial contamination of traditional and yoba *mutandabota*

Independent experiments were done with five strains of each pathogen; accordingly five strains of *L. monocytogenes* were mixed together in BHI broth and an experiment done, then five strains of *E. coli* O157:H7 were mixed together in BHI broth and an experiment done, and so on until the independent experiments were completed for the five strains of *B. cereus* in BHI broth and five strains of *C. jejuni* in BI broth. The three strains of *Salmonella* subspecies Enteritidis, a strain each of *Salmonella* subspecies Paratyphi B and *Salmonella* subspecies Typhimurium were mixed together in BHI broth and an experiment done. Each cocktail of each pathogen in the respective broth was serially diluted in PPS, with the final dilution 10^{-3} done in UHT full-fat cow's milk. Of this, 1 mL was inoculated into 79g UHT full-fat cow's milk that was used to prepare traditional, and 1 mL was inoculated into 78g UHT full-fat cow's milk that was used to prepare yoba *mutandabota*. In both traditional and yoba *mutandabota*, the initial concentration of the pathogenic cocktail suspension was approximately 5.5 log cfu/mL. For yoba *mutandabota*, inoculation of the pathogenic cocktail suspension was done simultaneously with *L. rhamnosus* yoba inoculation. Independent triplicate experiments were done with each cocktail suspension. Non inoculated samples (negative controls inoculated with 1 mL full-fat UHT milk) were also tested using the above procedure to confirm that no naturally occurring bacterial pathogens under test, or organisms giving similar colonial morphologies to those added to the inoculated products, were present.

2.6 Sampling, incubation and enumeration of bacterial strains

In Southern Africa *mutandabota* is consumed within 24 h after preparation. Therefore the survival of pathogens in both yoba and traditional *mutandabota* was determined over a potential consumption time of 24 h at 25 ^{o}C, to simulate the average ambient temperature in Southern Africa where the product is consumed. For traditional *mutandabota*, sampling was immediately done after mixing of ingredients and the inoculum (defined as

t=0), and at approximately 3 h intervals until the end of the 24 h storage at t=24. For yoba *mutandabota* (Fig. 5.1), sampling was immediately after mixing of ingredients and the inoculum (defined as t= -24), and at approximately 3 h intervals throughout the 24 h fermentation time and the subsequent 24 h storage time until t=24. Sequential serial dilutions were made in PPS and plating was done on selective media followed by incubation under appropriate conditions. For *L. monocytogenes*, plating was on Agar Listeria Ottavani & Agosti (bioMerieux, Marcy I'Etoile, France) and incubation was aerobically at 37 ºC for 24 h. For *Salmonella* spp., plating was on Xylose-Lysine-Desoxycholate Agar (Oxoid) and incubation was aerobic at 37 ºC for 24 h. For *E. coli* O157:H7, plating was on MacConkey agar (Oxoid) and incubation was aerobic at 37 ºC for 24 h. For *B. cereus* plating was on Mannitol Egg Yolk Polymyxin Agar (Oxoid) and incubation was aerobic at 30 ºC for 24 h. For *C. jejuni* plating was on Campy Food Agar (bioMerieux) and incubation was microaerobic at 41.5 ºC for 48 h. For *L. rhamnosus* yoba, plating was on de Man, Rogosa and Sharpe Agar [MRSA: 12 g agar bacteriological (Oxoid), added to 52.2 g de Man, Rogosa and Sharpe broth (Merck, Darmstadt, Germany) in 1 litre distilled water] and incubation was under microaerobic conditions at 37 ºC for 24 h. Colonies were enumerated and results were expressed in log cfu/mL of either traditional or yoba *mutandabota.* The detection limit of the plating method was 100 cfu/mL.

2.7 Lactic acid determination

Lactic acid was determined by HPLC. Briefly, samples of traditional and yoba *mutandabota* were deproteinated by adding 0.25 mL cold Carrez A solution (Sigma-Aldrich, Steinheim, Germany) (42.2 g $K_4Fe(CN)_6.3H_2O$ per 1 L demineralized water) to 0.5 mL sample of traditional or yoba *mutandabota* in an Eppendorf tube and mixed, then 0.25 mL of cold Carrez B solution (Sigma-Aldrich, Steinheim, Germany) (57.5 g $ZnSO_4.7H_2O$ per 1 L demineralized water) was added and mixed. The Eppendorf tubes

with samples were centrifuged and the supernatant was taken for HPLC analysis (Ultimate 3000, Dionex), using an Aminex HPX-87H 300x7.8mm column with a pre-column (Biorad). The eluent was 5 mM H_2SO_4 at a flow rate of 0.6 mL/min at 40˚C. Detection was done by refractive index (Shodex RI 101). Sample volume was 10 µL and the run time was 30 min.

2.8 pH measurement

The pH values during the experiments were measured using a combined glass electrode pH meter (WTW, Weilheim, Germany) that was calibrated using standard buffer solutions (Merck, Darmstadt, Germany) at pH 4.0 and 7.0. The pH was determined each time a sample was taken for microbial analysis at times indicated above.

2.9 Statistical analysis

Independent experiments including inoculum preparation, product making and storage were performed in triplicate. Data points were represented by the mean, with the standard deviation indicated by error bars. The mean values of pH of all samples as well as mean log counts of the bacterial pathogens and *L. rhamnosus* yoba were compared using a one-way ANOVA and Tukey's post-hoc tests. Statistical analysis was done using SPSS 13.0 for Windows (Apache Software Foundation, Forest Hill, Maryland, USA) and Microsoft Excel.

3. Results and Discussion

3.1 pH changes in traditional and yoba *mutandabota*

The time course of acidification in traditional *mutandabota* was similar in all experiments with the 5 cocktails of bacterial pathogens (Figs 5.2 to 5.6). The pH was 3.5±0.01 (*n*=15) immediately after preparation when *mutandabota* was ready for consumption at time 0 h (t=0). It remained rather constant at this pH throughout the 24 h potential consumption time, also regarded as the storage period, and at t=24 the pH was 3.4±0.1. The low pH could be attributed to the acidic nature of dry baobab fruit pulp. Airan and Desai (1954) and Carr (1955) 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. For yoba *mutandabota* at the simultaneous inoculation (t= -24h) with *L. rhamnosus* yoba and the pathogenic bacteria cocktail into the 4 % pulp-milk mixture, the pH was 4.2±0.1 (*n*=15). After 9 h the pH had dropped to 3.7±0.01. At t=0, signalling the end of the fermentation stage, the pH was 3.3±0.1. The lowering of pH from 4.2 to 3.3 during the fermentation phase could be attributed to organic acids such as lactic acid produced by the fermenting *L. rhamnosus* yoba. When the remaining 10 % pulp and 7 % sugar were added to get the standard constituents of *mutandabota*, the pH remained stable at 3.3±0.1. Yoba *mutandabota* was then ready for consumption. The pH remained rather constant at 3.3±0.1 throughout the 24 h storage period from t=0 to t=24 in probiotic *mutandabota* (Figs 5.2 to 5.6). The pH in the control experiment was 4.2 at time t= -24. At t=0, the pH was still 4.2. When the remaining 10 % pulp and 7 % sugar were added to get the standard constituents of *mutandabota*, the pH remained stable at 3.4±0.1.

3.2 Growth of *L. rhamnosus* yoba in yoba *mutandabota* with pathogens

L. rhamnosus yoba, an isolate of the probiotic bacterium *Lactobacillus rhamnosus* GG (LGG) (Kort and Sybesma, 2012) was chosen for this study. LGG is widely prescribed for treatment of acute diarrhea in children in Italy, its efficacy was evaluated by in vivo studies (Canani et al., 2007; Grandy et al., 2010; Hojsak et al., 2010). The growth of *L. rhamnosus* yoba in yoba *mutandabota* followed a similar pattern in each experiment (Figs 5.2 to 5.6). Generally, from an inoculation level of 5.5±0.1 log cfu/mL, *L. rhamnosus* yoba showed robustness and increased counts in the presence of pathogens, in some instance, even with increasing pathogen concentration, such as *Salmonella,* which increased by 2 log units during the first 9 h of incubation (Fig. 5.6). *L. rhamnosus* yoba reached 6.6±0.2 log cfu/mL at t= -15, and further increased to 9.1±0.4 log cfu/mL at t=0, which was the end of the fermentation process. At this time the remaining baobab fruit pulp and sugar were added to reach the standard 14 % pulp and 7 % sugar in yoba *mutandabota*. At the end of the storage time, t=24, *L. rhamnosus* yoba was at 8.5±0.9 log cfu/mL. In the control experiment no growth was observed.

3.3 Survival and decline of pathogens in traditional and yoba *mutandabota*

The loss in viability of *B. cereus* inoculated into traditional and yoba *mutandabota* is depicted in Fig. 5.2. The harsh effect of the acidic environment with a pH of 3.5 was pronounced. From an inoculation level of 5.6 log cfu/mL in both traditional and yoba *mutandabota*, no *B. cereus* could be detected 3 h after inoculation in both types of *mutandabota. B. cereus* does not have a marked tolerance for pH below 4.5. Hassan et al. (2010) noted that vegetative cells of *B. cereus* rapidly die in yoghurt, a comparable product to probiotic *mutandabota*, hence

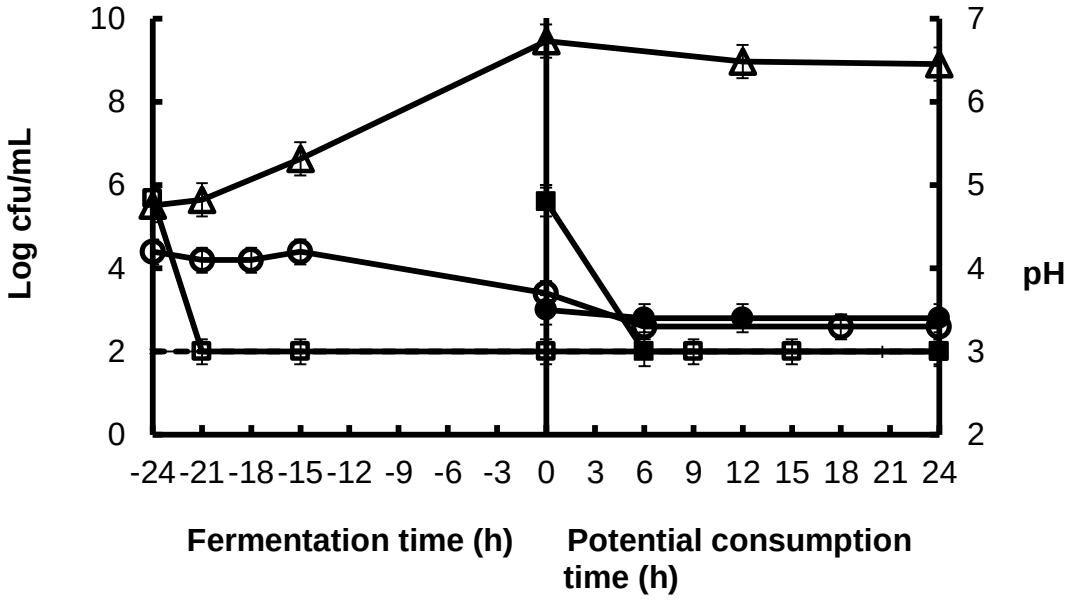

Fig. 5.2: Kinetics of *Lactobacillus rhamnosus* yoba and *Bacillus cereus* during production of traditional and yoba *mutandabota*. Fermentation takes 24 h, 0 h is the start of potential consumption time; (open triangle) *Lactobacillus rhamnosus* yoba in yoba *mutandabota*, (closed square) *B. cereus* in traditional *mutandabota*, (open square) *B. cereus* in yoba *mutandabota*, (closed circle) pH changes in traditional *mutandabota*, (open circle) pH changes in yoba *mutandabota*. Points represented on the detection limit indicate results below the detection limit (no detection).

acidification is a common method of preservation. Working on yoghurt, Ayoub et al. (2003) found that 20 % of examined yoghurt samples were tested positive for *B. cereus*. Lower incidence of *B. cereus* were reported by Hassan et al. (2010), who found 2 % (1 out of 50) of yoghurt samples positive for *B. cereus*. Conversely, Khalil (1997) and Abdel-Khalek (2002) did not detect *B. cereus* in yoghurt. The lower incidence or absence of *B. cereus* in yoghurt was attributed to the inhibitory effect of lactic acid bacteria on *B. cereus*. Control of *B. cereus* in *mutandabota* and other dairy products is important because this bacterium, when producing toxins, endangers public health upon consumption of contaminated foods. It can cause two types of food-borne illnesses, namely a diarrhoea syndrome because of its ability to produce three different

enterotoxins, first described by Hauge (1955), and an emetic syndrome because of its ability to produce an emetic toxin (Granum and Baird-Parker, 2000). Both syndromes have occasionally been associated with milk products (Christiansson et al., 1999). When stored, both traditional and yoba *mutandabota* are unlikely to be sources of *B. cereus* infection because of the inherent low pH of 3.5.

The *C. jejuni* cocktail was inoculated at a level of 5.7 log cfu/mL in both traditional and yoba *mutandabota*. No *C. jejuni* cells could be detected 3 h after inoculation (Fig. 5.3). A similar result was obtained by Simango and Rukure (1991) with *mahewu*, a traditional fermented cereal beverage in Zimbabwe. They showed that none of the 4 strains of *C. jejuni* tested survived for 30 min in *mahewu* (pH 3.6) although high inocula of 6 to 7 log cfu/mL were used. *C. jejuni* was also shown to die rapidly within 30 min in yoghurt (Cuz et al., 1987) and within 2 h at pH 4 in the presence of formic acid (Chaveerach et al., 2003). Rahimi et al. (2013), investigating the prevalence of *Campylobacter* spp. in milk and dairy products, isolated *C. jejuni* from raw cow's milk, goat's milk and traditional cheese made from raw milk. No *C. jejuni* was isolated from pasteurised milk, yoghurt and commercial dairy products. Boiling milk or pasteurisation is believed to be sufficient to inactivate *C. jejuni* (D'Aoust et al., 1988). Inactivation of *C. jejuni* in *mutandabota* is of interest because this bacterium is one of the most important causes of diarrhoea in infants under 2 years of age in Southern Africa (Simango and Rukure, 1991; Kotloff, 2013).

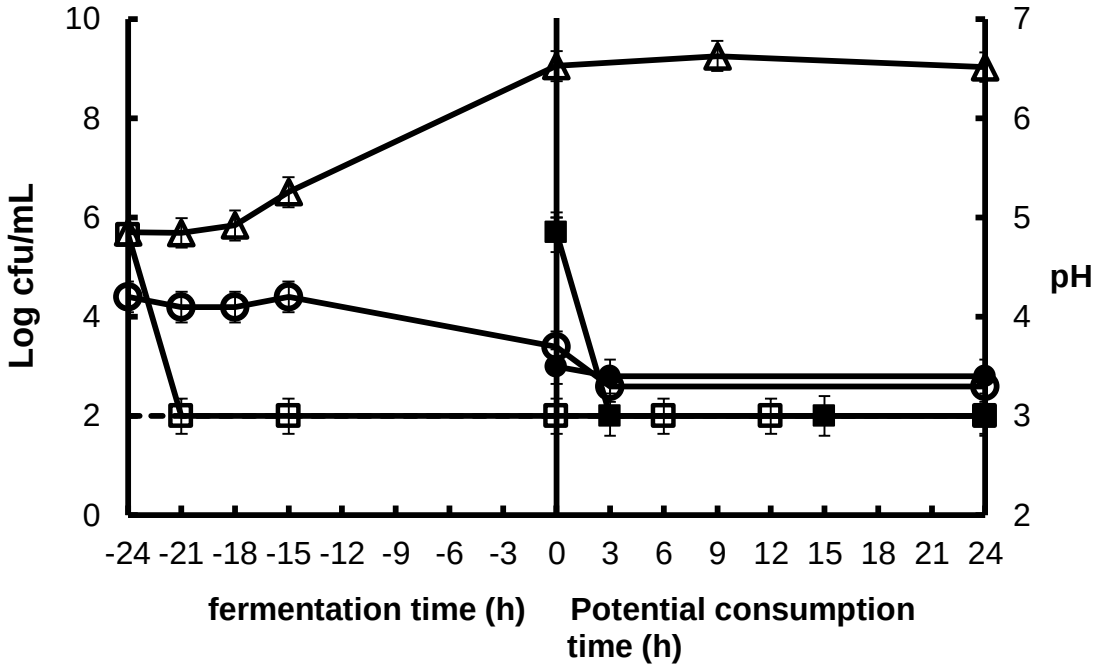

Fig. 5.3: Kinetics of *Lactobacillus rhamnosus* yoba and *Campylobacter jejuni* during production of traditional and yoba *mutandabota*. Fermentation takes 24 h, 0 h is the start of potential consumption time; (open triangle) *Lactobacillus rhamnosus* yoba in yoba *mutandabota*, (closed square) *C. jejuni* in traditional *mutandabota,* (open square) *C. jejuni* in yoba *mutandabota,* (closed circle) pH changes in traditional *mutandabota*, (open circle) pH changes in yoba *mutandabota*.

The disease caused by *C. jejuni* usually manifests itself as diarrhoea, fever, malaise and severe abdominal pain. More recent studies suggest that *C. jejuni* infections can lead to inflammatory bowel diseases such as Crohn's disease (Horrocks et al., 2009). The combination of temperature, organic acids and low pH of both types of *mutandabota* could explain the failure of *C. jejuni* to survive in the product during production and the period of potential consumption. In conclusion, both traditional and yoba *mutandabota* are unlikely to be sources of *C. jejuni* infection.

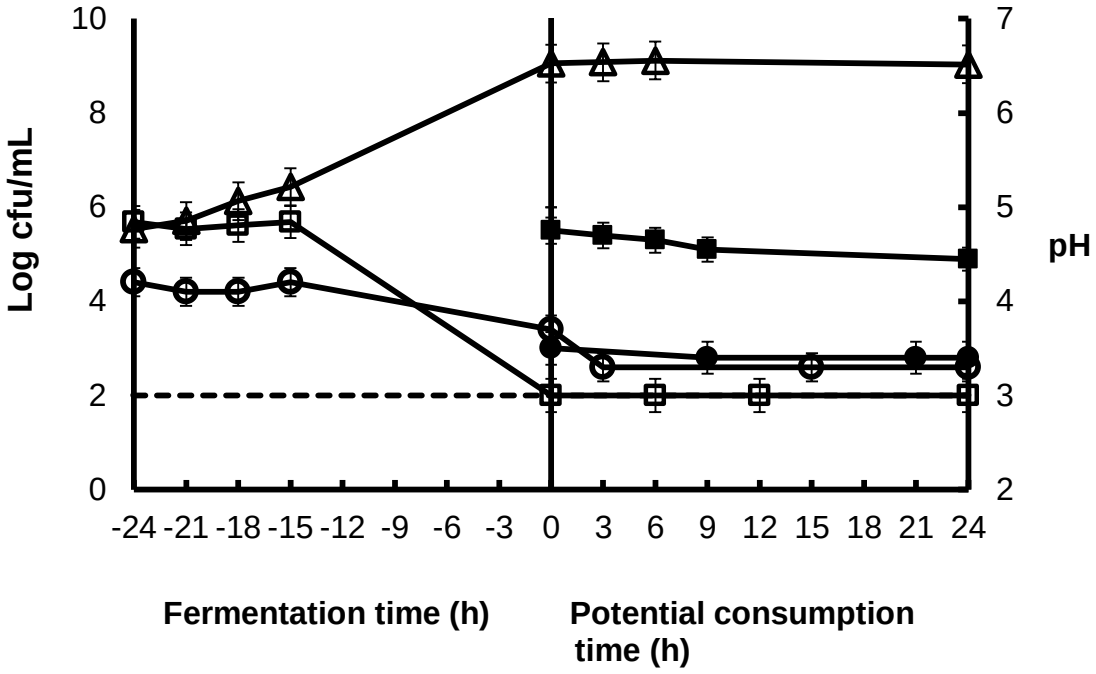

Fig. 5.4: Kinetics of *Lactobacillus rhamnosus* yoba and *Listeria monocytogenes* during production of traditional and yoba *mutandabota*. Fermentation takes 24 h, 0 h is the start of potential consumption time; (open triangle) *Lactobacillus rhamnosus* yoba in yoba *mutandabota*, (closed square) *Listeria monocytogenes* in traditional *mutandabota,* (open square) *Listeria monocytogenes* in yoba *mutandabota,* (closed circle) pH changes in traditional *mutandabota*, (open circle) pH changes in yoba *mutandabota.*

In traditional *mutandabota* (Fig. 5.4), *L. monocytogenes* from an inoculation level of 5.5 log cfu/mL, was at 5.1 log cfu/mL after 9 h, and 4.9 log cfu/mL at the end of the period of potential consumption at t=24, a significant ($P<0.05$), but limited decline. Although contamination is unlikely to reach the levels used in this study, it is evident that low pH alone (3.5±0.1) will not completely inactivate *L. monocytogenes* and will not guarantee microbiological safety of traditional *mutandabota.* It must be accompanied by other measures, such as controlled fermentation, to produce a fermented product with antimicrobial properties. The ability of *L. monocytogenes* to survive in traditional

mutandabota is of public health significance. *L. monocytogenes* can cause meningitis, encephalitis, abortion, premature birth, stillbirth, and gastroenteritis (Seeliger and Jones, 1986; Siegman-Igra et al., 2002). The worldwide case fatality rate for listeriosis is estimated to be as high as 30 % (Siegman-Igra et al., 2002; Swaminathan and Gerner-Smidt, 2007). In yoba *mutandabota* (Fig. 5.4), *L. monocytogenes* remained rather constant at the inoculated level of 5.7 log cfu/mL until t= -15. However, no *L. monocytogenes* could be detected at t=0, the end of fermentation. The decrease of *L. monocytogenes* corresponded to an increase in *L. rhamnosus* yoba from 5.5 log cfu/mL at t= -24, 6.4 log cfu/mL at t= -15 and 9.1 log cfu/mL at t=0 at the end of the fermentation (Fig. 5.4). When the remaining pulp and sugar were added to prepare *mutandabota* for consumption, yoba *mutandabota* was microbiologically safe to consume. Similar observations were made by Dalu and Feresu (1995), working on traditionally fermented unpasteurized and pasteurized milk and on an industrially fermented milk marketed in Zimbabwe. Their results indicated that *L. monocytogenes* was inactivated at different levels during fermentation and storage of all the three fermented milk products (pH 4.5 and stored at 20 ^{o}C). The current study demonstrated that inactivation of *L. monocytogenes* in yoba *mutandabota* was probably due to a combined effect of a low pH of 3.4, acids and possibly other fermentation products produced by the fermenting *L. rhamnosus* yoba, since in traditional *mutandabota* (pH 3.5), *L. monocytogenes* survived rather well.

In traditional *mutandabota* (Fig. 5.5), *E. coli* O157:H7 was inoculated at a level of 5.9 log cfu/mL. *E. coli* survived the first 3 hours and then declined to 4.7 log cfu/mL after 9 h, and finally 2.6 log cfu/mL at t=24, the end of the period of potential consumption for traditional *mutandabota*. Since *E. coli* decreased by only 1 log cfu/mL to 4.7 log cfu/mL at t=9, and was still at 2.6 log cfu/mL at the end the storage period, it suggests that traditional *mutandabota,* if contaminated with *E. coli* and depending on levels of contamination, might not be safe for consumption. In yoba *mutandabota, E. coli* was

rather stable at 5.9 log cfu/mL during the first 9 hours of fermentation, but was below the detection limit at the end of fermentation at t=0, before addition of the 10% pulp and 7% sugar (Fig. 5.5). The decrease corresponded to an increase in counts of *L. rhamnosus* yoba.

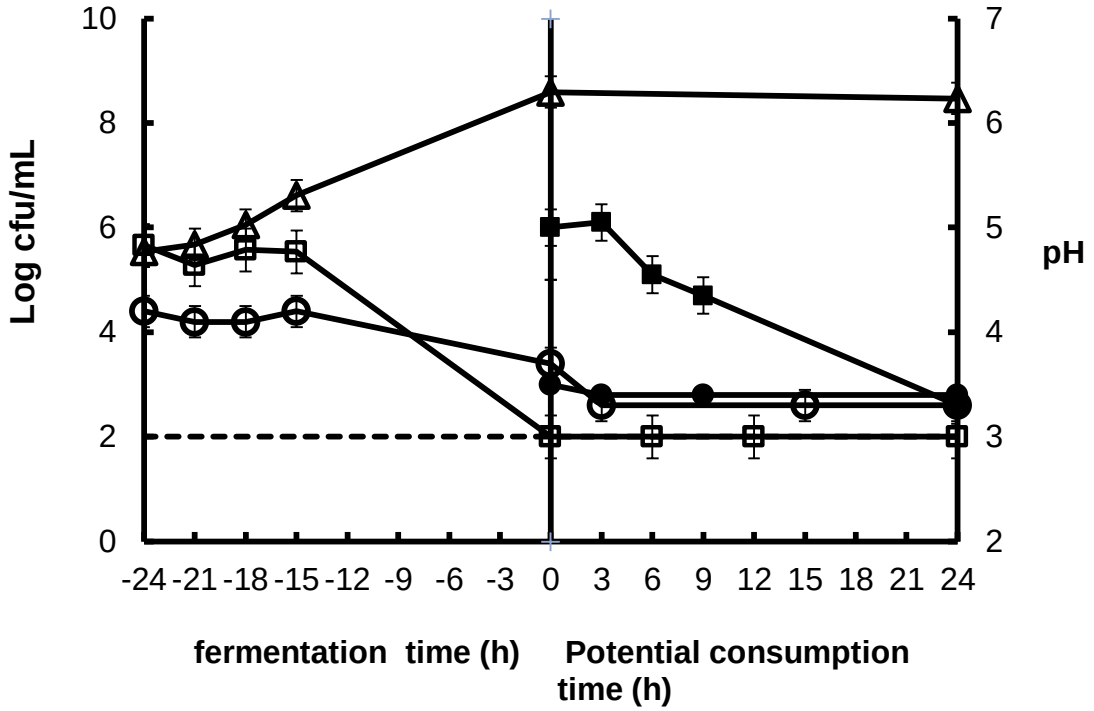

Fig. 5.5: Kinetics of *Lactobacillus rhamnosus* yoba and *Escherichia coli* O157:H7 during production of traditional and yoba *mutandabota*. Fermentation takes 24 h, 0 h is the moment of start of potential consumption time; (open triangle) *Lactobacillus rhamnosus* yoba in yoba *mutandabota*, (closed square) *E. coli* O157:H7 in traditional *mutandabota,* (open square) *E. coli* O157:H7 in yoba *mutandabota,* (closed circle) pH changes in traditional *mutandabota*, (open circle) pH changes in yoba *mutandabota*.

In a study to investigate the survival of bacterial pathogens that had been associated with childhood diarrhoea in Zimbabwe, Simango and Rukure (1991), showed that starting with a high inoculum of 6 to 7 log cfu/mL in *mahewu*, all strains of enteropathogenic and enterotoxigenic *E. coli* were detected in *mahewu* (pH 3.6) after 24 h of storage at 25°C.

Most of the *E. coli* strains showed very little change in numbers of surviving cells. The low infective dose of verotixin-producing *Escherichia coli* (VTEC) and the severity of the infection and the apparent increase in incidence of infection by *E. coli* O157:H7 and emergence of other VTEC sero-groups (e.g. O111 and O26), does give cause for concern, especially for susceptible consumer groups, such as children and elderly (Baylis et al., 2004). Yoba *mutandabota* is safer than traditional *mutandabota*.

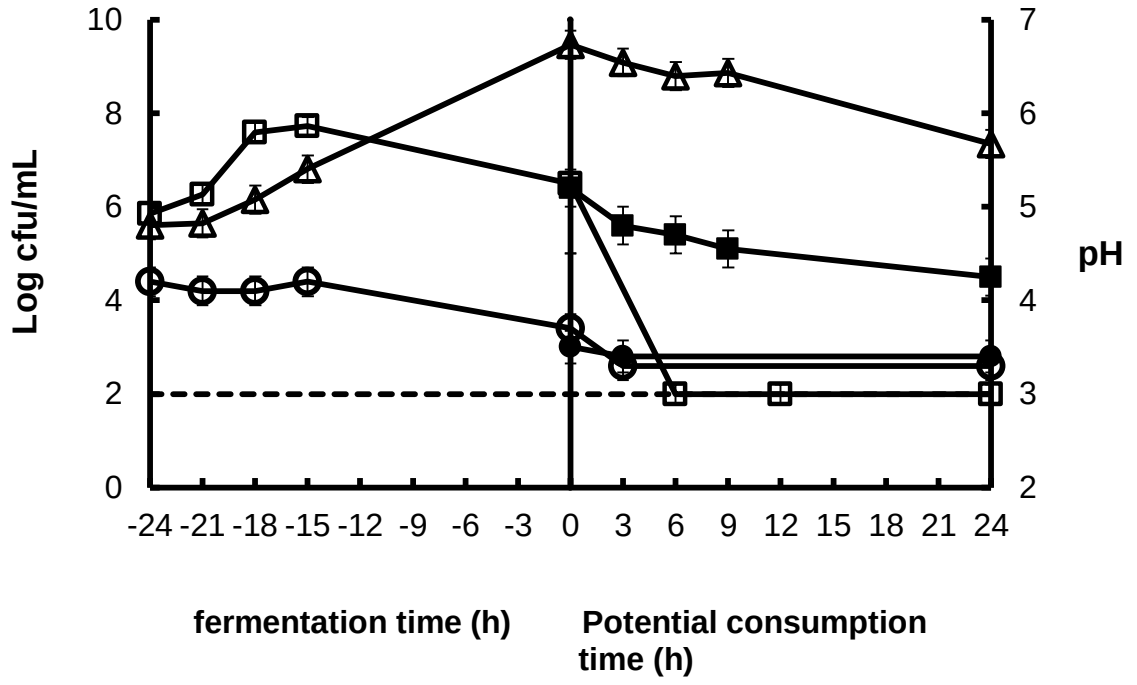

Fig. 5.6: Kinetics of *Lactobacillus rhamnosus* yoba and *Salmonella* spp. during production of traditional and yoba *mutandabota*. Fermentation takes 24 h, 0 h is the moment of start of potential consumption time; (open triangle) *Lactobacillus rhamnosus* yoba in yoba *mutandabota*, (closed square) *Salmonella* spp. in traditional *mutandabota*, (open square) *Salmonella* spp. in yoba *mutandabota*,(closed circle) pH changes in traditional *mutandabota* over a period of 24 h, (open circle) pH changes in yoba *mutandabota*.

In traditional *mutandabota*, there was a decrease of *Salmonella* spp. throughout the potential consumption period of 24 h (Fig. 5.6). From an inoculation level of 6.4 log cfu/mL, the *Salmonella* counts decreased to 5.1 log cfu/mL at t=9, and 4.5 log cfu/mL at

the end of the potential consumption time, t=24, a significant decrease ($P<0.05$) by 2 log units. However, the level still remained above the detection limit. This reflects that once contaminated with *Salmonella* spp., traditional *mutandabota* could be a health hazard. Meanwhile in yoba *mutandabota*, *Salmonella* spp. increased from an inoculation level of 5.8 log cfu/mL at t= -24 to 7.7 log cfu/mL at t= -15, giving higher levels than *L. rhamnosus* yoba during the first 9 h of fermentation (Fig. 5.6). This suggests that with a pH of 4.2, the milk-pulp mixture provided a conducive environment for proliferation of *Salmonella*. The *Salmonella* spp. then decreased to 6.5 log cfu/mL at the end of fermentation at t= 0. The remaining 10 % pulp and sugar were then added, subsequently within 6 h, no *Salmonella* could be detected (Fig. 5.6). This suggests that *Salmonella* spp. could not withstand the additional hurdle due to the added pulp. It should be noted that the other tested pathogens in this study did not need this extra hurdle to be inactivated below the detection threshold. Mufandaedza et al. (2006) observed a similar trend with *S.* Enteritidis in naturally fermented milk and industrially fermented milk (pH 4.4). *S.* Enteritidis grew from 7 log cfu/mL to reach high populations of about 9 and 8.8 log cfu/mL respectively, after 18 h. But *S.* Enteritidis could not be recovered from the cultures after 48 h. The inhibitory effect was associated with fast acid production by the fermenting lactic acid bacteria, which resulted in a pH reduction. Several investigations have demonstrated that *Salmonella* spp. can survive in acidic foods at lower pH values for longer periods of time. Indeed, declining numbers of viable cells have been detected up to 12 weeks in apple, orange, pineapple and white grape juice concentrates (Oyarzabal et al., 2003; Parish et al., 1997) and 10 weeks in yoghurt (El-Gazzar and Marth, 1992). Mugochi et al. (1999) found that within 30 min of inoculation at 6 to 7 log cfu/mL, there were no viable *Salmonella* group B and *Salmonella* Enteritidis in the fermented *mapfura* (*Sclerocarya birrea* subsp. *caffra*) juice (pH 3.4), whilst in the unfermented juice, more than 4 log cfu/mL were still viable after 8 h (pH 3.4). However, none were still present after 24 h. They attributed the disappearance of *Salmonella* to antimicrobial substances in the fermented *mapfura* juice. In the control experiment no growth was observed.

The inactivation of pathogens in yoba *mutandabota* was clearly enhanced by adding *L. rhamnosus* yoba to *mutandabota*. The cocktails of bacterial pathogens could not survive in yoba *mutandabota* during the potential consumption time. This was probably due to lactic acid and other organic acids produced by *L. rhamnosus* yoba during the fermentation process. Yoba *mutandabota* is thus safer than traditional *mutandabota* during the potential consumption time. The amount lactic acid in yoba *mutandabota* was 2.2±1.2 g/L. No lactic acid was detected in traditional *mutandabota*. This is on the lower side when compared with studies done by Østlie et al., (2003), in which lactic acid produced by *L. rhamnosus* GG (ATCC 53103) was 7 g/L after 24 h of fermentation in UHT milk supplemented with 0.75 % (w/v) fructose. While some *Lactobacillus* strains such as *L. acidophilus* and *L. casei* produce bacteriocins, *L. rhamnosus* GG is not known to produce bacteriocins (Avonts et al., 2004)

4. Conclusion

The study investigated whether five important pathogens, namely, *L. monocytogenes*, *Salmonella* spp., *C. jejuni, E. coli* O157:H7 and *B. cereus*, could impose a foodborne risk to consumers of both traditional and yoba *mutandabota.* In traditional *mutandabota* (pH 3.4±0.1) no viable *B. cereus* and *C. jejuni* were detected 3 h after inoculation. However, *L. monocytogenes*, *Salmonella* spp., and *E. coli* O157:H7 significantly declined ($P<0.05$), but could still be detected at the end of the storage period. In yoba *mutandabota*, the pH dropped from 4.2±0.1 to 3.3±0.1 after 24 h of fermentation, and remained rather constant at pH 3.3±0.1 throughout the 24 h storage period. *L. rhamnosus* yoba showed robustness in yoba *mutandabota* and grew from 5.6±0.1 to 9.1±0.4 log cfu/mL in the presence of pathogens during fermentation. None of the bacterial pathogens tested survived during production and/or storage of yoba *mutandabota.* Yoba *mutandabota* was made from pasteurised milk, and in practice, this will also largely reduce the risk of contaminated milk with pathogens. Our findings demonstrate that yoba *mutandabota* fermented with *L. rhamnosus* yoba has antimicrobial properties against the tested bacterial pathogens and

can thus be regarded as a safer product compared to its traditional counterpart. Inactivation of pathogens in *mutandabota* is of public health significance because food-borne pathogens endanger public health upon consumption of contaminated *mutandabota*. Improving safety of *mutandabota* should also be achieved by providing information to food handlers and consumers on food hygiene to reduce the risk of contamination of *mutandabota*.

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Chapter 6

GENERAL DISCUSSION

1. Introduction

Tropical fruit trees are important crops that supplement and improve the quality of diets in rural communities. Many species have multipurpose uses as food and non-food products such as fuel, timber, fodder, medicines and industrial products. The baobab tree is in the top of the list. Many authors have described the immense contribution of the baobab to livelihoods in Africa. The National Research Council (2008) captures it well by describing it as 'Africa's Tree of Life' because of its food and non-food contribution to daily needs. By far the most valued component of the baobab tree is the fruit. Nutritionally, pulp from a baobab fruit can be considered as nature's gift to natural food fortification (National Research Council, 2008). The dry pulp provides a way to add protein, carbohydrate, energy, fiber, provitamin A, vitamin C, several B vitamins, calcium, phosphorus and iron to other foods (Glew et al., 1997; Osman, 2004). Moreover, its protein has an excellent amino acid profile, including essential ones such as lysine, methionine, cysteine and tryptophan (Osman, 2004; Sena et al., 1998).

The baobab tree grows in hot arid regions (precipitation <600 mm/annum) with poorly drained soils (Wickens, 1982). Perhaps to sustain their well-being in harsh climatic conditions where the baobab flourishes, inhabitants had to exploit the baobab fruit as their main forest-food resource. In Southern Africa, processing milk and the baobab fruit to produce *mutandabota* ensured a constant supply of proteins, micronutrients, vitamin C and minerals to the diet. *Mutandabota* also provides fibre to the diet, which evidently has potential health benefits. A number of scientific studies have shown a link between eating a high fibre diet and a range of health benefits such as cardiovascular health (Pereira et al., 2004), digestive health (Jefferson, 2005), reducing risk of diabetes (Liu et al., 2003), reducing risk of cancer (Bingham, 2006) and weight loss (Ludwig et al., 1999). Milk, the other raw material in *mutandabota* production, is one of the most nutritious food known

endowed with nutritional components that positively influence human health (Millsa et al., 2011; Wong et al., 2006).

Fermented milk products can exhibit health benefits besides basic nutrition, leading to the concept of "functional foods". This concept is a practical approach to achieve optimum health status by promoting the state of well-being and possibly reducing the risk of disease (Schrezenmeir and de Vrese, 2001; Stanton et al., 2001). Probiotics, "the live microorganisms which, when administered in adequate amounts confer a health benefit on the host" have rapidly gained interest as functional foods (FAO/WHO, 2001; Kopp-Hoolihan, 2001). Probiotic bacteria are increasingly used in food and pharmaceutical applications to balance disturbed intestinal microbiota of the human gastrointestinal tract (Kailasapathy and Chin, 2000) and are well tolerated and extremely safe without adverse effects (Reid, 2006). Probiotic products have been widely used in Japan and Europe for decades and are an important category of food supplements in the United States (Lin, 2003). However, in developing countries, malnutrition and enteric infections frequently lead to complex diarrhoeal disorders. Probiotics such as *Lactobacillus rhamnosus* GG can reduce diarrheal disorders (Guandalini et al., 2000; Grandy et al., 2010). Therefore, people in developing countries might benefit the most from probiotics (Monachese, 2011; Reid et al., 2005; Sleator, 2010). The use of indigenous foods as potential vehicles for probiotics is an option with great potential in developing countries. Indigenous foods are made from locally available resources. These indigenous foods not only have a long history of safe use, they can also be afforded by local communities.

This thesis's overall objective was to develop a probiotic dairy product on the basis of an indigenous dish called *mutandabota* and to improve quality, safety and functionality of *mutandabota* under local conditions. The specific objectives outlined in chapter 1 were:

1. To document qualitatively and quantitatively the technology of *mutandabota* production.

2. To determine the chemical and microbiological composition, and the socio-economic significance of *mutandabota*.

3. To isolate and identify predominant microbes from *mutandabota* during preparation.

4. To incorporate *Lactobacillus rhamnosus* yoba, an isolate of *Lactobacillus rhamnosus* GG, in *mutandabota* and produce a locally sustainable probiotic food.

5. To evaluate consumer preference for yoba *mutandabota*.

6. To investigate inactivation of pathogens in both traditional and yoba *mutandabota*.

The major conclusions of the thesis are:

- The baobab tree is ranked first in usefulness, mainly because its fruit is used in *mutandabota* preparation, and because different parts of the tree are processed into different foods and non-food items. Fruits are sold to generate income and used in barter trade.

- *Mutandabota* is part of the food cultural heritage of Zimbabwe. Its high protein content, in addition to micronutrients, vitamins, and minerals, gives it potential usefulness as a food protein source in Sub-Saharan Africa where child malnutrition needs to be combated and eradicated.

- The high microbial load of milk used for *mutandabota* processing and the correspondingly high microbial load in *mutandabota* pose a hygiene challenge.

- No pathogens were isolated in traditional *mutandabota*, however, the properties of isolated microorganisms from traditional *mutandabota* suggest that several microbial species survive the acidity and low pH of 3.5 in *mutandabota*.

- Baobab fruit pulp added to milk successfully promoted growth of *L. rhamnosus* yoba.

- Yoba *mutandabota* was produced at the village level with viable probiotic cells in excess of recommended daily intake levels. This was meant to generate access to probiotics by a rural population using their local traditional food as carrier matrix.

- The pH of yoba *mutandabota* was pH 3.5. This makes it a relatively low risk food product for pathogenic contamination, which increases its appeal in regions where a cold chain is not available.

- Yoba *mutandabota* inactivated all tested food borne bacterial pathogens during potential consumption time. Traditional *mutandabota* was unable to completely inactivate some food-borne pathogens. This demonstrates that yoba *mutandabota* can be safer stored than traditional *mutandabota*.

- Yoba *mutandabota* was appreciated by consumers at the same level of acceptance as traditional *mutandabota*.

The following sections discuss the progress made in accomplishing the overall objective and provide recommendations for further research.

2. Discussion and recommendations

2.1 Processing, composition, and socio-economic significance of *mutandabota*

Traditional and indigenous food systems, once lost, are hard to restore, underlining the imperative for timely documentation and the use of food culture for promoting sustainable diets. Focus group discussions and interviews revealed that the baobab tree was the most valued indigenous tree in terms of usefulness in Binga, Zimbabwe. This was mainly because its fruit was used as a food resource, especially in *mutandabota* preparation. Fruits were also sold to generate income and used in barter trade in exchange for the staple pearl millet grains (*Pennisetum glaucum*). Dry baobab fruits for preparing *mutandabota* are collected from the wild. The fruits ripen and dry while they are still on trees. Harvesting used to be done by collecting fruits from the ground. Nowadays because of demand, fruits are dislodged from trees by bad harvesting techniques such as stoning. A sustainable harvesting mechanism needs to be developed that does not damage the trees. This could be a long pole with finger-like structures at one end that can be manipulated by the operator.

The microbiological load of raw cow's milk on processing to produce *mutandabota* was high and exceeded the Zimbabwe regulatory limit for milk intended for processing. The main cause for the high microbial counts was poor hygiene leading to contamination. The milk used in preparing *mutandabota* could be pasteurized to ensure a safe product. Introduction of fermentation technology in *mutandabota* production could be beneficial, since this leads to improved nutritional value, digestibility of food, microbiological safety and is an inexpensive technology that can be applied successfully in rural communities. Another area needing attention is storage of baobab fruits to ensure a constant supply when they are out of season. Some farmers claimed that if well preserved the fruit can be stored for a whole year without use of artificial preservatives, this needs research and confirmation. It is desirable to store baobab as a whole fruit, rather than as extracted pulp.

No chemicals are used in storing the whole fruit. The tendency nowadays is to go natural and avoid use of artificial preservatives.

Mutandabota is of social, economic and nutritional significance in the livelihoods of people living in Binga and many other Zimbabwean communities. The whole community is involved in production of *mutandabota*. This brings the family together, fostering family and community cohesion. It enhances social networks. The dominance of women (80 %) in *mutandabota* production indicates that it is a gendered activity dominated by women (Mpofu et al., 2014a). In recognition of the prominent role of women in the whole process, it is envisaged that any technological intervention has to prioritize the role of women, and the effect of the technology on their livelihoods. The difficult processing operation needing special attention was pulp extraction done by pounding in a mortar with a pestle. It is a tedious manual operation. A mechanical pulp extractor, as wished for by the local population, would significantly contribute to a sustainable solution that saves time and improves the livelihoods of processors.

2.2 Isolation and identification of microorganisms in *mutandabota*

Mutandabota is a non-fermented food made from raw unpasteurised milk, so monitoring its microbiological quality is crucial in controlling the quality, spoilage, safety and protecting consumers. Mpofu et al. (2014a) recorded high microbial counts in *mutandabota*. Mesophilic bacteria counts were 4.7±1.2 log cfu mL^{-1}, lactic acid bacteria were 5.3±2.1 log cfu mL^{-1} and yeast and moulds were 5.0±1.3 cfu mL^{-1}. This was however, not surprising because *mutandabota* is prepared at household level in a shaded open space using domestic utensils and without reference to aseptic techniques.

Lactic acid bacteria (LAB) isolated in *mutandabota* were identified as *Lactobacillus plantarum, Lactobacillus alimentarius, Lactobacillus crustorum, Lactobacillus fermentum, Leuconostoc lactis, Leuconostoc garlicium, Leuconostoc mesenteroides* subsp. *mesenteroides, Microbacterium testaceum, Microbacterium arborescens, Lactococcus lactis* subsp. *lactis, Enterococcus faecium, Enterococcus hirae, Weissella paramesenteroides* and *Pediococcus pentosaceous*. Most of the LAB in *mutandabota* were thought to have originated from the unpasteurised raw milk that was used for producing the product.

Enterococcus faecium is considered as one of the most suitable indicators of faecal pollution (Suzuki et al., 2012). The presence of *E. faecium* indicates possible faecal contamination of water used to produce *mutandabota*. The microorganisms are likely to have been brought into the product from water used for cleaning teats when milking and water droplets on utensils. Binga is located in an arid region (precipitation <600 mm/ annum). Water is scarce and fetched from unprotected sources such as rivers, which can carry faecal matter from animals. *E. faecium* has been previously isolated in river water (Suzuki et al., 2012). Measures should be implemented for provision of safe water and to improve hygiene standards during production processes.

Isolates of aerobic mesophilic bacteria were identified as *Bacillus aryabhattai, Bacillus megaterium, Bacillus oleronius, Bacillus niacin, Staphylococcus heamolyticus, Morganella morganii* and *Pseudomonas oryzihabitan*. Strains of *B. megaterium* have been shown to produce novel heat-stable toxins with similar physical characteristics to the *Bacillus cereus* emetic toxin, cereulide (Taylor et al., 2005). Vegetative cells of *B. megatarium* are capable of spoiling high acid fruit products (Silva and Gibbs, 2004). *Morganella morganii*, a species of the family of *Enterobacteriaceae*, is reported to

contaminate food through processing equipment and food handlers (McCarthy and Burkhardt, 2012), thus emphasising the need for improved hygiene.

Yeasts and moulds in *mutandabota* were identified as *Aureobasidium pullulans, Cryptococcus flavescens, Candida parapsilosis, Aspergillus tubingensis, Trichosporon asahii, Clavispora lusitaniae,* and *Penicillium pinophillum.* The yeasts and moulds were thought to originate from milk and baobab fruit pulp, the major raw materials in *mutandabota* preparation. *Candida parapsilosis* is a normal human commensal that is most frequently isolated from human hands. It could have contaminated *mutandabota* from processors. This yeast has been isolated from a number of environmental sites and animals, in addition to being recovered from clinical specimens obtained from human patients (Merz et al., 1992). Some yeast isolated in *mutandabota* such as *Clavispora lusitaniae* and *Trichosporon* are usually associated with fruits (Adikhari et al., 2003; Heras-Vazquez et al., 2003) In *mutandabota*, these could have originated from the baobab fruits. The inside of a healthy baobab fruit is supposed to be sterile, however microorganisms may enter the fruit through minute cracks in the hard shell. These cracks may develop on the shell when the falling dry fruit hits the ground. Feeding insects piercing the fruit whilst it is still fresh can also introduce microorganisms into the fruit.

The identified microorganisms indicate that various microorganisms survive the acidity and low pH of 3.5 in *mutandabota*. All microorganisms isolated in *mutandabota* are capable of spoiling the product, thus making it undesirable and shortening its shelf-life. From a food safety perspective, it is important that producers of *mutandabota* avoid milk contamination by taking pre- and post-milking measures. Such practices are expected to reduce the incidence of food borne diseases. Village processors need training in food and personal hygiene. No pathogens were isolated in *mutandabota*, however some of the isolates such as *Candida parapsilosis* and *Trichosporon asahii* are known aetiological

agents in immuno-compromised individuals (Ebright et al., 2001; Trofa et al., 2008). Some of the species isolated from *mutandabota* are being used in biotechnological applications. For that reason, the *mutandabota* isolates could be investigated to explore their industrial relevant properties. *Bacillus megaterium* has been shown to produce a bacteriocin that displayed a wide spectrum antimicrobial activity against food-spoilage microorganisms and possessed a bactericidal mode of action (Khalil et al., 2009). The species may have a potential use as a food bio-preservative because of its thermo-stability and broad antimicrobial spectrum (Khalil et al., 2009). *Penicillium pinophilum* has been shown to produce cellulases useful for commercial applications. There is world-wide interest in the potential commercial applications of cellulases to generate glucose feedstock that can be used for further chemical and biological conversion (Béguin and Auberta, 1994).

2.3 Development of a locally sustainable functional food based on *mutandabota*

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) published an Expert Panel Report back in 2001 stating that ‘‘adequate scientific evidence exists to indicate that there is potential for the derivation of health benefits from consuming food containing probiotics’’ (FAO/WHO, 2001). They went on to say that ‘‘The health benefits for which probiotics can be applied include conditions such as gastrointestinal infections, certain bowel disorders, allergy, and urogenital infections which afflict a large portion of the world’s population”. To enhance access to probiotics in Southern Africa, a probiotic dairy product was developed on the basis of a traditional dish called *mutandabota*. Traditional *mutandabota* is widely consumed in rural southern Africa, making it an ideal food matrix to carry probiotics.

L. rhamnosus yoba, a strain of *L. rhamnosus* GG (Kort and Sybesma, 2012) was propagated successfully in milk supplemented with 4 % baobab fruit pulp. For good growth in milk, *L. rhamnosus* needs extra carbohydrates. Baobab fruit pulp provides the much needed carbohydrates, which promotes growth of the bacterium. Baobab fruit pulp is rich in sugars such as glucose, galactose, sucrose, maltose, and raffinose (Odetokun, 1996, Nour et al., 1980; Osman, 2004). It is possible that baobab fruit pulp can also electively enhance growth of other lactobacilli or bifidobacteria populations in the gut, thus exhibiting prebiotic properties. Prebiotics are non-digestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favourable growth or activity of a limited number of indigenous bacteria. Recently, prebiotics were shown to have significant impact in reducing the pro-inflammatory state of people living with HIV (Gori et al., 2011). The potential prebiotic properties of baobab fruit pulp in yoba *mutandabota* could be of immense value for people with HIV/AIDS. In 2004, a community kitchen was established in Mwanza, Tanzania, primarily for the production of yogurt supplemented with *Lactobacillus rhamnosus* GR-1 for people living with HIV (Wenner, 2009). This intervention has been associated with an increase in CD4 count among the consumers living with HIV. CD4 are a type of white blood cells that fights infection. The CD4 count indicates the stage of an individual's HIV disease (Irvine et al., 2010). The kitchens producing the product have now expanded to Kenya and Rwanda. The pH of yoba *mutandabota* was pH 3.5, which ensures microbiological safety of the product. Most food pathogens do not survive or grow at such a low pH (International Commission on Microbiological Specifications for Foods, 2002).

Consumers' knowledge and awareness of health effects of newly developed functional foods are usually limited, therefore there is a need for specific communication to consumers in this respect. The message should be communicated via credible media in a relatively simple way, so that it can be understood easily by consumers. The viable plate count of *L. rhamnosus* yoba in yoba *mutandabota* at the moment of consumption met the

criterion to have a viable count of the probiotic bacterium in excess of 6 log cfu/mL of a product (Adikhari et al., 2003; Kajander et al., 2007; Shah, 2000). Upon consumption, yoba *mutandabota* is expected to improve the population's intestinal health, which is especially relevant for vulnerable target groups such as children and elderly people.

2.4 Inactivation of bacterial pathogens in yoba *mutandabota*

With a new or modified product, it is necessary to assess the microbiological hazards before such a product is promoted. This was particularly important with *mutandabota* because no information is available on the behaviour of microorganisms in this product. Henceforth, five important bacterial pathogens were used in microbial challenge testing of both traditional and probiotic *mutandabota*, namely *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter jejuni*, *Escherichia coli* O157:H7 and *Bacillus cereus*.

The advantage of a low pH of around 3.4-3.5 in both traditional and yoba *mutandabota* is that it enhances food safety. In traditional *mutandabota* (pH 3.4±0.1), no surviving *B. cereus* and *C. jejuni* were detected 3 h into consumption time. However, *L. monocytogenes*, *Salmonella* spp., and *E. coli* O157:H7 survived in traditional *mutandabota* but significantly declined ($p<0.05$) in numbers. This indicated that in traditional *mutandabota* some food-borne pathogens survive the low pH. Interestingly, in yoba *mutandabota*, (pH 3.3±0.1), none of the tested strains representing the five species of food pathogens, were detected 6 h into consumption time. The inactivation of pathogens in yoba *mutandabota* clearly showed the benefits of fermenting milk with *L. rhamnosus* yoba.

L. rhamnosus yoba in yoba *mutandabota* was not affected by the behaviour of pathogens in the product as it grew from 5.6±0.1 to 9.1±0.4 log cfu/mL within the standard 24 h fermentation time. Yoba *mutandabota* was thus safer than traditional *mutandabota* during the potential consumption time. Inactivation of pathogens in *mutandabota* is of public health significance because food-borne pathogens endanger public health upon consumption of contaminated food, especially in Southern Africa where there are many vulnerable consumers of *mutandabota* such as children, elderly and immuno-compromised people with HIV/AIDS. In producing yoba *mutandabota* good hygienic practice and HACCP are systems that can be instituted to further prevent, eliminate or control pathogens.

2.5 Sensory properties and consumer preference of yoba *mutandabota*

The difference concerning health benefits of probiotic and conventional non-probiotic food is not always clear to consumers. Preference for a product ultimately depends on many factors of which the most important is sensorial quality (Majchrzak et al., 2010). There was no significant difference ($p=0.31$) in consumers' preferences for either traditional or yoba *mutandabota*. Probiotic *mutandabota* was thus appreciated by consumers at the same level of acceptance as traditional *mutandabota*. However, the sensory properties of the two products differed significantly ($p<0.001$), probably because *mutandabota* is a non-fermented product whilst probiotic *mutandabota* is a fermented product.

The main sensory descriptors used by assessors in their evaluations were "a sweet taste" and "a milky taste". Several participants mentioned sweet *mutandabota*. This is important because children tend to prefer a sweet taste, and yoba *mutandabota* is primarily meant to benefit children in improving nutrition and gastrointestinal health. Yoba *mutandabota* was accepted by its regular consumers as a local, functional food with the recommended

concentration of probiotic bacteria that can deliver health benefits. This meant that yoba *mutandabota* can be introduced to the target community without further product development.

3.0 Concluding remarks

Poverty alleviation has been placed high on the international development agenda since adoption of the United Nations Millennium Development Goals in 2000. Producing yoba *mutandabota* at village level contributes to the first and foremost of these goals: Goal 1 "to eradicate extreme poverty and hunger by 2015" (UN Millenium Declaration, 2000). Yoba *mutandabota* processing and trading may provide a pathway out of poverty for some individuals and their households through improvement in livelihoods and income. On realizing that livelihoods can be improved by benefits derived from the baobab tree, communities are inclined to conserve the trees and thus the environment, this is the concept of benefit driven natural resource management. Beyond all that, fruit trees hold fragile lands together, combating deforestation, soil erosion, water pollution, desertification and perhaps even climate change. Thus evidently this work also contributes to Millennium Development Goal 7: Ensuring environmental sustainability.

3.1 Sustainable production of the *L. rhamnosus* yoba for *mutandabota*

To ensure sustainability in producing yoba *mutandabota*, two areas need attention: the first is sustainable local production of the probiotic bacterium. The second is a sustainable supply of the baobab fruits in anticipation of the perceived increased demand. The *L. rhamnosus* yoba used in this study was provided free of charge by Yoba for Life Foundation, Amsterdam, the Netherlands, www.yoba4life.com. This foundation supports the use of probiotics in Africa. It is recommended that local industries in Southern Africa embark on producing the probiotic bacteria. This leads to establishment of enterprises

with the added benefits of capacity building, knowledge transfer, financial security, and promotes a sense of community empowerment. Currently Yoba for Life Foundation is undertaking studies on supplying *L. rhamnosus* yoba in a freeze dried form in sachets to developing countries. Our experiments (unpublished results) confirmed that the viability of *L. rhamnosus* yoba in fresh state was the same as in a freeze dried form. This is important because the freeze dried *L. rhamnosus* yoba is convenient in remote areas where the cold chain is not available.

Village production of yoba *mutandabota* will start by having one producer in the village propagating *L. rhamnosus* yoba in milk supplemented with 4 % baobab fruit pulp (Mpofu et al., 2014b). This culture will act as an inoculum that will be distributed to other villagers, who would, in turn, use the inoculum to produce their own yoba *mutandabota* for consumption and marketing. One or two cycles of back-slopping are recommended, more cycles increases the chances of contamination from the surroundings. A training program for villagers is recommended to allow knowledge and skills acquisition in producing the product. Qualified personnel have to monitor production, quality control and safety practices in each production site until producers are competent enough to produce a safe and beneficial product. In such a community-based initiative, it is important in later stages to study nutrition, health and socio-economic outcomes and assess the impact of the initiative on the community, including economy and quality of life. It is our view that such a scientific approach can provide significant benefits and bring to the fore the advantages and limitations of probiotic initiatives.

Producing yoba *mutandabota* can deliver a range of financial and nonfinancial benefits. Trading could provide women, the target group in this project, with their own independent source of income. Several women revealed how producing and selling a product would be important for their psychological well-being, independence, self-

esteem, skills and in providing them with a way to use their time productively and expanding their social networks. This investment may contribute to intergenerational poverty reduction.

3.2 Sustainable supply of baobab fruits

The second area needing attention is the sustainable supply of the baobab fruits in the light of the expected increased demand. It is vital to ensure an adequate supply of fruits throughout the year for processing. Whilst current supplies can adequately sustain operations, the expected increased demand needs to be properly planned for. Moreover, exports in baobab fruit pulp are picking up ever since the European Commission authorized marketing of dried baobab fruit pulp as a novel food ingredient (Vassiliou, 2008). To meet future demand, baobab tree plantations have to be established in a similar way as has been done for the comparable slow-growing indigenous *marula* tree (*Sclerocarya birrea* subsp. *caffra*) in South Africa, where orchards have been established (von Teichman, 1982).

Farmers mentioned that for processing, they preferred less-bitter fruits from good trees identified over the years (Mpofu et al., 2014a). Individual tree specimens with the largest number of desirable qualities will have to be identified. With those in hand, selection, clonal propagation, breeding, germplasm conservation and other horticultural manipulations can quickly transform the baobab's prospects for commerce and for national nutrition. The baobab tree may be difficult to grow, slow to mature, and susceptible to grazing, but once established it becomes nearly indestructible, and can provide its multifarious benefits for generations to come.

Mali shows the way: according to the National Research Council (2008), in Mali, baobabs are often planted in courtyards, carefully grown for 5-6 years, and then transplanted to family owned fields, where the trees are protected from roaming animals. There are already many examples of baobab "orchards" planted in the periphery of Malian towns and cities like Bamako and Mopti. Those orchards are all harvested for leaves rather than fruit. Leaves are more abundant and harvesting can commence at a much earlier stage than in the case of fruits. In Mali, local agroforestry research has perfected grafting techniques with close to 100 percent success rate. Already more than 5,000 trees in over 100 farmer orchards have been grafted with stock from trees with extremely high vitamin C content (National Research Council, 2008).

The outcome of this work was a safe, healthy, optimum-quality product of relevant nutritional value. Although this work focused on growth of *L. rhamnosus* yoba in *mutandabota*, the potential exists to apply this approach to other traditional foods worldwide as a low-cost method which, while improving dietary quality by consuming more of the nutritious product, it also benefits the gastro-intestinal health of communities.

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Summary

Research and development of underutilised foods in Sub Sahara Africa provides a viable option to combat increasing hunger and malnutrition. According to The Food and Agriculture Organization of the United Nations and the World Health Organization, the Sub-Saharan population of Africa, notably women and children, suffer from insufficient intake of protein and energy, and a lack of micronutrients. Malnourished individuals are more susceptible to disease, may suffer cognitive impairment, have poorer educational outcomes and are likely to experience reduced productivity in their lives. Improvement in nutrition in Southern Africa is needed to reverse this trend. An option with great potential is to improve nutrition at community level through the research and development of indigenous foods. Indigenous foods are made from locally available resources. These traditional foods have a long history of safe use, and local communities can afford them. *Mutandabota* or *umlondo* is one underutilized food product found in arid areas of Southern Africa. *Mutandabota* is consumed on a daily basis as a major source of proteins and micronutrients, and is sometimes used as a complimentary food for infants. The product is made by mixing raw cow's or goat's milk with 14 % (wt/vol) dry pulp of the baobab fruit (*Adansonia digitata* L.) and 7 % sugar. Producing a probiotic variant of *mutandabota* at village level in a locally sustainable way would be more beneficial. It would ensure access to probiotics by resource-poor communities in Southern Africa. Ingestion of probiotics is associated with health benefits. *Lactobacillus rhamnosus* GG is one of the most thoroughly studied probiotics. It is a lactic acid bacterium that meets the United Nations standard for probiotics and the requirements for clinical trial documentation. Evidence exists of beneficial effects of *L. rhamnosus* GG derived from clinical trials with double-blind and placebo-controlled cross-over designs for prevention and treatment of diarrhoea and gastrointestinal and upper respiratory tract infections in children and inhibiting growth and adhesion of enteropathogens. Incorporation of *L. rhamnosus* GG in *mutandabota* would produce a probiotic variant of *mutandabota* that will improve the population's gastrointestinal health or would restore it when it is transiently affected, in addition to the nutritional benefits of the product.

The overall objective of this study was to improve quality, safety and functionality of *mutandabota* produced under local conditions. The specific objectives were: (i) To document qualitatively and quantitatively the technology of traditional *mutandabota* production, (ii) To determine the chemical and microbiological composition, and the socio-economic significance of *mutandabota*, (iii) To isolate and identify predominant microbes in *mutandabota* during preparation, (iv) To incorporate *Lactobacillus rhamnosus* yoba in *mutandabota* and produce a locally sustainable probiotic food based on *mutandabota*, (v) To investigate inactivation of bacterial pathogens in probiotic *mutandabota*, and (vi) To determine consumer acceptance of yoba *mutandabota*.

Chapter 1 formulated the justification and relevance of the research, its objectives and outline. It provided information on the two major raw materials for *mutandabota* preparation, namely the baobab fruit and milk. Nutritionally, baobab fruit pulp can be considered as nature's gift to natural food fortification. The dry pulp provides a way to add protein, carbohydrate, energy, fiber, provitamin A, vitamin C, several B vitamins, calcium, phosphorus and iron to other foods. Moreover, its protein has an excellent amino-acid profile, including essential ones such as lysine, methionine, cysteine and tryptophan. Milk on the other hand, has been considered a close to perfect food, endowed with nutritional and immunological components that positively influence human health. Furthermore this chapter provides a summary of relevant literature on the probiotic bacterium *Lactobacillus rhamnosus* GG.

Chapter 2 documents the current practice of processing milk and baobab fruit pulp into *mutandabota*. Moreover, the chapter evaluates the chemical and microbiological composition of the finished product as well as its socio-economic significance. Focus group discussions and interviews revealed the dominance of women (80 %) in

mutandabota production, indicating that it is a gendered activity. The baobab tree was the most valued indigenous tree in terms of usefulness in Binga, Zimbabwe. This was mainly because it had both food and non-food uses, its fruit is being used as a food resource, especially in *mutandabota* preparation. Fruits are also sold to generate income and used in barter trade. The nutrient content of *mutandabota* was found to be (g 100 g^{-1} w.b) protein 4.8 ± 1, fat 2.8 ± 0.9, fiber 1.1 ± 0.4, ash 0.9 ± 0.2, carbohydrates 20 ± 1.7, moisture 70.4 ± 3.7, and vitamin C 80 ± 25 mg/100g. The high protein content of *mutandabota*, in addition to other nutrients, vitamin C, and minerals, as recorded in this study, demonstrates it's potential usefulness as a protein source in Southern Africa. *Mutandabota* also provides fibre to the diet, which evidently has potential health benefits, in particular for preventing diabetes, cardiovascular diseases, various cancers and constipation.

Microbiological load (expressed in log cfu mL^{-1}) in *mutandabota* was high, with 4.7 ± 1.2 mesophilic bacteria, 5.3 ± 2.1 lactic acid bacteria, and 5.0 ± 1.3 yeasts and moulds. The pH of *mutandabota* was found to be low (pH 3.5 ± 0.1). This was considered a major benefit to the product in the enhancement of microbiological safety. To reduce the high microbial load in *mutandabota*, general hygienic practices should be investigated and improved, from milking through all the processing stages. The difficult stage needing attention during *mutandabota* preparation was pulp extraction done by pounding in a mortar with a pestle. A mechanical pulp extractor, as wished for by the local population, would significantly contribute to a sustainable solution that saves time and improves the livelihoods of processors. Another area needing attention is storage of baobab fruits to ensure a constant supply when they are out of season. The locals claimed that if well preserved, the fruit can be stored for a whole year without use of artificial preservatives, this needs research and confirmation.

Evaluating microbiological quality of *mutandabota* is crucial in controlling the quality, safety and protecting consumers. Lactic acid bacteria isolated from *mutandabota* were identified as *Lactobacillus plantarum, Lactobacillus alimentarius, Lactobacillus crustorum, Lactobacillus fermentum, Leuconostoc lactis, Leuconostoc garlicium, Leuconostoc mesenteroides* subsp. *mesenteroides, Lactococcus lactis* subsp. *lactis, Enterococcus faecium, Enterococcus hirae, Weissella paramesenteroides* and *Pediococcus pentosaceous*. Isolates of aerobic mesophilic bacteria were identified as *Bacillus aryabhattai, Bacillus megaterium, Bacillus oleronius, Bacillus niacin, Staphylococcus heamolyticus, Morganella morganii, Microbacterium testaceum, Microbacterium arborescens,* and *Pseudomonas oryzihabitan*. Yeasts and moulds in *mutandabota* were identified as *Aureobasidium pullulans, Cryptococcus flavescens, Candida parapsilosis, Aspergillus tubingensis, Trichosporon asahii, Clavispora lusitaniae* and *Penicillium pinophillum.* This result indicates that several microorganisms survive the acidity and low pH of 3.5 in *mutandabota*. All microorganisms isolated in *mutandabota* are capable of spoiling the product, thus shortening its shelf-life. No pathogens were isolated in *mutandabota*. However some of the isolates such as *Candida parapsilosis* and *Trichosporon asahii* are known to cause health problems in immuno-compromised individuals. The presence of *E. faecium* indicates possible faecal contamination of water and milk used to produce *mutandabota.* Measures should be implemented for provision of safe water and to improve hygiene standards during milking and preparation.

The research described in **Chapter 3** focused on developing a probiotic variant of *mutandabota* as a sustainable, nutritious and health-promoting food that can be produced at village level. In producing probiotic *mutandabota*, raw cow's milk was boiled and subsequently cooled to ambient temperature (25 °C). Next, dry baobab fruit pulp was added to the milk at a concentration of 4% (wt/vol). This mixture was inoculated with the probiotic *Lactobacillus rhamnosus* yoba, a single colony isolate of a culture of

Lactobacillus rhamnosus GG, and left to ferment for 24 h. Final ingredients were then added to produce yoba *mutandabota* that had 14% (wt/vol) baobab fruit pulp and 7% (wt/vol) sugar in cow milk. The pH of yoba *mutandabota* was pH 3.5, which ensured microbiological safety. 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 in the product. Baobab fruit pulp at 4% promoted growth of *L. rhamnosus* yoba with a maximum specific growth rate (μ_{max}) of 0.6 ± 0.2/h at 30 °C. It is possible that baobab fruit pulp can electively enhance growth of other lactobacilli or bifidobacteria populations in the gut, thus exhibiting prebiotic properties. Upon consumption, yoba *mutandabota* is expected to improve the population's intestinal health.

In **Chapter 4** it is noted that in producing yoba *mutandabota* a new process was designed based on the traditional *mutandabota* preparation procedures. This could significantly alter its organoleptic properties and thus its acceptability by consumers. Sensory evaluation to determine acceptability of the probiotic product was conducted by a panel consisting of 100 regular consumers of *mutandabota*. The findings of the paired difference test indicated that there was no significant difference ($p=0.31$) in consumers' preferences between traditional and probiotic *mutandabota*, despite a significant difference ($p<0.001$) in sensorial properties of the two products. The main sensory descriptors used by assessors in their evaluations were "a sweet taste" and "a milky taste". Several participants mentioned sweet *mutandabota*. This was important because children tend to prefer a sweet taste, and yoba *mutandabota* is primarily meant to benefit children. Yoba *mutandabota* was thus accepted by its regular consumers as a local, sustainable functional food with the recommended concentration of viable *L. rhamnosus* yoba cells that can deliver health benefits. This meant that probiotic *mutandabota* can be introduced to target communities without further product development.

With a new or modified product, it is necessary to assess the microbiological hazards before such a product is promoted. This is particularly important with *mutandabota* because no information is available on the behaviour of pathogens in it. **Chapter 5** documents challenge tests to evaluate the impact of *L. rhamnosus yoba* on competing pathogens. Five important bacterial pathogens were selected for use in challenge experiments, namely, *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter jejuni*, *Escherichia coli* O157:H7 and *Bacillus cereus*. In traditional *mutandabota* (pH 3.4±0.1) no strains of *B. cereus* and *C. jejuni* were detected 3 h after inoculation, whilst *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella* spp. significantly ($p<0.05$) declined in numbers during the potential 24 h consumption time, probably due to the effect of acid and low pH of the product. Therefore traditional *mutandabota* was unable to completely inactivate some food-borne pathogens. In yoba *mutandabota*, the pH dropped from 4.2±0.1 to 3.3±0.1 after 24 h of fermentation, mainly due to organic acids produced during fermentation. The pH remained constant at pH 3.3±0.1 throughout the 24 h consumption time. None of the tested pathogens were detected after 6 h into consumption time of yoba *mutandabota*. *L. rhamnosus* yoba showed robustness and grew from 5.5±0.1 log cfu/mL to 9.1±0.4 log cfu/mL within 24 h in the presence of pathogens in yoba *mutandabota*. Inactivation of pathogens in yoba *mutandabota* clearly showed the benefits of fermenting milk with *L. rhamnosus* yoba. The study demonstrated that yoba *mutandabota* can be safer stored than traditional *mutandabota*.

Chapter 6 presents a general discussion of results together with concluding remarks on how far this thesis has realised its objectives. Furthermore recommendations are given and an outline of work currently being done to make production of yoba *mutandabota* sustainable. Yoba *mutandabota* processing and trading may provide a pathway out of poverty for some households through improvement in livelihoods and income, thereby

contributing to the United Nations Millennium Development Goal 1 on eradication of extreme poverty and hunger by 2015. Baobab fruit trees hold fragile lands together, combating deforestation, thus contributing to MDG 7 on ensuring environmental sustainability.

The *L. rhamnosus* yoba used in this study was provided free of charge by Yoba for Life Foundation, Amsterdam, the Netherlands, www.yoba4life.com. This foundation supports the use of probiotics in Africa. It is recommended that local industries in Southern Africa embark on producing the probiotic bacteria. This leads to establishment of enterprises with the added benefits of capacity building and knowledge transfer. Currently Yoba for Life Foundation is undertaking studies on supplying *L. rhamnosus* yoba in a freeze dried form in sachets to developing countries. Our experiments confirmed that the viability of *L. rhamnosus* yoba in fresh state was the same as in a freeze dried form. This is important because the freeze dried *L. rhamnosus* yoba is convenient in remote areas where the cold chain is not always available. Whilst current supplies of baobab fruits can adequately sustain operations, the perceived increased demand needs to be properly planned to ensure sustainable utilisation. Baobab tree plantations have to be established in a similar way as has been done for the comparable slow-growing indigenous *marula* tree (*Sclerocarya birrea* subsp. *caffra*) in South Africa, where orchards have been established. Individual baobab tree specimens with the largest number of desirable qualities will have to be identified and propagated to transform the baobab's prospects for commerce and for national nutrition.

The outcome of this work was a safe, healthy, optimum-quality product of relevant nutritional value. Although this work focused on growth of *L. rhamnosus* yoba in *mutandabota*, the potential exists to apply this approach to other traditional foods

worldwide as a low-cost method to improve dietary quality and gastro-intestinal health, thereby enhancing access to probiotics for communities who might need them most.

Zviri mubhuku rino muchidimbu

Mutandabota kudya kunowanikwa munyika dziri muAfrica, kunyanya muZimbabwe. Mutandabota unodyiwa zuva rogaroga sechikafu chinovaka muviri uye chinopa simba. Unogadzirwa nomukaka mumbishi wemombe kana mbudzi, zvikamu makumi monomwe kubva muzana, munyepfu wemauyu zwikamu guminena kubva muzana, uye tswigiri zvikamu zvinomwe kubva muzana. Hupfu hwemauyu hunotorwa mumuchero wemuti womuuyu. Mutandabota unonyanya kupa maproteins uye vitamin C kumuviri, une zvakare maminerals akawanda anosimbisa mapfupa, uye anoshanda mukugadzirwa kweropa mumuviri. Mumutandabota munowanikwawo fibre, iyo yakanakira utano, pamusana pokuti mafibre anodzivirira zvirwere zvesugar, mwoyo, nhuta uye kusanetseka kana munhu achizwibatsira. Basa rino rakaitwa kuongorora mutandabota nechinangwa chekunadzurudza chikafu ichi kuti chinakire munhu wese kunyanya pwere, madzimai akazvitakura uye chembere neharawa. Basa iri rakaitirwa pachikoro chikuru cheChinhoyi University of Technology, iyo inounyanzwi munyaya dzezweummhizha uye ruzivo rweScience, Mukubata basa iri vepaChinhoyi vakashanda nechikoro chukuru chinonzi Wageningen University chinowanikwa kunyika ye Netherlands. Vashandi, mukuita basa iri vaibatsirana navagari vemu Binga, zvimbo inowanikwa kudunhu reMatebeleland South, muZimbabwe. Mukutaurwa zvinonzi kuBinga ndiko kwakatanga kugadzirwa mutandabota.

Zwakabuda mubasa iri zvinosanganisira kuti pH, kana kuti kuvavira kwe mutandabota kwaive 3.4±0.1. Kunyange zvazvo pasina kuwanikwa utachiwana ungakonzerese kurwara, humwe utachiwana hwema bacteria nama yeast akawanikwa mumutandabota zvinoratidza kuti anogona kukanganisa chikafu ichi, nokudaro panodiwa basa rekuchenesa, kunhadzurudza uye kuvandudza chikafu ichi, izwi ndizvo zvimwe zwakaitwa, zwirikutsanangurwa mubhuku rino.

Papedza kuongororwa kugadzurudzwa kwechikafu ichi, zwakaonekwa zwakakodzera kuti mamwe mabaccteria anobatsira anonzi *Lactobacillus rhamnosus* yoba aiswe muchikafu ichi. Nokudaro mutandabota, uyu wakatumidzwa zita rokuti yoba mutandabota , wakagadzirwa. zvinounyanzwi. *L. rhamnosus* yoba yakatinakira zvikuru kana tikaidya muchikafu. Inoita kuti tiwane utano hwakazara kubva muchikafu ichi. Inodzivirira manyoka kunyanya kuvanavadiki, harawa nechembere. Inoita kuti muviri uvenemasoja akasimba uye akawanda ayo anonyanya kubatsira kunavaya vanezvirwere zwakaita seshuramatongo nenhuta. Vaya vasingafarire mukaka mumbishi vanobatsirikana mukudya yoba mutandabota, pamusana pokuti mukaka mumbishi unenge wakodzwa panenge pachikura *L. rhamnosus* yoba mumutandabota. Zwakaongororwawo zvikaonekwa kuti mutandabota nouyu we yoba mutandabota waidiwa nevanhu zwakafanana. Zvakawanikwawo mukuongorora kuti utachiona hwema bacteria akaita se *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter jejuni, Escherichia coli* O157:H7 ne *Bacillus cereus* hunopera kufa kana huchinge whapinda muyoba mutandabota.

Saka zwakabuda mubasa rino rakanyatsokuitwa nokunyorwa zwinobatsira kunyanya zvizwarwa zwemuAfrica. Pasi rose rinogona kushandisa unyanzwi hwakaitwa mukugadzira mutandabota kugadzurudza nokunatsa zvikafu zvinogadzirwa munzwimbo dzakasiyana siyana. Uye kugadzira mutandabota inzira yakanaka yapachena ingagona kushandiswa nani nani zwake pasi pose kugadzira chikafu chakanaka uye chinovaka muviri. Vagari vemunharaunda munogadzirwa mutandabota vanogona kugadzira yoba mutandabota vachibva vautengesa vachizowanawo mari yekuzwiraramisa nayo.

Nokudaro zvinokurudzirwa kuti tirambe tichivandudza zvokudya zvedu, kunyanya zvechinyakare, tichishandisa chizvinoizvino kuti tiyenderane nenguva, uye kuti chikafu chedu chitambirike kunemumnhu wesewese. Zvinhu zwakanakawo kuti tiongorore kuti

tingarime sei miti yamauyu. Kunyika dziri muno muAfrica dzakaita seMali vava kutorima miti yemauyu. *L. rhamnosus* yoba yatinoshandisa pakugadzira yoba mutandabota, zwakanaka zwakare kuti tigone kuigadzira mumaindusitiri edu, izwi zwakatinakira pamusana pokuti zvino kurudzira umhizha weScience, uye vana vanowanawo mabasa.

Samenvatting

Mutandabota is een traditioneel zuivelproduct uit Zuidelijk Afrika. Het product wordt gemaakt door rauwe geitenmelk of koeienmelk te verrijken met droge pulp van vruchten van de apenbroodboom (baobab of *Adansonia digitata* L.)en suiker.*Mutandabota* heeft een hoog eiwitgehalte, is rijk aan vitamine C, mineralen en bevat veel vezels. De hoge voedingswaarde maakt dit product tot een belangrijk onderdeel van het dieet van velen op het platteland van Zuidelijk Afrika. Hoofdstuk 1 van dit proefschrift geeft algemene informatie over de twee belangrijkste ingrediënten van *mutandabota*: melk en baobab vruchten. In hoofdstuk 2 wordt verder ingegaan op het productie proces van *mutandabota*, relevante fysische parameters en de chemische en microbiële samenstelling. Door de hoge concentratie van baobab vruchtpulp (140 g/L) is het product zuur met een pH van ongeveer 3,4. Ondanks deze lage pH waarde werden diverse bacteriën en gisten in het vers bereide product aangetroffen en geïdentificeerd. Er werden echter geen pathogene micro-organismen gevonden in het verse product, hetgeen een aanwijzing is dat het product kort na bereiding microbieel relatief veilig is.

Naast levensmiddelentechnologische kennis is ook sociaaleconomische kennis van dit product van groot belang, onder meer voor het formuleren van een stappenplan om de kwaliteit en veiligheid van *mutandabota* als belangrijk lokaal gerecht te verbeteren en daarmee in Zuidelijk Afrika een bijdrage te leveren aan de bestrijding van de alomtegenwoordige ondervoeding. Onderzoek beschreven in dit proefschrift heeft uitgewezen dat *mutandabota*in het rurale gebied van Zimbabwe voornamelijk door vrouwen wordt gemaakt. Tegen deze achtergrond is er voor gekozen om een variant van *mutandabota* te ontwikkelen met daarin levende probiotische bacteriën. Hierbij is in beginsel voor een probioticum gekozen, te weten *Lactobacillus rhamnosus* GG; LGG, waarvan door uitgebreid en gedegen klinisch onderzoek is vast komen te staan dat consumptie ervan de duur en intensiteit van diarree bij jonge kinderen kan beperken.

Deze keuze kan eenvoudig worden gerechtvaardigd doordat diarree als gevolg van besmet voedsel en water bij jonge kinderen in Zuidelijke Afrika, en in het bijzonder in Zimbabwe, een groot probleem is dat samenhangt met ondervoeding.

In hoofdstuk 3 wordt de ontwikkeling van een proces beschreven voor de productie van een probiotische variant van *mutandabota*. Het proces is zodanig ontworpen dat het relatief eenvoudig in kleine plattelandsdorpen kan worden uitgevoerd. Hierdoor komt de technologie in handen van de arme bevolkingsgroepen.De eerste stap van het proces is een hittebehandeling van de melk (op houtvuur), waarna ongeveer een kwart van de totale hoeveelheid baobab pulp wordt toegevoegd. Het mengsel wordt vervolgens gedurende 24 uur gefermenteerd met *L. rhamnosus* yoba (een vrij beschikbare variant van *L. rhamnosus* GG). Tenslotte wordt de resterende hoeveelheid pulp en suiker toegevoegd, waarna het product gereed is voor consumptie. De probiotische variant van *mutandabota* heeft een pH van 3,5 en bevat 6.5 x 10^8 levende *L. rhamnosus* yoba cellen.

Om de acceptatie van dit nieuwe product te onderzoeken, is sensorisch veldonderzoek in Zimbabwe uitgevoerd waarbij de traditionele *mutandabota* werd vergeleken met de probiotische variant. Hierbij werd geen significant verschil in consumentenvoorkeur gevonden tussen beide producten (hoofdstuk 4). Tenslotte is door toepassing van zogeheten “challenge testen” de microbiële veiligheid van het nieuwe probiotische product onderzocht (hoofdstuk 5). In het probiotische product (pH 3,4) werden alle geteste voedselpathogenen geïnactiveerd terwijl onder dezelfde testomstandigheden in het originele product (ook pH 3,4) nog enkele pathogenen konden worden gedetecteerd. Dit betekent dat de microbiële veiligheid van de probiotische variant van *mutandabota* hoger is dan die van het originele product. Dit kan mogelijk worden verklaard door de antimicrobiële werking van *L. rhamnosus* yoba.

Het resultaat van dit promotieonderzoek is een veiligere en gezondere variant van de traditionele *mutandabota*. Hoewel het onderzoek zich richtte op de groei van *L. rhamnosus* yoba in *mutandabota*, bestaat de mogelijkheid om deze aanpak wereldwijd te vertalen naar andere traditionele voedingsmiddelen als een voordelige methode om de voedingskundige kwaliteit van een product en daarmee de gastro-intestinale gezondheid van de consument te verbeteren. De productie van probiotische *mutandabota* en het verhandelen van dit product kunnen het welzijn van huishoudens op het platteland verhogen door middel van verbeteringen in de gezondheidstoestand en levensstandaard.

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Augustine

List of publications

Full papers

1. **Mpofu A.,** Linnemann A.R., Sybesma W., Kort R., Nout M.J.R., Smid E.J., 2014. Development of a locally sustainable functional food based on *mutandabota*, a traditional food in Southern Africa. Journal of Dairy Science 97, 2591-2599.
2. **Mpofu A.,** Linnemann A.R., Nout M.J.R., Zwietering M.H., Smid E.J., 2014. *Mutandabota*, a food product from Zimbabwe: Processing, composition, and socioeconomic aspects. Ecology of Food and Nutrition 53, 24-41.
3. **Mpofu A.,** den Besten H.M.W., Linnemann A.R., Nout M.J.R., Zwietering M.H., Smid E.J., 2015. Inactivation of bacterial pathogens in probiotic *mutandabota*, a dairy product with *Lactobacillus rhamnosus* yoba. International Journal of food Microbiology (*accepted*).
4. **Mpofu A.,** (2013) Sustainable production of functional foods in Southern Africa. Journal of Probiotics and Health 1, 65.
5. **Mpofu A.,** Kock J.L.F., Pretorious E.E., and Pohl C.H., Zvauya R., 2008. Identification of yeasts isolated from *mukumbi*, a Zimbabwean traditional wine. Journal of Sustainable Development in Africa 10, 88-102
6. **Mpofu A.** and Zvauya R. 2002. Microbial and biochemical changes occurring during production of *mukumb*i from *marula* (*Sclerocayrra birrea* subspecies *caffra*). Advances in Food Sciences 24, 116-120.
7. Mugochi T., Wilson P. **Mpofu A.** Simango C. and Zvauya R., 1999. Survival of some species of *Salmonellae* and *Shigella* in *mukumbi*, a traditional Zimbabwean wine. International Journal of Food Science and Nutrition 50, 451-455.
8. **Mpofu A.,** 2004. Post Harvest Technology in Zimbabwe's small-scale farming sector. The New Farmer 9, 111-112.

Abstracts, presentation, posters

9. **Mpofu A.,** Sustainable local production of a functional food based on *mutandabota*, a traditional dairy product in Southern Africa. 2nd International Conference and Exhibition on Probiotics and Functional Foods, Holiday Inn Orlando International Airport, Orlando, Florida, USA, October, 2013.
10. **Mpofu A.,** Linnemann A.R., Nout M.J.R., Zwietering M.H., Smid E.J., 2011. *Mutandabota*, a traditional dairy product from Zimbabwe, as carrier food for *Lactobacillus rhamnosus* GG. Food Congress, Copenhagen, Denmark, September 2011.
11. **Mpofu A.** Linnemann A.R., Nout M.J.R. And Zwietering M.H. Processing of Baobab fruit products in Southern Africa, University of Lausanne, Switzerland, October 2010.

12. **Mpofu A.** and Nyakudya E. Guidelines for small-scale industrial fermentation of *marula* (*Scherocarya birrea* subspeceis *caffra*) fruit juice to produce *mukumbi*, a traditional Zimbabwean wine, First International Conference on Appropriate Technology, National University of Science and Technology, Zimbabwe, July 2004.
13. **Mpofu A.** Identification of yeasts isolated from *doro* and *mukumbi*, two traditional alcoholic beverages: The *22nd International Specialised Symposium on Yeasts*, South Africa, March 2002.

CURRICULUM VITAE

Augustine Mpofu was born on 17 July 1971 in Bulawayo, Zimbabwe. He did his education at Chirenje Primary School, Beatrice Secondary School and Matopo High School, all in Zimbabwe. From 1990 to 1994 he did his BSc in Applied Biology and Biochemistry at the National University of Science and Technology, Zimbabwe. In 1995 he was employed as a teacher at Muchinjike Secondary School in Mrewa, Zimbabwe. In 1996 to 2002 he was employed as a technician at the University of Zimbabwe, where in 2000 to 2002 he did an MPhil in Food Biotechnology with the same institution. In 2003 he moved to Chinhoyi University of Technology to establish the Department of Food Science and Post Harvest Technology where he worked as Programme Co-ordinator, and later as Chairman of Department and Lecturer. In 2001 he was a UNESCO MIRCERN Biotechnology Fellow at the University of the Free State, Republic of South Africa. In 2005 he completed an International Training course on Application of Biotechnology in Food Industries at China National Research Institute of Food and Fermentation Industries, Beijing. In 2006 Augustine got a NFP Fellowship in Food Industry and Agribusiness Wageningen International, The Netherlands. In 2007 to 2009 he led the Faculty of Agricultural Sciences and Technology at Chinhoyi University of Technology. In 2011 he spent his contact leave at the University of Venda, South Africa. In 2010 Augustine started his sandwich PhD degree with the Laboratory of Food Microbiology and the Food Quality and Design group, Wageningen University, The Netherlands. He carried out his research from 2010 to 2013 in Zimbabwe and the Netherlands. The Title of the project was "Development of a locally sustainable functional food based on *mutandabota*, a dairy product incorporating *Lactobacillus rhamnosus* yoba". The results of the project are described in this thesis. His PhD was sponsored by Netherlands University Foundation for International Cooperation (Nuffic), Grant award number CF6631/2010. The field work was partly sponsored by Chinhoyi University of Technology (SRC grant number 03/08/10). He was also awarded a LEB foundation sponsorship for a trip to the USA. Augustine is married to Susan, they have three children, Tinomutenda, Anotidaishe and Tovimbanashe. Currently he is working at Chinhoyi University of Technology as Lecturer and Head of Department.

Overview of completed training activities

Discipline specific activities
Courses
Management of Microbiological Hazards in Foods, VLAG, 2010
Food and Bio-refinery Enzymology, VLAG, 2011
Food Fermentation, VLAG, 2012
Reaction Kinetics in Food Science, VLAG, 2012
Genetics and Physiology of Food Associated Microorganisms, VLAG, 2013

Meetings
Two days work at the fungal biodiversity centre CBS, CBS, 2010
Food Denmark PhD Congress, 2011
Conference: Food in Africa (Poster presentation), Wageningen, 2012
Probiotics and prebiotics International Conference (Oral presentation), USA, 2013
General courses
Information literacy, including introduction to end note, WU, 2010
Statistics for Life Sciences, WIAS, 2010
Presenting skills, WGS, 2011
Scientific writing, WGS, 2011
Grant proposal writing, WGS, 2011
Mobilising your - scientific – network, WGS, 2012
Communication in Interdisciplinary Research, WGS, 2013
Career assessment, WGS, 2013
Scientific integrity, WGS, 2013
Fraud, plagiarism, and co-authorship: Moral dilemmas in daily scientific practice, WGS, 2013

Other activities
Preparation PhD research proposal (2010)
PhD trip Switzerland, Food Microbiology, 2010
PhD Trip Japan, Food Microbiology, 2012
Laboratory of Food Microbiology seminars (2010-2014)
Food Quality and Design seminars (1010-1013)

Children enjoying a dish of *mutandabota* in Binga, Zimbabwe