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Cereal-Based Functional Foods

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1. Introduction

Functional foods are defined as foods that, in addition to their basic nutrients, contain biologically active components, in adequate amounts, that can have a positive impact on the health of the consumer [1, 2, 3, 4]. Such foods should improve the general and physical conditions of the human organism and/or decrease the risk of occurrence of disease [5]. Functional foods have also been referred to as medicinal foods, nutritional foods, nutraceuticals, prescriptive foods, therapeutic foods, super-foods, designer foods, foodceuticals and medifoods [4]. These foods generally contain health-promoting components beyond traditional nutrients [1]. Various criteria for defining functional foods have been mooted by [6] and a number of published reports have indicated the benefits of functional foods to the consumer [7, 8].

One way of creating a functional food is by inclusion of ingredients such as probiotics and prebiotics to levels that enable the consumer to derive optimal health benefits [2]. Probiotics are defined as live microorganisms which upon ingestion in adequate numbers impart health benefits to the host animal beyond inherent basic nutrition [4, 9,10]. Most of the probiotic species belong to the genera *Lactobacillus* and *Bifidobacterium* [11, 12,13]. Benefits of probiotic intake include prevention and treatment of infantile diarrhoea, travelers' diarrhoea, antibiotic induced diarrhoea, colon cancer, constipation, hypercholesterolaemia, lactose intolerance, vaginitis and intestinal infections [14, 15, 16]. Prebiotics, on the other hand, are non-digestible food ingredients that affect the host by selectively targeting the growth and/or activity of one or a limited number of beneficial bacteria in the colon, and thus have the potential to improve health [2, 7, 17, 18, 19]. Potential benefits of prebiotic intake include reduction of cholesterol absorption, control of constipation, bioavailability of minerals and reduction in blood glucose levels when used to replace sucrose in diabetic diets [8, 15, 20, 21]. The main aim of this chapter is therefore to discuss the possibility of converting cereal-based fermented foods into functional foods similar to the existing commercial dairy products. The fermentation of cereal based foods and the beneficial

attributes of such foods will be discussed. The latter attributes include the use of such foods as delivery vehicles for probiotic bacteria to the consumer.

2. Fermentation of cereal based foods

Generally, fermentation is a food preservation method intended to extend shelf-life, improve palatability, digestibility and the nutritive value of food [22, 23, 24]. Lactic acid fermentation comprises of the chemical changes in foods accelerated by enzymes of lactic acid bacteria resulting in a variety of fermented foods [11, 25]. Lactic acid fermentation processes are the oldest and most important economical forms of production and preservation of food for human consumption ([11, 23, 26, 27]. It is, therefore, not surprising that fermented foods and beverages make a big contribution to people's diets in Africa [28]. It is reported that fermented foods globally contribute 20 to 40% of the food supply and usually, a third of the food consumed by man is fermented [29]. This renders fermented foods and beverages a significant component of people's diets globally. It is estimated that the largest spectrum of lactic acid fermented foods occurs in Africa [23, 30]. However, in Africa, fermented foods and beverages are often prepared by employing spontaneous fermentation processes at household level or by small-scale industries using maize, sorghum and millet as the main cereals [11, 31, 32]. In sections 3 and 4 of this chapter, a description will be given of acid-fermented cereal-based foods and beverages and the major bacteria involved in the fermentation of such foods. In section 5 of this chapter, probiotic cereal beverages will be dealt with.

2.1. Some beneficial attributes of African fermented cereal-based foods

Lactobacillus species are the predominant organisms involved in the fermentation of cereal-based foods and beverages in Africa (see section 4.1). These organisms are reported to have bacteriostatic, bactericidal, viricidal, anti-leukaemic and antitumor effects in the consumer [25, 28, 33]. Beneficial starter cultures are not usually used in the fermentation of traditional cereal-based foods and beverages. However, it is reported that fermented foods have a probiotic potential [34] due to the probiotic *Lactobacillus* species that may be contained in them, some of which are of human intestinal origin [11].

The quality of some traditional African fermented products (see section 3.2) can be enhanced using beneficial cultures. 'Dogik' for example is 'ogi' enhanced with a lactic acid starter culture reputed to have antimicrobial activities against diarrhoeagenic bacteria [11]. *Lactobacillus paracasei* ssp. *paracasei*, a probiotic *Lactobacillus* species [11] was present together with other LAB in *uji* [35]. Strains of *Lb. acidophilus*, which are probiotic, were also isolated from an African sorghum-based product in which accelerated natural lactic fermentation was observed [36].

Improved production of milk by nursing mothers has been attributed to consumption of fermented *uji*, one of the traditional fermented beverages in Africa. *Kanun-Zaki*, a fermented non-alcoholic cereal-based beverage widely consumed in Northern Nigeria is also popularly believed to enhance lactation in nursing mothers [37]. Restoration of the normal blood level

and resultant compensation for blood lost during traditional tribal circumcision operations in parts of Africa is attributed to drinking large quantities of fermented *uji* [38].

It is reported that several B vitamins including niacin (B3), panthothenic acid (B5), folic acid (B9), and also vitamins B1, B2, B6 and B12 are released by LAB in fermented foods. These vitamins are co-factors in some metabolic reactions, for instance, folates prevent neural tube defects in babies and provide protection against cardiovascular disease and some cancers [39].

2.1.1. Shelf-life extension and improved nutritional and sensory properties

Generally, shelf-life, texture, taste, aroma and nutritional value of food products can be improved by fermentation [11, 23, 25, 40, 41]. The metabolic activities of microbial fermenters are responsible for the improvement in taste, aroma, appearance and texture [23, 30]. During fermentation, there is production of lactic, acetic and other acids and this enhances the flavour and lowers the pH of the final product. The acids also prolong food shelf-life by lowering the pH to below 4 and this restricts the growth and survival of spoilage organisms and some pathogenic organisms such as *Shigella*, *Salmonella* and *E. coli* [11, 25, 28, 33, 42]. Fermented foods, unlike non-fermented foods, have a longer shelf-life, making fermentation a key factor in the preservation of such foods [23, 43]. Because fermentation improves keeping quality and nutritional value, it is a predominant food processing and preservation process [44, 45]. During fermentation, enzymes such as lipases, proteases, amylases and phytases are produced and these in turn hydrolyse lipids, proteins, polysaccharides and phytates respectively [46]. The released nutrients contribute to the enhancement of sensory quality and nutritional value of the product [46, 47].

2.1.2. Inhibition of pathogenic microorganisms in fermented foods.

Spontaneous fermentation may involve species of *Lactobacillus*, *Lactococcus*, *Pediococcus* as well as certain yeasts and moulds [48]. Lactic acid bacteria involved in fermentation are able to produce hydrogen peroxide, but lack the true catalase to break down the hydrogen peroxide. The hydrogen peroxide can, therefore, accumulate and be inhibitory to some harmful bacteria and to the LAB themselves [11].

The organic acids released (e.g. lactic, acetic, propionic and butyric acids), as by-products during lactic acid fermentation, lower the pH to levels of 3 to 4 with a titratable acidity of about 0.6% (as lactic acid) [23, 40, 48]. The undissociated forms of the acetic and lactic acids at low pH exhibit inhibitory activities against a wide range of pathogens [23 48]. This improves food safety by restricting the growth and survival, in fermented cereal beverages, of spoilage organisms and some pathogenic organisms such as *Shigella*, *Salmonella* and *E. coli* [11, 25, 28, 33, 43, 47]. Fermented maize gruel and high-tannin sorghum gruel at pH 3.8 inhibited *E. coli*, *Campylobacter jejuni*, *Shigella flexneri*, *Salmonella typhimurium* and *Staphylococcus aureus* [30]. When starter cultures were used to ferment sour maize bread, it was found out that *Lb. plantarum* lowered the pH to 3.05 [40]. The fermented maize dough

also showed growth inhibitory activity against *Salmonella typhi*, *S. aureus*, *E. coli*, and the aflatoxigenic *Aspergillus flavus* [40].

Although *Koko* sour water (KSW) fed to Ghanaian children did not seem to halt diarrhoea, improved well-being was claimed after 14 days of consumption of this product [44]. Conflicting results about the efficacy of fermented beverages against pathogens and diarrhoea is attributed to the unpredictable nature of spontaneous fermentation. Spontaneous fermentation results in a variety of species and strains with varying degrees of antibacterial activity and ability to adhere to intestinal membranes [44]. Other studies have however, reported positive outcomes of consuming fermented cereal beverages. It was reported that a fermented cereal gruel in Tanzania reduced diarrhoea by 40% in consuming children compared to those children that did not consume it over a period of 9 months [44, 48]. This was attributed to better beverage microbial safety as well as protection against intestinal enteropathogenic colonization [48]. In a review by [25] information gathered revealed that fermented cereal-based products which contained *Lactobacillus* spp. and lactic acid had viricidal, anti-leukemic, antitumor and antibacterial activities. .

Lactobacillus isolates including *Lb. fermentum*, and *Lb. plantarum*, from maize-based ogi (West Africa) and *Lb. fermentum*, *Lb. paracasei* and *Lb. rhamnosus* from maize-based boza (Eastern Europe) were active against potential pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Bacillus cereus* due to the low pH in these products and the production of bacteriocins by the *Lactobacillus* spp [49].

2.1.3. Production of bacteriocins by lactic acid bacteria

Bacteriocinogenic lactic acid bacteria (LAB) isolated from fermented foods produce proteinaceous, antimicrobial substances (Table 1) called bacteriocins [23, 31, 50, 51]. It was reported that bacteriocinogenic LAB prevent the growth of pathogens such as *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus* and *Clostridium difficile* [23].

Bacteriocins have the ability to form pores in the membrane of target bacteria, in this way exerting bactericidal and bacteriostatic effects against the growth of pathogens in the intestinal tract [52]. Bacteriocins also reduce or prevent post-production microbial contamination of feed and food fermentation products in the food chain [51]. It was observed that bacteriocins from *Lb. plantarum* and *Lb. casei* isolated from fermented maize products, *kenkey* and *ogi* respectively inhibited and acted against a number of food borne pathogens [51]. However, bacteriocins have a narrow antimicrobial spectrum and of all bacteriocins, nisin produced by *Lactococcus lactis* is the only one generally used as a preservative by food manufacturers [46, 50]. A range of characterized bacteriocins that have potential benefits, have been reported to be produced by the *Lactobacillus* spp. and these are referred to in Table 1. While some LAB may show bacteriocin-linked inhibition of food spoilage and pathogenic bacteria *in vitro* in laboratory media, inhibitory activity in the food matrices may not be equally effective. This may be due to poorer diffusion of the bacteriocin into the cells of pathogenic bacteria in the food matrix or be the result of bacteriocin inactivation by nutrient components in the food [53].

Bacteriocin	Bacterial Species	Active against
Bulgarican	<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i>	Broad, including G (-).
N.N	<i>Lb. fermentum</i>	Broad G (+) incl <i>Listeria</i> spp
Acodophillin	<i>Lb. acidophilus</i> DDS 1	Disease-causing M/Os
Lactocidin	<i>Lb. acidophilus</i>	Disease-causing M/Os
Acidolin	<i>Lb. acidophilus</i>	Disease-causing M/Os
Lactobacillin	<i>Lb. acidophilus</i>	Disease-causing M/Os
Lactacin B	<i>Lb. acidophilus</i>	LAB
Nisin	<i>Lactococcus lactis</i>	Broad G(+) incl <i>Listeria</i> spp
Lactabacillin	<i>Lb. brevis</i>	LAB
Brevicin	<i>Lb. brevis</i>	LAB
Caseicin 80	<i>Lb. casei</i>	<i>Lb. brevis</i>
Plantaricin A	<i>Lb. plantarum</i>	LAB
Reuterin	<i>Lb. reuteri</i>	Broad G (+), G (-) and fungi

Source: [22, 27, 52, 119], G+, Gram positive bacteria; G-, Gram negative bacteria; MOs, microorganisms

Table 1. Some of the bacteriocins produced by lactic acid bacteria (LAB)

2.1.4. The effect of fermentation on toxic, antinutritional and indigestible compounds in cereal foods

During fermentation, microbial activity may lead to the elimination of toxic compounds from food products [28, 31]. For example it was reported that fermentation with *Lb. plantarum* starter cultures significantly reduced the cyanogenic glucoside content of cassava [23]. High cyanide content in a diet can cause acute poisoning, tropical ataxic neuropathy and konzo (a paralytic disease). It may also exacerbate iodine deficiency resulting in goitre and cretinism [54]. During 'gari' and 'lafun' production from cassava, the cyanogenic glucoside, linamarin, is hydrolysed by the linamarinase enzyme to glucose and cyanohydrin. The latter product is then broken down to acetone and hydrocyanic acid by hydroxynitrile lyase at pH 5-6 and the free cyanide is released faster by gentle heating [25, 55]. If the cyanogenic glucoside linamarin were to be hydrolysed in the gastro-intestinal tract (GIT), the released cyanide anion would be absorbed and halt the functioning of cytochrome oxidase enzymes in the body [23, 29].

Legumes and cereals contain indigestible oligosaccharides such as stachyose, verbascose, and raffinose which cause flatulence, diarrhoea and digestion problems [23]. The α -D-galactosidic bonds in the above-mentioned sugars are relatively heat-resistant, but they can be degraded by the galactosidase enzymes of some LAB including strains of *Lb. fermentum*, *Lb. plantarum*, *Lb. salivarius*, *Lb. brevis*, *Lb. buchneri* and *Lb. cellobiosus* [23]. During fermentation, the microorganisms disintegrate these flatulence-causing and indigestible oligosaccharides into utilisable di- and mono-saccharides [25, 29, 53].

Phytic acid, tannins and phenolic acids are polyphenols that are considered to be antinutritional factors (ANFs) and are found in cereals and legumes and the foods

prepared therefrom [56]. The ANFs contribute to malnutrition and reduced growth rate due to the promotion of poor protein digestibility and by limiting mineral bioavailability [23, 46, 56, 57]. Phytic acid in cereals and legumes, for example, (Table 2) affects the nutritional quality due to chelation of phosphorus and other minerals such as Ca, Mg, Fe, Zn, and Mo [41, 56, 58, 59]. The resultant low mineral bioavailability can result in mineral deficiency [47, 59]. Deficiency in a mineral such as iron can result in anaemia, a decrease in immunity against disease and impaired mental development. Poor calcium bioavailability on the other hand prevents optimal bone development and can cause osteoporosis in adults. Insufficient zinc brings about recurring diarrhoea and retarded growth [59].

Product	Range (%)
Sorghum	0.57-0.96
Maize	0.44-1.2
Millet	0.85-1.1
Cowpeas	0.89-1.5

Adapted from reference [30]

Table 2. Approximate phytate content of sorghum, maize, millet and cowpeas

Other negative effects of the presence of phytate in the diet, include the reduction of the activity of digestive enzymes such as trypsin, alpha-amylase and beta-galactosidase in the GIT. This is due to the formation of complexes of phytate with the enzymes and other nutrients that negatively affect digestive processes [57, 58]. Similarly tannins and polyphenols are enzyme inhibitors of plant origin that form complexes with proteins, resulting in deactivation of digestive enzymes, reduction in protein solubility and digestibility and reduction of absorbable ions [57, 60, 61]. The enzymes inhibited by tannins and/or polyphenols include pepsin, trypsin, chymotrypsin, lipases, glucosidase and amylase [57, 62]. Inhibition of the amylase enzymes results in low starch breakdown and hence, less sugar release in the GIT [117]. In fermented products this amylase inhibition by tannins impairs microbial proliferation [83]. This in turn decelerates pH decrease and acidity production in the medium [83].

Fermentation, by certain LAB and yeasts, removes or reduces the levels of antinutritional factors such as phytic acid, tannins and polyphenols present in some cereals meant for weaning purposes [23, 31, 41, 47, 53, 56, 59, 63]. During fermentation, optimal pH conditions prevail for enzymatic degradation of the antinutritional factors. This results in better bioavailability of minerals such as iron, zinc and calcium [11, 23]. Strains of *Lb. plantarum* degraded phytic acid in the cereals after incubation at 37 °C for 120 hours [23]. This degradation can be ascribed to the hydrolysis of the phosphate group by phytases from the raw cereal substrate and produced by the fermenting microorganisms [46, 47, 57]. Fermentation alone reduced the phytate content by 39%. The combined effect of fermentation plus the addition of exogenous phytase, resulted in a reduction of 88% of the phytates in tannin sorghum gruel [47].

Fermentation reduced phenolic compounds and tannins in finger millet by 20% and 52% respectively [60]. Fermentation coupled with methods such as decortication, soaking and germination reduced the tannins in sorghum, other cereals and in beverages made from these cereals [57, 60, 61, 62, 83]. Fermentation of porridges from whole and decorticated tannin sorghum led to significant reduction of total phenols [61].

The use of *Rhizopus oligosporus* to ferment cooked soybean in *tempe* production reduced residual trypsin inhibitor activity (TIA) by 91% in addition to the 86.4% reduction attributed to steaming [57]. The reduction of the TIA was ascribed to hydrolysis of the trypsin inhibitor by the fungi fermenting the *tempe* [57]. In another study [63], *Lb. brevis*, *Lb. fermentum*, *Streptococcus thermophilus* and *Pediococcus pentosaceus* were observed to have improved the nutritional quality of fermented sorghum products. Table 3 shows that some strains of LAB significantly degraded trypsin inhibitors. This illustrates the possibility that using carefully selected probiotic bacteria to ferment cereal foods may reduce the antinutritional factors in such products.

Fermentation can also decrease the activity of the proteinase and amylase inhibitors in cereals resulting in an increase in the availability of starch and essential amino acids such as lysine, leucine, isoleucine and methionine [23, 46, 53]. The protein quality and nutritive value of fermented products such as *kenkey*; *iru*; and *ugba* [25] and *ogi* [64] was improved during fermentation due to either microbial protein synthesis or loss of non-protein material. In support of the above, [39] reported that fermenting with *Lb. plantarum* OG 261-5 significantly improved the levels of tryptophan, lysine and tyrosine even though other amino acids such as isoleucine, leucine, valine and phenylalanine decreased.

LAB isolate	Reduction of TI (mg)	Percent reduction
<i>Lb. plantarum</i> 91	2.41	48.0
<i>Lb. fermentum</i> 103	1.22	24.4
<i>Pediococcus</i> sp. 90	0.89	17.8
<i>Pediococcus</i> sp. 19	1.08	21.6
<i>Leuconostoc</i> sp. 106	2.68	53.6
<i>Lactobacillus</i> sp. 41	0.65	13.0
<i>Lactobacillus</i> sp. 17	1.86	37.2
<i>Lactobacillus</i> sp. 62	1.34	26.8

Adapted from references [23, 30]; **Aflata* is a gelatinized maize paste intermediate in *kenkey* production.

Table 3. Degradation of trypsin inhibitor (TI) by lactic acid bacteria isolated from **aflata* in Ghana

Fermentation in many instances results in an increased vitamin content of the final product [23]. Lactobacilli involved in fermentation may require vitamins for growth, but several of them are capable of bio-synthesizing B-vitamins in excess. It is reported that several B vitamins including niacin (B3), panthothenic acid (B5), folic acid (B9), and also vitamins B1, B2, B6 and B12 are released by LAB in fermented foods [39]. Cereal-based products such as *ogi*; *mageu*; and *kenkey* have thus been reported to have an improved B-vitamin content [25, 29]. Fermentation therefore improves the nutritive value of cereal foods.

2.1.5. Reduction, binding or detoxification of mycotoxins in fermented foods

Maize (*Zea mays*), sorghum (*Sorghum vulgare*), pearl millet (*Pennisetum glaucum*) and finger millet (*Eleusine coracana*) constitute the most important cereals for the preparation of fermented foods in the developing world [41, 65, 66, 67]. These cereal grains are however, exposed to pre- and post-harvest mycotoxin contamination which end up in the fermented foods [23, 54, 67]. Among the cereals, maize is the most prone to mycotoxin contamination [66].

Mycotoxins are secondary metabolites released into cereal grains and legume seeds by species of the genera *Aspergillus*, *Fusarium* and *Penicillium* [54, 66]. Aflatoxins and fumonisins are the mycotoxins, in cereals, of major health and economic concern in the developing world [23, 24, 48, 54, 66, 68, 69]. Table 4 shows the deaths linked to mycotoxins in foods. Aflatoxin B1 (AFB1) is toxic, carcinogenic, mutagenic and teratogenic [45, 69]. Fumonisin have been linked to oesophageal cancer in South Africa and liver cancer in China [66, 68]. Kwashiorkor in children is aggravated by long term exposure to aflatoxin [66]. The development and propagation of cereal-based probiotic and/or synbiotic (prebiotics and probiotics combined) beverages may consequently, to some extent, be hampered by mycotoxin-contamination of the cereals used in making such beverages.

Bacterial and fungal (biological) decontamination is one of the mycotoxin-reducing strategies that have been and are being investigated [24]. *Flavobacterium aurantiacum* (*Nocardia corynebacterioides*), *Corynebacterium rubrum*, *Saccharomyces cerevisiae*, *Candida lipolitica*, *Candida krusei*, *Aspergillus niger*, *Mucor* spp., *Rhizopus* spp., *Nurospora* spp., *Amillariella tabescens*, and *Trichoderma viride* are bacterial and fungal species reported to have the capability to degrade mycotoxins enzymatically ([23, 24, 45, 69]. Extracellular extracts of *Rhodococcus erythropolis* reduced Aflatoxin B1 (AFB1) by 66.8% after 72 hours of incubation [69]. Fermentation by *R. oryzae* and *R. oligosporus* was reported to reduce aflatoxins to aflatoxicol A which, under conditions created by organic acids, gets permanently converted to aflatoxicol B [54]. It was claimed that aflatoxin B1 is 18 times more toxic than aflatoxicol B and it is also possible that the former, during lactic acid fermentation to pH < 4.0, gets transformed into a less toxic isomer, aflatoxin B2 [54].

A heat-treated *Saccharomyces* yeast species was said to absorb more than 90 % (w/w) of ochratoxin A in grape juice while live cells could only bind 35 % (w/w) [24, 45]. Other workers have indicated that binding of Aflatoxin B1 was better at low pH and when cells were subjected to acid or heat treatment [24]. The implication is that food beverage preparation, which involves cooking after fermentation, together with the highly acidic conditions of the fermented food beverage, may physically alter the microbial cell structure thereby increasing the binding sites for AFB1 [45]. This provides a way of reducing aflatoxins in African fermented foods and beverages. However, some of the microorganisms indicated in the above paragraphs may not necessarily be GRAS (generally recognised as safe) in the human GIT.

Country	Year	Food source	Mycotoxin content	Percentage of samples contaminated	Mycotoxin	Deaths	Case patients
India	1974	maize	NA	NA	Aflatoxin B1	106	397
Kenya	1981	maize	NA	NA	Aflatoxin B1	NA	20
Kenya	2004	maize	~4400ppb	NA	Aflatoxin B1	215	317
Nigeria	2005	maize	NA	NA	Aflatoxin B1	100	NA
Kenya	2005	maize	NA	NA	Aflatoxin B1	30	8
Kenya	2006	maize	NA	NA	Aflatoxin B1	9	NA
Kenya	NA	3 maize brands	0.4-2.0 µg/Kg	NA	Aflatoxins	NA	NA
South Africa	NA	Peanut butter	< 300 ppb	NA	Aflatoxin B1	NA	NA
Togo, Benin	NA	Household maize	NA	30%	Aflatoxin B1	NA	NA
Nigeria	NA	Maize samples	NA	33%	Aflatoxin B1	NA	NA
Benin	NA	Agro-zone sample	> 5 µg/Kg	9.9 - 32.2%	Aflatoxins	NA	NA
Ghana	NA	Maize silos	20-335 µg/Kg	NA	Aflatoxins	NA	NA
Togo, Benin	NA	Maize samples	> 100 ppb	50%	Aflatoxins	NA	NA

Source: reference [66]

Table 4. Deaths and ill health linked to mycotoxin contamination of samples in African countries

Aflatoxin B1 could not be detected in fermented maize porridge (*amahewu*) that had been made from maize meal samples containing 0.55 and 0.84 µg/g aflatoxin B1. In the same study, the levels of fumonisin B1, in contaminated maize meal samples containing 12.1, 24.6, 4.1, 20.6, 47.2 µg/g of this mycotoxin, were drastically reduced in fermented maize porridge to levels of 1.4, 1.4, 0, 6.9, 6.3 µg/g respectively [46]. This exemplifies the detoxification potential for cereal beverages by lactic acid fermentation. The mechanism of mycotoxin removal from fermented food matrices is not clear.

Without forgetting the above paragraph relating to the effect of probiotic fermentation on mycotoxin levels, some reports on fermentation-linked reduction of aflatoxins in cereal food matrices are controversial. There are reports indicating no significant aflatoxin reduction during fermentation [54]. It was observed that fermentation only enabled a reduction of 18% and 13% of aflatoxin and fumonisin respectively in *ogi* [68]. It was reported that under acidic conditions, aflatoxins persist due to aflatoxin precursors and on the other hand, aflatoxin only undergoes reformation but not reduction under acidic conditions created by organic acid metabolites of LAB [68]. There are also fears that fumonisin binds with starch to form an undetectable complex and besides this, they may react with reducing sugar (D-glucose) to form sugar adducts or are hydrolysed to aminopolyols AP1 and AP2 [68].

The foregoing findings indicate that mycotoxin-reduction in fermented cereal food matrices has not yet been properly elucidated. It is therefore necessary to screen probiotic microbial isolates to find those strains that have a definite potential to degrade aflatoxins during fermentation in food matrices. Such mycotoxin-degrading species need to be fully compatible with the human GIT ecosystem. Some workers recommended the use of probiotic microorganisms with high aflatoxin B1 binding capability in fermented foods [24].

However, binding is not degradation and the binding probiotic cells are consumed along with the food matrix. The fate of bound toxins in fermented food matrices needs to be investigated. Probiotics and/or LAB suitably screened for their biological mycotoxin degradation, among other technological and health benefits could be better applied in human food fermentation, even though, prevention of mycotoxin contamination is the better option. Besides fermentation and contamination-preventive measures, it was noted that processing operations including sorting, winnowing, washing, crushing and dehulling [68] significantly reduced mycotoxin levels in several cereal foods.

3. Cereal-based beverages with a probiotic potential

3.1. Selected non-African cereal foods

Most of the commercial products containing probiotics and prebiotics available today are dairy-based [70]. Several workers have, however, endeavoured to develop non-dairy, cereal-based probiotic and/or synbiotic products [4, 57, 70-76]. The following non-African fermented cereal beverages have a probiotic potential or in other words, the potential to be transformed into functional beverages.

3.1.1. *Boza*

Boza is consumed in countries of the Balkan region including Bulgaria, Romania, Albania and Turkey [4, 77]. Reports indicated that *boza* in Turkey contained 0.03-0.39% (w/v) alcohol but the country's national regulations allow beverages with an alcohol content of not more than 5.0 g/L to be considered non-alcoholic [78].

Boza is a highly viscous traditional fermented product, made from millet, maize, wheat, rye, or rice and other cereals mixed with sugar [79, 78, 80]. In the preparation of *boza*, the milled cereals are mixed in water and then cooked in an open or steam-jacketed boiler. The gruel is cooled and strained to remove the bran and hull. Sugar is added and then fermented at 30 °C for 24 hours by back-slopping or use of sourdough and/or by adding yoghurt starter cultures [78]. Fermented *boza* is then cooled to refrigeration temperatures and distributed into 1L plastic bottles to be consumed within 3-5 days [78]. *Boza* is popularly accepted in the countries referred to above due to its pleasant taste, flavour and nutritional value [4].

Spontaneous fermentation involves LAB and yeasts [80]. Lactic acid bacterial species isolated from *boza* included *Leuconostoc paramesenteroides*, *L. mesenteroides* subsp. *mesenteroides*, *L. mesenteroides* subsp. *dextranicum*, *L. oenus*, *L. raffinolactis* *Lb. coryniformis*, *L. confusus*, *L. sanfrancisco*, *Lb. fermentum* *Lb. plantarum*, *Lb. acidophilus*, *Lb. coprophilus* and *Lb. brevis* [4, 79, 80]. The yeast isolates included *Saccharomyces cerevisiae*, *Candida tropicalis*, *Candida glabrata*, *Geotrichum penicillatum* and *G. candidum* [4, 80]. The microflora in *boza* [4, 80] can vary depending on the region and/or country as well as the combination of cereals used and other factors. Only three species were however recommended for inclusion in a mixed starter culture for *boza* production namely: *S. cerevisiae*, *L. mesenteroides* subsp. *mesenteroides* and *L. confusus* [80].

3.1.2. *Kvass*

Kvass is a non-alcoholic fermented cereal-based beverage made from rye and barley malt, rye flour, stale rye bread, and sucrose and is most often consumed in Eastern Europe [81]. *Kvass* is manufactured using two techniques. One technique involves the use of stale dough bread in which the sugars for the yeast fermentation are obtained from the bread-making process, while the second technique involves the use of malt enzymes to hydrolyse the gelatinized starch [81]. Before fermentation is initiated by the addition of baker's yeast or back-slopping, sucrose is added to the *kvass* wort [81]. The fermentation process is terminated by cooling the *kvass* to 4 °C and the product contains proteins, amino acids, vitamins and organic acids either from the raw materials or from the activity of the fermenting microorganisms [81].

The *kvass* alcohol content is less than 1% while the carbohydrate components predominantly include maltose, maltotriose, glucose and fructose [81]. Maltose and maltotriose components are categorized as isomalto-oligosaccharides that are not completely broken down by digestive enzymes in the GIT [81]. Isomalto-oligosaccharides can hence serve as bifidogenic (prebiotic) factors for the proliferation of probiotic bifidobacteria in the intestines [81].

The predominant microorganisms in *kvass* fermentation were found to be *Lb. casei*, *L. mesenteroides* and *S. cerevisiae*. *Kvass* is not heat-treated after fermentation and as a result high counts of viable cells can be found in the beverage [81]. The isolation of *Lb. casei* from *kvass* (in which it was highly viable), is indicative of the potential of cereal-based beverages such as this to be used as alternatives to milk products in the delivery of probiotics and other functional ingredients to the consumer in the developing world [81].

3.1.3. *Pozol*

Pozol is a traditional fermented maize dough consumed in South-eastern Mexico [4]. *Pozol* is made mainly by Indian and Mestizo populations of Mexico [82]. During *pozol* preparation, maize grains are cooked in lime water to obtain nixtamal (nixtamalization is a process in which maize (corn), or other grains are treated by soaking and cooking in limewater). This results, *inter alia*, in the grain being more easily ground and the nutritional value being improved). The nixtamalized product is then cleaned by washing in water to separate the husks. The grains are ground, moulded into balls, then wrapped in banana leaves and spontaneously fermented at room temperature for about 7 days [82]. The pH of *pozol* is usually in the range of 3.7-4.7 after 48 hours of fermentation [82]. *Pozol* balls at different stages of fermentation can be mixed with water to make a gruel of desired viscosity and then consumed as a beverage by adults, children and infants [82]. Although African fermented maize gruels are not nixtamalized, *pozol* is similar to African traditional products such as *mageu/mahewu*, *ogi*, *kenkey* and *koko* that will be discussed in the next section of this chapter.

Escherichia coli was isolated from *pozol* after 48 hours of fermentation [82]. This was linked to the high pH in the initial stages of fermentation and the possibility of the presence of high pH-localities in the dough after 48 hours even though the measured pH was 3.4-4.7 [82]. It is

also possible that acid fermented doughs can harbor some pathogenic bacterial strains resistant to high acidity and/or strains adapted to low pH [82].

3.2. African traditional fermented foods

In Table 5 a number of African traditional lactic acid-fermented cereal-based foods and beverages and the major lactobacilli involved in fermentation are listed. Cereals including maize, sorghum and millet have been used individually or in combination in the preparation of a variety of fermented beverages in Africa [83].

3.2.1. *Ben-saalga*

Ben-saalga is a pearl millet (*P. glaucum*)-based fermented beverage mainly consumed in Burkina Faso [41, 43, 84]. It is popularly consumed by the young, elderly, the sick and the general populace [41, 84]. The traditional way of producing *ben saalga* involves washing the pearl millet, soaking, wet-milling, kneading and sieving moistened flour, and fermenting the settled, but diluted slurry prior to cooking. This then becomes the *ben-saalga* beverage [41, 43, 84]. The pH decreases from 6 to to a pH of 3.6 – 4.0 during a 24-hour fermentation period [84, 85]. In terms of the LAB responsible for the fermentation, spontaneously fermented *ben saalga* is dominated by *Lb. fermentum*, *Lb. plantarum* and *Pediococcus pentosaceus* [41]. Ethanol, lactic acid and acetic acid were the main products of fermentation in *ben saalga* [84].

Ben saalga has a solids content of 8-10 g/ 100 mL and like other cereal beverages discussed in this chapter, it has a poor energy density and nutrient content [41]. However, the preparation of *ben-saalga* results in a reduction of millet's antinutritional factors, such as phytic acid, by about 50% [41]. Thirty three of the 99 bacterial isolates from *ben-saalga* showed antimicrobial activity against at least one of the indicator pathogens used in the study [43]. Seven of the isolates, identified as *Lb. plantarum*, were bacteriocinogenic against indicator pathogens which included *Escherichia coli* U-9, *Listeria monocytogenes* CECT 4032, *L. innocua*, *Salmonella typhimurium*, *S. aureus* CECT 192 and *B. cereus* LWL1 [43]. These findings indicate the probiotic and/or the prophylactic and the therapeutic potential of intake of this fermented cereal beverage. These characteristics may even be improved by using selected starter cultures that can benefit the health of the consumer and enhance the preservation and safety of the food.

3.2.2. *Dégué*

Dégué is a millet-based fermented food consumed in Burkina Faso [86]. Preparation of *dégué* involves dehulling and grinding of the millet grains, modeling into balls with water and steam cooking to produce gelatinized balls. The balls are then stored to allow a further 24-hour spontaneous fermentation [86]. The pH of *dégué* is usually in the range of 4.57-4.72 and the following microorganisms have been found in the product: *Lb. fermentum*, *Lb. brevis*, *Lb. gasseri*, *Lb. casei*, *E. coli* and *Enterococcus* sp. [86].

Fermented food product name	Raw materials	Lactobacilli involved	Nature of use	Country or region	References
Ogi, Ogi-baba	Maize, millet or sorghum	<i>Lb. plantarum</i>	Paste as staple, breakfast or weaning food	Nigeria, W. Africa	[11, 26, 99]
Uji	Maize, millet or sorghum	<i>Lb. plantarum</i>	Porridge	Uganda, Kenya, Tanzania	[11]
Koko	Maize	<i>Lb. plantarum</i> , <i>Lb. brevis</i>		Ghana	[11]
Kenkey	Maize	<i>Lb. fermentum</i> <i>Lb. reuteri</i>	Mush steamed, eaten with vegetables	Ghana	[11]
Kwunu-Zaki	Millet, sorghum or maize	LAB*	Paste used as breakfast cereal	Northern Nigeria	[37]
Mahewu	Maize, sorghum, millet	<i>Lb. delbrueckii</i> , <i>Lb. bulgaricus</i> <i>Strep. lactis</i>	Gritty gruels, Solid staple	S. Africa	[28, 99]
Mawe	Maize	LAB*	Basis of preparation of many dishes	S. Africa, Togo	[11]
Mangisi	Millet	Unknown	Sweet-sour non-alcoholic drink	Zimbabwe	[11]
Munkoyo	Sorghum, millet or maize plus munkoyo roots	Unknown	Liquid drink	Zambia, Africa	[11]
Mutwiwa	Maize	LAB*	Porridge	Zimbabwe	[11]
Tobwa	Maize	LAB*	Non-alcoholic drink	Zimbabwe	[11]
Togwa	Sorghum, millet, maize		Acid fermented gruel for refreshment and weaning	Tanzania	[34]
Liha	Maize	Unknown	Sweet-sour non-alcoholic drink	Ghana, Togo, Benin, Nigeria	[118]

Table 5. African acid-fermented non-alcoholic cereal-based foods and beverages and the lactic acid bacteria involved in the fermentation (LAB*, lactic acid bacteria)

3.2.3. *Kanun-Zaki*

Kanun-zaki is a non-alcoholic fermented cereal-based beverage consumed in Northern Nigeria [11, 37]. *Kanun-zaki* can be prepared from pearl millet, sorghum or maize ([37]:49). This product is popularly served as a breakfast dish [25]. In the preparation of *Kanun-zaki*, the kernels are washed and dried in the sun, then coarsely ground in a mortar and pestle. The flour is then mixed with hot water to form a paste which is spontaneously fermented for 1-3 days resulting in a sour beverage [25]. It was reported that this beverage is nutritionally, medically and economically important in the regions where it is widely consumed [39].

3.2.4. *Kenkey*

Kenkey is a fermented maize dough product eaten by the people of Ghana, primarily the Gas, Fantis and Ewes [38, 41]. The preparation of the two main types of *kenkey* (*Ga-kenkey* and *Fanti-kenkey*) was described in reference [41]. The Fanti people's name for *kenkey* is *dokon* interpreted to mean 'mouth-watering' because of its pleasant odour and flavour [38]. Similar products to *kenkey* made from sour maize dough include *akasa*, *koko*, *banku*, *abele*, *akple*, and *kpekpe* though these are not as popular as *kenkey* [38]. *Kenkey* fermentation is spontaneous and is dominated by lactic acid bacteria, particularly *Lb. fermentum* and *Lb. reuteri*, and yeasts that include *C. krusei* (*Issatchenkia orientalis*) as the dominant yeast species, while *S. cerevisiae* also contributes to the flavour [11, 41]. Apart from improvement in the protein content from 1.3 to 3.3 g per 16 g nitrogen in ready-to-eat *kenkey*, the *kenkey* flavour is attributed to the formation of flavour compounds, during fermentation, such as 2,3-butanediol, butanoic acid, lactic acid, 3-methylbutanoic acid, octanoic acid, 2-phenylethanol, and propanoic acid [41].

3.2.5. *Koko*

Koko is a millet-based spontaneously fermented beverage mainly consumed in Northern Ghana [44]. The predominant microbial species during fermentation are *Lb. fermentum* and *Weissella confusa* [44]. It was reported that isolates from *koko* showed good antimicrobial activity, tolerance to 0.3% oxgall bile and acid resistance at pH 2.5, which are characteristics of good probiotic strains [44].

3.2.6. *Mageu (mahewu)*

Mageu is a non-alcoholic largely maize-based beverage popular among the indigenous people of Southern Africa, but is also consumed in some Arabian Gulf countries [4, 74, 83]. It is consumed at schools and mines and on farms. It is a refreshing drink and a traditional weaning beverage for infants. *Mageu* is prepared by using 8% to 10% (w/v) maize flour as the major solid substrate in aqueous suspension. Wheat flour or maize bran is added to initiate the lactic acid fermentation [32]. Some ethnic groups also use sorghum and millet flours instead of maize flour and *mageu* is known by different names (Table 6) among the

ethnic groups in Southern Africa. Acceptable *mageu* contains 0.4 – 0.5% lactic acid corresponding to an average pH of 3.5 [87, 88, 89].

Several studies have been conducted on *mageu*. One of these included an investigation of the survival of bacterial enteric pathogens in fermented *mageu*, from which it was concluded that fermented *mageu* had bacteriostatic and bactericidal properties [33]. Another study targeted the growth and survival of *Bacillus cereus* in fermented *mageu* in which growth inhibition of the organism was observed [32]. Studies on the development of a starter culture for *mageu* [88, 90, 91] led to the production of mahewu on a commercial scale [92].

Ethnic group	Local name of product	Reference
Zulu	Amahewu	[91]
Swazi	Emahewu	[89]
Xhosa	Emarewu	[91]
Venda	Mabundu	[70]
Pedi	Mapotho	[70]
Sotho	Machleu	[89]

Table 6. Local names for sour maize porridge (*mageu*) in Southern Africa

3.2.7. *Matwe*

This is fermented maize dough consumed in the form of a variety of dishes in Togo, Benin and Nigeria [68]. Making the *matwe* (maize dough) involves washing, wet extraction of the endosperm and kneading to a dough which is then spontaneously fermented for about 3 days [41]. In Benin, *matwe* dough is used for the preparation of cooked beverages (*koko*), stiff gels (*akassa*, *agid* and *eko*) and steam cooked bread (*ablo*) [41]. The predominant LAB in the fermented *matwe* dough included *Lb. fermentum*, *Lb. cellobiosus*, *Lb. brevis*, *Lb. curvatus*, *Lb. buchneri* and *Weissella confusa*. Other microorganisms in the dough included pediococci and yeasts such as *Candida krusei*, *C. kefir*, *C. glabrata* and *Saccharomyces cerevisiae* [41]. It was reported that in a study of *matwe* production using starter cultures, *C. krusei*, stimulated the growth of *Lb. fermentum* and *Lb. brevis* [41]. Fermentation of this product offers a number of benefits that include flavour enhancement, nutrient bioavailability (including that of some proteins, minerals and B vitamins) as well as protection against some pathogens due to reduction of the pH to 3.5-4.0 [41]. Maize products are however, deficient in some amino acids such as lysine, tryptophan and methionine, which are found more abundantly in legumes such as cowpeas and soybeans. Co-fermentation with legumes can therefore be expected to improve the quality of the protein and protein levels significantly.

3.2.8. *Munkoyo*

Munkoyo is a traditional fermented maize-based beverage popularly consumed in Zambia and the Democratic Republic of Congo's Katanga province in the south [93, 94]. In Zambia, tree species of *Eminia*, *Vigna* and *Rhynchosia*, generally referred to as *munkoyo*, are extracted and the extract, high in α - and β -amylases, is used for the liquefaction of maize porridge gel

[93, 94]. The thinned porridge is then spontaneously Fermented, mainly by LAB, for 24-48 hours at room temperature. The sweet-sour *Munkoyo* flavoured drink has a mean pH of 3.5 due to organic acids produced during fermentation, but alcohol (14-26 g/kg) is also detectable. The beverage is consumed by people of all ages [93].

Introduction of *Rhynchosia heterophylla* root extract, *Lb. confusus* LZ1 and *Sacchromyces cerevisiae* YZ20 to the fermentation mix, resulted in a *munkoyo* beverage of pH 3.3, 60 mmol/l lactic acid and an ethanol content of 320-410 mmol/l [93]. The workers observed that a ratio of not more than 1:1000 (yeast: LAB starter culture) fermented for not more than 24 hours resulted in an acceptable *munkoyo* beverage [93]. *Munkoyo* was found to have antibacterial activities. Total coliforms in the *munkoyo* mash initially were 10 cfu/mL but were absent when tested after 15 hours of fermentation due to acidification of the product [94]. The microorganisms in *munkoyo* were not recognised probiotics and it was therefore recommended that the incorporation of probiotic starter cultures producing D (+) lactate be investigated to improve the nutritional, sensory and health benefits of *munkoyo* [94].

3.2.9. *Obushera* (*bushera*)

Obushera fermented spontaneously from malted sorghum or millet flour is consumed by young people and adults in Western Uganda [95]. *Obushera* is prepared using sorghum or millet flour. The flour is mixed with water and cooked into a thin porridge and then mixed with a portion of previously fermented porridge. The added fermented portion acts as a 'starter culture' for fermentation to commence and the result is the '*obushera*' beverage consumed by people of any age [48]. *Obushera*, produced on a small commercial scale, can be used as a thirst quencher, social drink, energy drink and weaning food [95]. The household *bushera*, with a pH in the range 3.7-4.5, had LAB counts varying from 7.1 to 9.4 log₁₀ cfu/mL and coliform counts that were in the range of <1 to 5.2 log₁₀ cfu/mL [96]. The LAB species from household *bushera* included *Lb. plantarum*, *Lb. paracasei* subsp. *paracasei*, *Lb. fermentum*, *Lb. brevis*, *Lb. delbrueckii* subsp. *delbrueckii* and *Streptococcus thermophilus*. The isolates from laboratory fermented *bushera* belonged to the genera *Lactococcus*, *Leuconostoc*, *Lactobacillus*, *Weissella* and *Enterococcus* [96]. This is indicative of the probiotic potential of *obushera*.

3.2.10. *Ogi*

Ogi is another traditional African acid-fermented cereal gruel prepared from maize, although sorghum and millet flours are also used [11, 25]. During fermentation, *Lb. plantarum* is the predominant microorganism although bacteria such as *Corynebacterium* spp hydrolyse the corn-starch following which yeast genera such as *Saccharomyces* and *Candida* contribute to the flavour [11, 27]. *Ogi* is traditionally produced by washing the grains, steeping for 12 to 72 hours, wet-milling, wet-sieving and sedimenting the filtrate for 1-3 days to obtain sour *ogi* [64, 97]. The pH of *ogi* is 3.0 – 4.0 after fermentation depending on the time of fermentation and the presence of LAB [64, 68]. *Ogi* has a sour flavour and a characteristic aroma [25, 38, 98]. In Nigeria the name of '*ogi*' depends on the locality and the

type of cereal. *Ogi* is the generic name in the Western states of Nigeria where it is usually processed from white maize. *Ogi* from sorghum is known as 'ogi-baba' [99] while 'ogi-gero' is prepared from millet. In Northern Nigeria, *ogi* is known as 'akamu' or 'eko gbona', while in the Republics of Togo, Benin and Ghana, *ogi* from maize is known as 'koko' [38, 98]. *Ogi* is the major traditional weaning food commonly served to babies in West Africa. It is also eaten as a breakfast meal and it is a food of choice for the sick [25, 31, 64].

It was observed that use of *Lb. brevis* alone to ferment sterile maize slurry for *ogi* production rapidly reduced the pH to 3.0 in 48 hours compared to the sterile slurry fermented by *S. cerevisiae* [64]. In this study, it was illustrated that it is possible to use starter cultures, such as *Lb. brevis*, to produce *ogi* without compromising its acceptability [64]. The use of starter cultures results in rapid drop in the pH of the food matrix [40]. Rapid pH decline may imply significant increase in the *Lactobacillus* population and increased concentration of organic acids can be indicative of the anti-pathogenic and/or prophylactic and therapeutic potential of *ogi* or other fermented cereal beverages.

3.2.11. *Poto poto*

This is a traditional fermented maize dough used in homes by the people of the Congo for weaning and for other purposes [86, 100]. *Poto poto* is prepared by soaking maize kernels for about 55 hours followed by milling and sedimentation of the paste in water [86]. The paste is fermented for about 11 hours and then cooked to produce maize gruel [86, 100]. The fermented paste can be made into *poto poto* balls for selling to make *poto poto* gruel through addition of water and sugar [86, 100]. The pH of *poto poto* samples was found to be in the range 3.48-3.66 [86].

When DNA bands from TTGE gels of *poto poto* extracts were sequenced, the following microorganisms were observed to be present in the fermented product namely: *Lb. plantarum* (predominant), *Lb. gasseri*, *Enterococcus* sp., *E. coli*, *Lb. acidophilus*, *Lb. delbrueckii*, *Lb. reuteri* and *Lb. casei* [86]. It was established that *Lb. plantarum* and *Lb. fermentum* isolated from *poto poto* produced bacteriocins that were variably inhibitive against strains of *E. coli*, *Salmonella typhi*, *Enterobacter aerogenes*, *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Enterococcus faecalis* [100]. The *E. coli*, *B. cereus* and other food pathogens reported to be in *poto poto* can consequently be inactivated by the bacteriocin-producing LAB from the same food source and make it safer for human consumption [86, 100].

3.2.12. *Thobwa*

This is a non-alcoholic thin porridge drink prepared from sorghum in Malawi and is popularly consumed by people of all demographics in the country. It is important to note however, that there is an alcoholic version of the *thobwa* in Malawi [67]. *Thobwa* may be similar to *togwa* reportedly made from maize or cassava flour and finger millet malt and consumed in Southern Tanzania [4].

3.2.13. *Ting*

Ting is a fermented traditional sorghum food of Botswana and South Africa [101, 102]. *Ting* is prepared by combining sorghum flour (40-45%, w/v) with warm water and the slurry formed is kept in a warm place (~30-37 °C) for spontaneous fermentation to take place over a period of 2-3 days [102]. *Bogobe* and *motogo* (stiff and soft porridge respectively) are the two types of porridge that can be prepared and/or cooked from *ting* previously soured to pH 3.5-4.0 mainly by LAB and yeasts [102]. *Motogo* (soft) is usually consumed for breakfast and administered to weaning infants while *bogobe* (stiff) is consumed at lunchtime and supper by adults [101, 102]. In recent studies, the dominant microbiota during *ting* fermentation consisted of *Lb. reuteri*, *Lb. fermentum*, *Lb. harbinensis*, *Lb. plantarum*, *Lb. parabuchneri*, *Lb. casei* and *Lb. coryniformis*, *Lb. rhamnosus*, *Lb. curvatus* and *Weissella cibaria* [101, 102]. The presence of these microorganisms and the low pH (3.5-4.0) inhibits proliferation of a number of pathogens, in this manner maintaining the safety of the food. Fermentation of sorghum for *ting* production improves nutrient levels and reduces antinutritional factors thus increasing the bioavailability of macro- and micronutrients as well as enhancing the sensory attributes [101].

3.2.14. *Uji*

Uji is a non-alcoholic beverage consumed widely in East Africa (Uganda, Kenya and Tanzania). It is usually prepared from maize [41, 103] although sorghum and/or millet could be mixed with the maize flour [35, 41]. There are two types of *uji*, fermented and unfermented. The unfermented *uji* is prepared by boiling water and adding the flour while stirring to obtain the desired drinkable viscosity [41]. Fermented *Uji* can be obtained by fermenting before or after cooking the porridge [38, 41].

Finely ground cereal is slurried with water at a concentration of about 30% w/v. The slurry is spontaneously fermented for two to five days at room temperature (25 °C). During fermentation of *uji*, *Lb. plantarum* has been found to be the dominant *Lactobacillus* species [35] while *Lb. fermentum*, *Lb. cellobiosus* and *Lb. buchneri*, *Pediococcus acidilactici* and *P. pentosaceus* are also reported to be part of the fermenting microorganisms in *uji* [41]. The pH of *uji* decreases to 3.5 to 4.0 whereas total acidity (as lactic acid) reaches 0.3 to 0.6% in 32 to 40 hours [38]. After fermentation, *uji* is diluted to about 8 to 10% solids and brought to boil. It is further diluted to 4-5% solids and then sweetened by the addition of 6% sucrose and consumed while still warm [38]. Like other maize beverages, *uji* is of low energy density and is deficient in essential amino acids. Fortification with legumes can improve the protein quality and content while the involvement of α -amylase-rich malt flour and/or fermenting with starch-hydrolyzing starter cultures can increase the rate of fermentation [41]. Fermented and non-fermented *uji* is mainly consumed by rural and urban housewives. Non-fermented cooked *uji* is also consumed in boarding schools, hospitals and hostels. As is the case with *mageu* in South Africa [89], *uji* is also known by different names in different localities in Kenya (see Table 7).

Ethnic group	Local name of product
Embu	Ucuru
Kamba	Uccu
Luo	Nyuka
Luhya	Obusera
Swahili	Uji ^a

Source: reference [38], ^a the common name of sour porridge in East Africa

Table 7. Local names for sour porridge in Kenya

4. Microorganisms involved in cereal-based food fermentations

4.1. Lactic acid bacteria (LAB) involved in African food fermentations

Microorganisms of major importance in lactic acid fermentations belong to the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Pediococcus* [30, 31]. Others include *Streptococcus*, *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Tetragenococcus*, *Weisella* and *Vagococcus* [42]. These genera are lactic acid bacteria (LAB) that are widely used in the production of fermented food [39, 52]. The LAB are described as Gram positive, catalase-negative non-sporing rods and cocci, which are usually non-motile [31]. The LAB starter cultures are significant in the production of desired preservative organic acids in the food product during food fermentation [52]. Starter cultures are, however, not usually employed in food fermentations in Africa. Table 8 below shows the lactic acid bacterial species that are dominant in the spontaneous fermentations of several African traditional foods.

Product name	Dominant bacteria	Reference
Fufu	<i>Lb. plantarum</i>	[26]
Gari	<i>Lb. plantarum</i>	[27]
Mageu	<i>Lactococcus lactis</i>	[99]
Mawe	<i>Lb. fermentum</i> , <i>Pediococcus pentosaceus</i> , <i>Lactococcus lactis</i>	[31]
Ogi	<i>Lb. plantarum</i>	[26]
Ogi-baba	<i>Lb. plantarum</i> , <i>Lactococcus lactis</i>	[99]
Togwa	<i>Lb. plantarum</i>	[34]
Uji	<i>Lb. plantarum</i>	[35]

Table 8. Lactic acid bacteria (LAB) dominant in the spontaneous lactic acid fermentation of African traditional foods

Strains of *Lb. plantarum*, *Lb. fermentum*, *Lb. brevis*, *Pediococcus pentosaceus* and *P. acidilactici* are reported to be among the most predominant species in most African cereal-based fermented beverages [23, 39]. The strains of some of these species have several reported probiotic

properties and/or characteristics. Species such as *Lb. plantarum* and *Lb. fermentum* are characterized by being less fastidious, relatively acid resistant, bile tolerant and can thrive on the substances provided in the cereal matrices [39]. It was reported that *Lb. plantarum* showed rapid acidification and produced inhibitory compounds that were active against *Penicillium* and *Aspergillus* strains [40].

Although most of the lactobacilli are generally poor starch fermenters [104], *Lb. plantarum* and *Lb. fermentum* are reported to be the most dominant bacterial species in acid-fermented cereal-based foods. This can be attributed to the degree of acid tolerance and superiority of these species in the utilization of starchy substrates [34, 39]. *Lactobacillus plantarum* isolates from starchy foods such as 'togwa' [34], 'ogi' [104] and cassava [34, 104] have been shown to have good starch-fermenting abilities. The fact that several cereal-based beverages are high in starch, has resulted in several α -amylase-containing lactic acid bacteria, termed amyolytic LAB, becoming sought-after in Africa and elsewhere globally. It has been reported that several strains of *Lb. plantarum*, *Lb. fermentum*, and *Lb. manihotivorans* with amyolytic capabilities have been isolated from maize-, cassava-, sorghum- and millet-based fermentations [39, 42]. Such strains can ferment starch from a variety of different sources.

4.2. Other microorganisms and combinations of microbial species involved in cereal based food fermentations

Besides LAB, *Saccharomyces cerevisiae* is notable as a predominant yeast species involved in food fermentation in Africa [45]. However, it is important to note that there are several factors determining the predominant microbial species and these include the type of cereal, the geographical location or region, conditions in the fermentation medium, moisture content and the season of the year. Yeast species isolated from an ogi maize fermentation mix included *Geotrichum fermentans*, *G. candidum*, *Rhodotorula graminis*, *Saccharomyces cerevisiae*, *Candida krusei*, and *C. tropicalis* [97]. Further investigations revealed that *Candida krusei* was better than *S. cerevisiae*, but both species improved the growth of *Lb. plantarum* in maize slurry when each of the yeast species were in combination with the lactobacilli [97]. This was attributed to the capability of the two yeast strains to produce amyolytic enzymes which enabled starch breakdown into simpler sugars for the lactobacilli to metabolise into organic acids [97]. For the same reason, during the mixed culture fermentation of *mauwe*, *Candida krusei* improved the growth of *Lb. fermentum* and *Lb. brevis* [23, 41]. During yeast and *Lactobacillus* mixed culture fermentation, the yeasts were also able to provide vitamins and other nutrients for the metabolic activities of the lactobacilli [40].

Certain yeasts were important in producing enzymes such as lipase, esterase and phytase [97]. The lipolytic activity resulted in fatty acids which are precursors of flavour while esterase activity determined aroma and flavour. On the other hand, phytase, produced by these organisms, lowers phytic acid which can form complexes with minerals that in turn can negatively affect protein digestibility [97]. A mixture of *Lb. fermentum* and *Saccharomyces cerevisiae* as starters in the fermentation of *kenkey* and *koko* achieved more rapid pH reduction in 24 hours than spontaneously fermented preparations in 48 hours [39].

4.3. Safety concerns around the use of bacterial strains that could be used as probiotics

The cereal fermented foods and the predominant LAB are generally regarded as safe (GRAS, [23]). Some of the LAB in the fermented food beverages are of human origin and have been used for centuries knowingly or unknowingly [30]. The dominant microorganisms involved in the fermentation of cereal-based beverages have no reported health risk to human life [23]. It was however, noted that some strains of *Enterococcus faecium*, *E. faecalis*, and *Lb. rhamnosus* were isolated, highly questionable, cases linked to endocarditis [30]. *Escherichia coli* Nissle, *Saccharomyces boulardii*, *Streptococcus thermophilus*, *Enterococcus francium*, *Propionibacterium*, *Pediococcus* and *Leuconostoc* have also been categorized as probiotic species or genera [10].

Most of the bacteria used as probiotics, such as *Lactobacillus* and *Bifidobacterium*, are of human or animal origin and are generally recognized as safe [105]. Apart from *Lactobacillus* and *Bifidobacterium*, other genera such as *Enterococcus* have safety concerns as some of the species are pathogenic [10]. It was reported that even though some enterococci are of technological importance in cheese making, some clinical isolates are regarded as opportunistic pathogens [105]. On that basis LAB, but not enterococci, are generally regarded as safe (GRAS, [105]) and can be used in the preparation of cereal-based probiotic beverages.

4.4. Concerns relating to the isomeric type of lactic acid produced by lactic acid bacteria

The organic acids contribute to preservation and food safety, however, it is important to note the concerns relating to L (+) and D (-) lactic acid isomers. The LAB predominantly found in spontaneously fermented African cereal beverages produce lactic acid as one of the major organic acids. Lactic acid contributes to preservation, taste and safety of the fermented foods and beverages [46]. However, lactic acid can occur in two isomers namely L (+) and D (-) isomers and it is only the former isomer that can be degraded in the human system due to the presence of L-lactate dehydrogenase in the gastro-intestinal canal [27, 42, 94]. The genera *Streptococcus*, *Enterococcus*, *Lactococcus* and *Carnobacterium* mainly produce the L(+) isomer while *Leuconostoc* spp. and all subspecies of *Lb. delbrueckii* produce the D (-) isomer [23]. The *Weissella* species, *Lb. sakei* and heterofermentative lactobacilli produce a racemate (DL) of isomers [23]. Reports indicate that industrial production of mahewu, a fermented maize beverage, using *Lb. delbrueckii*, creates a challenge of D (-) lactate production [94]. The D (-) lactate producing *Lb. delbrueckii* (ID12441) was also the major fermenting organism isolated from *munkoyo* (see section 3.2.8) [94]. This is a concern since the organisms involved in spontaneous fermentation and the major lactic acid isomer produced in cereal beverages for weaning infants and children may not be known. Lactobacilli and pediococci produce lactic acid isomers that are species specific [23, 30]. In beverages used for weaning purposes, it needs to be established whether LAB strains produce the D (-) or the L (+) lactic acid isomer [53]. An acid-base imbalance can be induced in children consuming excessive amounts of beverages containing D (-) lactic acid and

therefore L (+) lactic acid is the most recommended isomer for man [94]. It is therefore necessary to screen any probiotic cultures used in foods due to the disadvantages (possible acidosis) of offering children foods containing D (-) lactic acid [53].

5. Probiotic cereal-based beverages

5.1. Introduction

It is estimated that over 60 million people use sorghum and millet as part of their staple food in Africa in the fermented or unfermented form [63]. This is in addition to maize which is a staple cereal for the majority of the people in Africa and elsewhere in the world. This extensive consumption of cereals is partially the basis for the mounting research into the development of non-dairy cereal-based probiotic beverages. Consumers are becoming more aware of the need to eat food for health reasons. This implies that apart from good taste and nutrients provided, food needs to impart additional health benefits to the consumer. Such benefits can be realized by processing the food in such a way that its functionality is improved, for example by incorporating ingredients such as prebiotics and probiotics.

Probiotic bacteria have several reported potential health benefits [70]. Besides probiotics, prebiotic oligosaccharides also impart reported health benefits to the consumer [70]. However, in terms of foods that are used to deliver probiotic bacteria to the consumer, milk and milk products are almost exclusively used for this purpose [4, 10]. Such dairy products however have limitations that include cost (especially in the developing world), allergens, cultural food taboos against milk consumption, requirement of cold-chain facilities, the need to use beverages that form part of the people's daily diets as well as the need to maintain viability of the probiotic bacterial population in excess of the physiologically required therapeutic minimum of 10^6 - 10^7 cfu/mL viable cells in the product when consumed [106].

Probiotic microorganisms need to be consumed regularly and adequately (10^6 cfu/mL per serving) to maintain the intestinal population and to ensure that health benefits will be derived by the consumer [105]. The increasing need to eat food for health reasons, the demand for vegetarian probiotic foods, the growing lactose intolerance in the world population, and the arguable concern about the cholesterol content of fermented dairy products, are other factors that increase the need for the development of non-dairy cereal-based foods [4, 10, 105]. The following paragraphs illustrate the investigations that have been directed towards cereal- and/or legume-based probiotic beverage development.

5.2. Oats-based probiotic beverages

5.2.1. *Proviva*

Proviva is known to be the first commercial oats-based probiotic food beverage [4]. *Proviva* is produced by Skane Dairy and it has been a commercial product in Sweden since 1994. *Proviva* has malted barley added as liquefying agent and the active probiotic component is

Lactobacillus plantarum 299v. The final product which is a mixture of fruit juice and 5% oat meal has a probiotic bacterial population count in the region of 5×10^{10} cfu/L [4, 76].

5.2.2. *Yosa*

Yosa is a probiotic oat snack food marketed in Finland and other Scandinavian countries. *Yosa*, which has a flavour and texture comparable to that of dairy yoghurt, is made by cooking the oat bran pudding in water and fermenting with lactic acid bacteria and bifidobacteria. The probiotic species are reported to be *Lb. acidophilus* LA5 and *Bf. lactis* Bb12 [11, 76]. Apart from probiotic bacteria, *yosa* also contains oat fibre, a source of β -glucan that has the potential to lower blood cholesterol and so reduce the chances of heart disease [11, 49].

5.2.3. Other experimental probiotic oats products

Several workers have endeavoured to develop non-dairy cereal-based probiotic food products. An oats-based synbiotic functional drink made by fermenting an oats substrate with *Lactobacillus plantarum* B28 was developed [4]. At the end of 21 days of refrigerated storage the bacterial cell counts were still at a level of 7.5×10^{10} cfu/ml. The drink was referred to as synbiotic due to the presence of β -glucan, a functional component in cereals and usually highest in oats and barley in addition to the probiotic organism [4, 105]. Oats therefore appears to be a suitable substrate for the growth of probiotic bacteria [71].

It is important, however, to take the probiotic species into consideration when developing cereal based probiotic beverages. The probiotic bacterial population levels were studied in an envisaged synbiotic oats beverage consisting of 5% oats, 2% inulin, 0.5% whey protein concentrate and 4% sugar [107]. After a storage period of 10 weeks at 4 °C the population levels for two probiotic species (*Lb. plantarum* B-28 and *Lb. paracasei* ssp. *casei* B-29) were $1.77 \times 10^6 - 1.29 \times 10^7$ cfu/mL and $7.39 \times 10^7 - 4.49 \times 10^8$ cfu/mL respectively. However when *Lb. acidophilus* ATCC 521 was inoculated into the same oats beverage, the initial population level of 6.77×10^7 cfu/mL declined to 1.55×10^5 cfu/mL by the 4th week of storage at 4 °C. This decline gradually continued during a subsequent storage period [107]. This tendency was confirmed by other workers [71] who also found that, *Lb. acidophilus* showed slower rates of pH reduction and lower viable counts in oats due to its higher requirement for nutrients in comparison with *Lb. plantarum* and *Lb. reuteri*. To be referred to as a probiotic beverage at the time of consumption such beverages should have a population level of at least 10^6 cfu/mL viable cells [107]. These findings illustrated that the survival of probiotics in cereal beverages is species and strain specific and this should be kept in mind in developing such products.

5.3. Probiotic beverages incorporating malted cereals and hydrolysates

The potential of four bifidobacterial species of human origin to ferment a barley malt hydrolysate similar to that obtained in the brewery was investigated [76]. These species

included *Bf. adolescentis* NCIMB 702204, *Bf. infantis* NCIMB 702205, *Bf. breve* NCIMB 702257 and *Bf. longum* NCIMB 702259. The workers found that the addition of yeast extract to the malt hydrolysate as a growth promoter was necessary for the population levels to increase by 1.5 - 2.0 log₁₀ cycles to 8.73 – 9.00 log₁₀ cfu/ml after 24 hours of fermentation at 37 °C. Their work illustrated the potential of using bifidobacteria to develop a probiotic malt-based beverage by way of looking at the population levels attained in the study [76]. The study did not include product characterisation to establish its sensory attributes neither was the acceptance of the product tested among the target consumers. In addition to this, shelf-life studies in terms of viable bacterial cells were not conducted. On the other hand the barley-malt hydrolysate used as the substrate may not be commercially feasible for use in the developing world and if it were, its protein deficiencies would have malnutrition implications for the African consumer [76].

In another study relating to barley malt, the potential of using *Lactobacillus reuteri* (probiotic) and yeast to develop a cereal-based probiotic drink by fermenting a 5% (w/v) malt suspension was investigated [75]. The workers observed that using a mixed culture of *Lb. reuteri* and yeast resulted in a better decrease in pH, increased lactic acid production and increased ethanol production compared to that observed with pure cultures. The protective effect of extracts of malt, barley and wheat on the bile tolerance of *Lactobacillus reuteri*, *Lb. acidophilus* and *Lb. plantarum* has also been investigated [108]. It was illustrated that the cereal extracts, particularly from malt, exerted a protective effect, against bile salts, on the studied lactobacilli. The protection was attributed to the presence, in cereal malt extracts, of non-reducing sucrose and soluble oligosaccharides (non-digestible carbohydrates) that have been reported to improve bile tolerance. The study indicated the potential of malt, barley and wheat extracts to offer protection against bile to the probiotics when ingested together.

The factors that influence the growth of selected potential probiotic lactobacilli (e.g. *Lb. fermentum*, *Lb. reuteri*, *Lb. acidophilus* and *Lb. plantarum*) in selected cereal substrates as a way of assessing the potential of producing a probiotic cereal-based beverage was investigated [72]. In their study, a malt medium enabled the tested lactobacilli to attain higher counts (8.10 – 10.11 log₁₀ cfu /mL) than in non-malted barley and wheat media (7.20 – 9.43 log₁₀ cfu /mL). The differences in counts were attributed to a higher level of sugars (15 g/L total fermentable sugars) and an increased free amino nitrogen concentration (80 mg/L) in malt medium than in the non-malted barley or wheat media (3 – 4 g/L total fermentable sugars and free amino nitrogen concentration of 15.3 – 26.6 mg/L). The sugars were present in the form of maltose, sucrose and also in the form of their monomeric components (glucose and fructose). Growth limitation was a result of either a low pH or a substrate deficiency. In malt medium, where sugars were abundant, the microbial growth was limited by low pH (3.40 – 3.77) while in barley and wheat media, growth was limited by insufficient fermentable sugars and free amino nitrogen. This was based on the observation that growth was halted at a higher pH (3.73 – 4.88) in barley and wheat media than in malt medium [72]. Barley is not abundant in the developing world and therefore a barley-malt probiotic beverage production would not be feasible [72] in this part the world.

5.4. Maize (corn)-based probiotic beverages

5.4.1. Synbiotic *mahewu* (*mageu*)

Mageu is commercially produced in South Africa which provides it with the potential to deliver probiotic bacteria to the consumers for whom it is part of their daily diets. The commercial *mageu* is prepared using *Lactobacillus delbrueckii* and the product is pasteurized after fermentation and it is therefore not a probiotic product. The possible enhancement of the functional quality of *mageu* was investigated [70]. To this end, six pure probiotic *Lactobacillus* starter cultures and prebiotic oligosaccharides in developing six fermented synbiotic maize-based *mageu*-like beverages were tested. The strains included *Lb. casei* BGP93, *Lb. casei* (Shirota strain), *Lb. rhamnosus* LRB, *Lb. paracasei* BGPI, *Lb. plantarum* BG112, *Lb. acidophilus* PRO and *Lb. delbrueckii* subsp. *lactis* C09 (used to prepare the control). The suitable prebiotic ingredient and the factors affecting the growth of these organisms in the maize gruel, as well as the sustained viability of these organisms in the product during extended refrigerated storage were investigated [70].

The viability of the probiotic strains, in terms of population level, in the fermented synbiotic maize-based beverages at the end of a 90-day storage period at 5 °C exceeded 7.5 log₁₀ cfu/mL [70]. This was well above the recommended therapeutic minimum of 6 log₁₀ cfu/mL at the time of consumption [109, 110]. Intake of a portion of 200 – 300 ml of the experimental synbiotic *mageu* products would potentially enable the consumer to derive 7 to 10.5 g d⁻¹ of prebiotic Raftiline® GR (inulin) and 2 × 10¹⁰ – 3 × 10¹¹ viable probiotic bacterial cells d⁻¹. A trained sensory panel found that the synbiotic maize-based beverages fermented by *Lb. acidophilus* PRO and *Lb. rhamnosus* LRB were the most similar to the control (*Lb. delbrueckii*). This was confirmed by a larger consumer acceptance panel [111]. This illustrated that *mageu* can be converted to an acceptable synbiotic beverage and that it was able to sustain a population of viable probiotic cells, exceeding the therapeutic minimum level, during an extended storage period.

5.4.2. *Mahewu* (*mageu*) with bifidobacteria

The survival of probiotic *Bifidobacterium lactis* DSM 10140 as harvested and inoculated free cells or as microencapsulated cells in mahewu (*mageu*) was studied [74]. The workers observed that the counts of free cells of *B. lactis* reduced significantly during the 21day storage at 4 °C and 22 °C both in the presence or absence of oxygen. Poor viability of *Bf. lactis* in mahewu was attributed to exposure to the low pH (3.5) of mahewu and the inadequate buffering capacity as a result of a low protein content (5.2 g/L) in a medium containing 78.4 g/L of carbohydrates [74]. The workers then recommended the use of microencapsulation coupled with storage at 4 °C as being optimal for the delivery of *Bf. lactis* to the consumer [74]. However, microencapsulation is not without its technological challenges and added cost. *Bifidobacterium lactis* has also been said to be closely related to *Bf. animalis* which is a probiotic of animal origin [112]. It is therefore important that the potential of using bifidobacteria of human origin as starters in combination with lactobacilli are investigated in providing a probiotic enhanced *mageu* product.

5.4.3. Fermented maize weaning porridge

In a fermented “maize porridge” (18.5% w/w maize meal) mixed with malted barley (1.5% w/w), the growth and metabolism of four strains of probiotic lactobacilli (*Lb. reuteri* SD 2112, *Lb. rhamnosus* GG, *Lb. acidophilus* LA5 and *Lb. acidophilus* 1748) were studied in terms of cell counts, pH and metabolites [73]. Bacterial cell counts attained maximum levels of 7.2-8.2 log₁₀ cfu within 12 hours of fermentation at 37 °C [73]. The lowest pH range attained after 24 hour fermentation period at 37 °C was 3.1-3.7 [73]. The products were of low viscosity that could be attributed to the use of the barley malt expected to be the source of amylase for the enzymatic hydrolysis of maize starch. Whereas the malt may have increased the level of fermentable sugars, it also led to a product of low viscosity (too watery) that may not have consumer appeal in the developing world either as porridge or a beverage. This product was not subjected to sensory evaluation, consumer preference evaluation or shelf-life testing. ‘Maize weaning porridge’ as it was referred to by the workers would not be nutritionally suitable for this purpose due to the inherent protein deficiency of maize that was the principal ingredient. It should also be noted that barley malt may not be readily available in the developing world.

5.5. Probiotic soy-based probiotic beverages

Soybeans and rice fermentation media are also reported to be suitable substrates for the growth of certain probiotic lactobacilli and bifidobacteria [49]. Soybean usage is however hampered by the presence of raffinose and stachyose, which can cause flatulence [105]. The non-inactivated lipoxygenase enzyme in the soybean is the causative agent of the beany off-flavour (as perceived in Western societies) in soy-containing products [105]. These limiting factors can, however, be significantly reduced by fermenting with technologically suitable LAB. Soy yoghurt and/or “sogurt” developed using soymilk, is characterized by a hard and coarse texture in addition to a beany “off-flavour”. Coupled with inadequate acid development, this has resulted in a lower sensory appeal of these products [105]. Reports indicate that inclusion of fructose, calcium, cheese whey proteins, gelatin and lactose as well as probiotic bacteria improved the textural and sensory properties of sogurt [105].

Soymilk is suitable for the growth of lactobacilli and bifidobacteria and a probiotic soymilk and soybean yoghurt with added prebiotic oligofructose and inulin was developed [4]. This was found to be the case with several lactobacilli that included *Lb. casei*, *Lb. fermentum*, *Lb. reuteri*, and *Lb. acidophilus* [49]. Probiotic bacteria were also introduced into a non-fermented vegetarian frozen soy dessert. This product was composed of a soymilk beverage, sugar, oil, stabilizer and salt. The probiotic organisms introduced included *Lactobacillus acidophilus*, *Lb. rhamnosus*, *Lb. paracasei* ssp. *paracasei*, *Saccharomyces boulardi* and *Bifidobacterium lactis*. Bacterial population levels after 6 months’ storage exceeded 10⁷ cfu/g for all species except for *S. boulardi* [49]. The population level of the yeast species was below the therapeutic minimum of 10⁶ cfu/g and this was attributed to the absence of ‘cell shielding’.

In summary it can be stated that generally speaking, cereals are good growth-substrates of probiotic bacteria [108]. This is illustrated by the Yosa oats-based product, which to date is the only cereal-based commercial product known to contain both LAB and bifidobacteria. Since cereal-nutrient components vary, growth rates of probiotic organisms may also vary. Further research is therefore imperative to investigate the growth factors that may enhance the growth and survival of lactobacilli and bifidobacteria in cereal-based gruels. The indigestible variable fractions of the cereals can be utilised as prebiotics by probiotics in the GIT of the host upon ingestion of the fermented cereal-based beverage and these should also be defined and tested.

5.6. Therapeutic minimum levels of bacterial species in probiotic beverages

The therapeutic minimum population level for bacterial species in probiotic beverages is recommended to be 10^6 cfu ml⁻¹. This is the lowest probiotic bacterial count in a probiotic product that may adequately impart prophylactic and therapeutic benefits to the host. In order to realize therapeutic effects of probiotic bacteria in a product, the bacterial counts should exceed 10^6 cfu ml⁻¹ [113]. Such a dose should be consumed regularly to ensure permanent colonisation in the small intestines. These high bacterial cell counts of probiotic bacteria are proposed to allow for the possible reduction in numbers during passage through the stomach and the intestines [114]. The need to have live probiotic cultures in products claimed to be probiotic has resulted in the formation of regulatory bodies and food legislation in some countries.

The Swiss Food Regulation and the International Standard of FIL/IDF require probiotic products to contain at least 10^6 cfu ml⁻¹ [115]. The Fermented Milks and Lactic Acid Bacteria Beverages Association of Japan specifies a minimum of 10^7 cfu ml⁻¹ to be present in fresh probiotic dairy products [114, 115]. Japan has the FOSHU (Foods for Specified Health Use) programme for approving functional foods for marketing. A product with a "FOSHU" tag is defined as a food, which is expected to have certain functional benefits and has been licensed to bear a label to that effect [1]. The USA's National Yoghurt Association (NYA) specifies a population level of 10^8 cfu/g of lactic acid bacteria, at the time of manufacture, before placing a "Live and Active Culture" logo on the containers of the product [14]. However, in the USA, no indication is given as to what the viable count should be at the end of shelf-life. In the South African context, the South African Food and Health Draft Regulation (regulation 63) stipulates that selected probiotic microbes must be present at levels of at least 10^6 cfu ml⁻¹ of product in order to exert a beneficial effect [110].

6. Conclusions and recommendations

Cereals and fermented cereal beverages can be advocated for use as delivery vehicles of health-benefiting functional ingredients such as probiotics and prebiotics. However, it is important to note some of the challenges associated with cereal grains and how they may be circumvented in improving probiotic cereal food delivery to masses in Africa and the

developing world. It was noted that there is no known distribution channel for starter cultures to small scale or household scale processors of cereal-based fermented beverages in Africa and the developing world [30]. The other bottleneck is the fact that probiotic strains that have been technologically used successfully in dairy products may not exhibit similar acceptable growth and viability in cereal beverages. This accentuates the need for doing further screening [105]. The developed plant-cereal-based synbiotic beverages may also not have the necessary acceptable sensory attributes [3, 105, 116]. In a recent study, the use of a strain of *Lb. paracasei* BGP1 in a maize based fermented synbiotic experimental product resulted in off-flavours detected by a trained sensory panel [70, 111].

The use of probiotic strains in a combination of cereals and legumes in fermented products needs to be based on a number of considerations including technological and functional properties; sensory properties, growth rate; capability to deal with antinutritional factors; reduction of toxic substances in cassava; reduction of mycotoxins in cereals; reduction of flatulence causing compounds in legumes; pathogen inhibitory capabilities; co-existence and growth in mixed cultures [30]. These determinations however are hampered by the lack of facilities, expertise and the cost-benefit ratio that, in most cases, is not favourable to small scale and household scale cereal beverage producers in the developing world [30].

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