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Lactic acid bacteria as a cell factory for the delivery of functional biomolecules and ingredients in cereal based beverages: a review

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Abstract:

In this review, we aim to describe the mechanisms by which LAB can fulfil the novel role of efficient cell factory for the production of functional biomolecules and food ingredients to enhance the quality of cereal based beverages. LAB fermentation is a safe, economical and traditional method of food preservation foremost, as well as having the additional benefits of flavour, texture and nutrition amelioration. Additionally, LAB fermentation in known to render cereal-based foods and beverages safe, in a chemical-free, consumer-friendly manner, from an antinutrient and toxigenic perspective.

Huge market opportunities and potential exist for food manufacturers who can provide the ideal functional beverage fulfilling consumer needs. Newly developed fermented cereal-based beverages must address markets globally including; high-nutrition markets (developing countries), lifestyle choice consumers (vegetarian, vegan, low-fat, low-salt, low-calorie), food-related non-communicable disease sufferers (cardiovascular disease, diabetes), and green label consumers (Western countries). To fulfil these recommendations, a suitable LAB starter culture and cereal-based raw materials must be developed. These strains would be suitable for the biopreservation of cereal beverages and, ideally, would be highly antifungal, anti-mycotoxigenic, mycotoxin-binding and proteolytic (neutralise toxic peptides and release flavour-contributing amino acids) with an ability to ferment cereals, whilst synthesising oligosaccharides, thus presenting a major opportunity for the development of safe cereal-based prebiotic functional beverages to compete with and replace the existing dairy versions.

Keywords: Lactic acid bacteria, fermentation, cereal-based functional beverages, biopreservation, exopolysaccharides, anti-allergenic, anti-mycotoxin

Introduction

In almost every country, the staple foods are comprised of cereals which are considered to be one of the most important sources of dietary nutrients like carbohydrates, proteins, vitamins, minerals and fibres. However, the nutritional quality of some cereals and the sensory properties of their products are sometimes inferior or poor compared to other principal dietary products (Blandino et al., 2003). Among the technologies used for cereal processing, fermentation represents one of the oldest and most economical methods of producing and preserving food (Stiles, 1996), thus illustrating why such a large proportion of cereals are processed in this way prior to consumption (Nout, 2009). Fermented foods encompass an estimated 25% of the European diet and 60% of that in developing countries (Holzapfel et al., 1995), some of which are cereal-based beverages, particularly in the latter population segment. These cereal beverages generally result from combined yeast and bacterial fermentations with the final product typically containing lactic acid bacteria (LAB). LAB are so named due to their production of organic acids during fermentation (Hugenholtz, 2008). Apart from the preservation effect, LAB fermentation provides a natural way to concentrate and enhance nutrients (vitamins and essential amino acid synthesis) by reducing cereal-food/beverage bulk (Holzapfel, 1997), destroying undesirable components (mycotoxin, antinutritional factors) and ameliorating sensory qualities (taste, aroma, texture, consistency, and appearance of the food). Additionally, these factors contribute to an easier preparation (reduced cooking times and lower energy consumption) and enhance product safety (microbial contaminants) (Leroy and De Vuyst, 2004; Simango, 1997). Moreover, during the last decade, consumer lifestyle choices (vegetarianism, veganism etc.), an increase in adverse reaction to foods (food intolerance, malabsorption and allergies), and diet-related non-

communicable diseases (cardiovascular disease, elevated blood pressure etc.) have led to an increasing demand for non-dairy based functional beverage substitutes with high acceptance and functionality (Zannini et al., 2011). Fermented cereal-based beverages have a huge potential to fill this gap in the consumer market acting as potential vehicles for the delivery of functional compounds such as antioxidants, dietary fibre, minerals, probiotics and vitamins (Kreisz et al., 2008). This review aims to highlight the mechanisms by which LAB can fulfil this novel role as efficient cell factories for the production of functional biomolecules and food ingredients to enhance the quality and safety of cereal based beverages.

Justification of porridge/gruel use as cereal beverage

Fermented beverages are of great significance because they provide and preserve vast quantities of nutritious food in a wide diversity of flavours, aromas and textures which enrich the human diet. They represent a significant proportion of all diets worldwide, typically providing about one-third of daily food intake (Geoffrey, 1994). LAB fermented cereal gruels range from thin beverages to thicker porridge type mixtures, which are consumed as beverages, breakfast foods or baked into breads depending on the local tradition and the desired end product. These acid-fermentates, of maize, sorghum, millet, or cassava (tuberous root vegetable), are still common around the world as staple foods, particularly in developing countries and are all made in a similar fashion, generally using spontaneous cultures; *ogi* (Nigeria), *uji* (Kenya), *koko* (Ghana); *togwa* (Tanzania), *obsuera* (Tanzania and Uganda), *bogobe* (Botswana), *nasha, aceda, raghida* or *medida* (Sudan), *ogibaba* (Nigeria), *kamu* (Nigeria), *mawe* (Benin), *ting* (South Africa), *fube* (Brazil), *chika* (Peru), etc.

Generally, porridge type cereal fermentates are too thick to feed babies and young children and there is a major problem in providing sufficient nutrient density of calories, proteins, and other factors within the consumption capacity of the child. As such, in many African countries the porridge is diluted with fermentation supernatant (for example *ogi*) or a portion of the cereal grain is malted/germinated and added to thicker cereal preparations transforming it to a liquid gruel in minutes (Steinkraus, 1995), thus allowing these fermented beverages to deliver the necessary functional biomolecules. By using a natural LAB starter culture and adding flour of germinated seeds (malt), it is possible to prepare liquid cereal gruels from maize, sorghum, and millet with up to 35% flour concentration (Lorri and Svanberg, 1993). This results in an enhanced *ogi* porridge which typically has a smooth creamy texture and a sour yogurt taste (Steinkraus, 1995).

Lactic acid bacteria

LAB constitute of a group of gram-positive bacteria characterised by their morphological, metabolic, and physiological characteristics. From a practical food-technology point of view, the following genera are considered to belong to LAB group: *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus*, *Vagococcus* and *Weissella*. The general description of the bacteria included in the group are gram-positive, catalase negative, non-motile, non-respiring and non-spore forming cocci or rods, which produce lactic acid as the major end product during the fermentation of carbohydrates. They have high acid tolerance and survive at pH 5 and lower giving them a competitive advantage over other bacteria.

LAB production from cereals

In addition to the well-studied dairy and meat LAB support substrates, cereal-based broths also deserve attention given their potential in the production of functional metabolites and foods. Cereal fermentations generally differ from most other fermentations because the final product is often not consumed but further processed to beer, bread, crackers etc. (de Valdez et al., 2010). Contrastingly, several cereals have been consumed for generations in traditional mixed fermentation staple food beverages worldwide from a variety of raw materials, all of which have an increased shelf-life, and improved taste and nutrition over their non-fermented counterparts (Table 1). Of these, both *tobwa* and *uji* non-alcoholic beverages are the only two solely fermented by LAB; the others represent various combinations of LAB, bacteria, yeasts and fungi (Blandino et al., 2003).

The aim of using a starter culture is to ferment sugars, improve nutritional attributes, decrease pH (through organic acid production) which, combined with the production of anti-microbial inhibitory components, can reduce or prevent of the growth of undesirable microflora. Additional organoleptic qualities arising from LAB cereal fermentation include flavour compound synthesis and protein hydrolysis which have direct impact on the texture and flavour.

By varying the starter culture, cereal substrate and other processing tools, the capability to create novel LAB fermented cereal beverages designed to target general or specific populations is significant. In particular, we have the opportunity to fulfil the needs of those populations which are specifically excluded from the current dairy- and meat-based fermented beverage and food segment. The potential consumer markets include the 1 in 226 worldwide Coeliac sufferers (Zannini et al., 2011), as well as the extended gluten-free market due to perceived gluten-intolerance and as a healthy life-choice, using non-allergenic cereals and pseudocereals such as

rice, sorghum, quinoa, buckwheat, teff, corn etc. (Zannini et al., 2011) (Table 2). Furthermore, the even larger population segment with lactose non-persistence, malabsorbtion or intolerance can enjoy dairy-free probiotic and increased nutrition cereal products. Prevalence of hypolactasia, including lactose intolerance, is approximately 70% worldwide with increased occurrence in certain populations (Lomer et al., 2008). Other target segments for cereal-derived fermented products include vegetarian, vegan, high-nutrition and low-calorie/-cholesterol consumers. Additionally, cereal derived functional beverages which have been LAB fermented to increase their nutritional composition would be poignant in the consumer markets for cardiovascular disease (CVD) and high blood pressure due to the low saturated fat contents of cereals and propensity to lower cholesterol levels (Smith and Tucker, 2011). CVD is currently the primary cause of mortality in the USA and Europe and is predicted to reach levels of 40.5% in the US by 2030 (Heidenreich et al., 2011). Thus, in contrast to the current mass consumption of fermented cereal beverages in developing countries, due to their affordability and nutritional properties, it is evident that the development of various combinations of gluten-free, lactose-free, vegetarian friendly or low cholesterol/fat and high nutrition fermented cereal products are of interest globally.

In addition to the technological applications of LAB incorporation into cereal beverages and foods, cereals are also ideal fermentable substrates for the growth of probiotic LAB considering their composition; substrate formulation, starter culture growth capability and productivity, the stability of the probiotic strain during storage and, the organoleptic properties and nutritional value of the final product (Nout, 2009). In addition, cereals are sources of non-digestible carbohydrates that selectively stimulate the growth of *lactobacilli* and *bifidobacteria* as

probiotics. Cereals contain water-soluble fibre, such as β-glucan (Patsioura et al., 2011) and arabinoxylan, galacto- and fructo-oligosaccharides and resistant starch, which have been linked to the prebiotic concept (Mei et al., 2011). Using these substrates to support LAB proliferation ensures the adaption of strains suitable for functional biomolecule delivery in fermented cereal beverages.

Oat, wheat, barley and malt substrates have previously been exploited for the cultivation of various LAB to probiotic levels. In a study by Charalampopoulos et al. (2003), malt, wheat and barley extracts were shown to exhibit a protective effect on the viability of probiotic *L. plantarum, L. acidophilus* and *L. reuteri* under acidic conditions due to the chemical compositions of these cereal broths. Many other examples of LAB proliferation and nutrient or beneficial metabolite production exist in cereal systems. These systems predominantly address sourdough microflora, however, from these we can draw many conclusions about LAB behaviour in cereal wort/broth beverages. In particular, gluten-free sourdough batters give us an insight into LAB behaviour in liquid cereal fermentations (Zannini et al., 2011). In traditional sourdough environments (wheat, rye and barley) highly adapted and competitive *lactobacilli* are predominant with yeasts also present. These LAB positively contribute to the final cereal product through flavour enhancement, processability, maltose and amino acid metabolism, use of electron acceptors (facilitating the heterofermenatative LAB dominance), and exopolysaccharide (EPS) formation (Vogel et al., 2002). According to this study, *Lb. sanfranciscensis, Lb. panis, Lb. pontis, Lb. reuteri, Lb. brevis, Lb. fermentum, Lb. frumenti, Lb. delbrueckii, Lb. amylovorus, Lb. acidophilus, Lb. amylolyticus* and *Lc. Lactis* are typical representatives of the Lactobacilli present in cereal fermentations (Vogel et al., 2002). Their contributions were dominant in the

system primarily due to their competitiveness, acid production and anti-microbial compounds (Corsetti et al., 1998; Lavermicocca et al., 2000; Ryan et al., 2011; Vogel et al., 2002).

LAB-mediated microbe inhibition through antibacterial compound production

Traditionally, a wide range of foods were fermented solely for bio-preservation purposes and initially this was thought to be entirely due to the lower pH and organic acid production during LAB fermentation. However, more recently it has become obvious that there are many antimicrobial (anti-fungal/-bacterial) compounds (Table 3) sharing the responsibility for this protective property of fermentation in the production of fermented cereal beverages. For example, research into the LAB present in *boza* has also lead to the discovery of several bacteriocin producing strains (Botes et al., 2007). Analyses of 10 *boza* samples from Turkey revealed further antimicrobial activities, to due acid and H_2O_2 production as well as proteinaceous bacteriocin-like metabolites, from species of *L. lactis* subsp. *lactis, Lc. citreum, L. brevis, L. plantarum, L. paraplantarum, E. faecium, L. graminis, Pediococcus* species and *L. paracasei* subsp. *paracasei* against reporter strains such as enterobacteriaceae and other gram-positive and gram-negative bacteria (Kivanç et al., 2011). The LAB population, isolated from a Bulgarian *boza*, included strains of *L. plantarum, L. pentoses, L. rhamonsus* and *L. paracasei*, which produced proteinaceous bacteriocins active against (gram-negative) bacteria *L. casei, E. coli, P. aeruginosa* and *E. faecalis* (Todorov and Dicks, 2006). Further work on *boza* LAB from the same research group revealed inhibitory activity against bacterial, fungal and viral species (Todorov, 2010; Todorov et al., 2008).

Microbiological research into the home produced *mahewu* beverage has also shown a bactericidal and/or bacteriostatic effect of LAB against negative pathogen strains *Aeromonas*,

Salmonella, *Shigella*, *Campylobacter* and *Escherichia coli*, suggesting that it is unlikely to pose bacterial health risks or transmit enteric pathogens (Simango and Rukure, 1991; Simango and Rukure, 1992).

LAB fermented *ogi* gruels were also found to inhibit gram-negative pathogenic bacteria such as enterotoxigenic *E. coli*, *Campylobacter jejuni*, *Shigella flexneri*, and *Salmonella typhimurium*, as well as gram-positive bacteria such as *S. aureus* (Lorri and Svanberg, 1993; Nout et al., 1989). The predominant organism in *ogi* fermentation is *L. plantarum* which is responsible for the production of lactic acid and is dominant due to its utilisation of corn dextrins after the depletion of the fermentable sugars. In one study, the shelf-life of an *ogi* preparation was extended by 4 days to 11 using a bacteriocin-producing *Lactobacillus* strain as a starter culture (Olasupo et al., 1997).

Similar enteropathogen inhibition of *C. jejuni* and *E. coli* by LAB fermentation, with dominant strains of *L. lactis*, *Lactobacillus* sp. and *Candida krusei*, were noted in *togwa* cereal gruel and it was concluded that regular consumption of fermented cereal weaning foods had the functional effect of potentially reducing transmission of enterotoxin-producing bacteria, and ingestion of enterotoxins (Kingamkono et al., 1995). An *in vivo* test, over a 9-month period showed that acid-fermented Nigerian *ogi* gruels reduced the incidence of diarrhoea in a group of school children from 2.1 to 3.5 per child (Lorri and Svanberg, 1993).

Several other fermented cereal sourdough studies have also illustrated the anti-microbial effects of LAB and their metabolites. There is an extensive knowledge about antibacterial compounds, especially bacteriocins, produced by LAB and their application in food preservation (Calo-Mata et al., 2008; Castellano et al., 2008; Dortu and Thonart, 2009; Galvez et al., 2008; Maqueda et

al., 2008; Papagianni and Anastasiadou, 2009; Rouse and van Sinderen, 2008; Settanni and Corsetti, 2008). A number of recent reviews exist on the topic (Dalié et al., 2010; Schnürer and Magnusson, 2005).

Production of antifungal compounds and mycotoxigenic activity

A hugely important aspect of cereal beverage production commences with germination and malting of the cereal starting substrate. As with other cereal processing steps, microbial contamination may cause substantial losses and influence malt safety and quality. However, microbes may also have a positive or functional effect even though the use of starter cultures in cereal beverage malting is a relatively new process. Different approaches were made in terms of the nature of starter cultures and desired goal of the application. The use of the *Pediococcus pentosaceus* and *Saccharomyces* spp. cultures inhibited moulds and coliforms in sorghum malting but did not influence the malt quality i.e. diastatic power (Lefyedi, 2007).

First approaches to use pure LAB starter cultures in malting were made in the 1990's (Haikara et al., 1993; Laitila et al., 1997). These authors reported that the beneficial effects on the malting. The addition of *Lb. plantarum* E76 culture, including cells and spent medium, have been reported to restrict the growth of indigenous *Fusarium* flora of naturally contaminated two-rowed barley in laboratory-scale malting. This was dependant on the contamination level and the fungal species/strains present on barley (Laitila et al., 2002). Continuous work with starter cultures of this strain and *P. pentosaceus* E390, which was added to the steeping water of normal malting barley, showed that yeast growth was promoted while the growth of harmful bacteria and *Fusarium* fungi were restricted. The authors attributed some of the beneficial effects to the presence of lactic acid and the low pH (Laitila et al., 2006). Liske et al. (2000) reported that

strains of the species *Streptococcus alactolyticus, Lb. pontis, Lb. sanfranciscensis, Lb. salivarius, Lb. reuteri* and *Weissella paramesentoides* were able to reduce the growth of *F. culmorum* under malting conditions.

There are increasing numbers of published studies on the identification of antifungal compounds produced by LAB. A summary of publications on antifungal LAB and the compounds they produce are presented in Table 3. Aside from the antifungal properties of the main metabolites; lactic and acetic acid, LAB produce other compounds like other organic acids, acetoin, carbon dioxide, diacetyl, hydrogen peroxide, caproic acid, 3-hydroxy fatty acids, phenyllactic acid, cyclic dipeptides, reuterin, fungicins and other proteinaceous compounds exhibiting antifungal potential. The exact mechanism of antimicrobial action can often not be explained due to the complex interactions and synergistic effects which are frequently observed between compounds involved in antimicrobial action (Corsetti et al., 1998; Dal Bello et al., 2007; Dalié et al., 2010; Niku-Paavola et al., 1999; Yang and Chang, 2010). A comprehensive overview about individual substances and their possible antifungal mechanism was recently reviewed by Dalié et al. (2010).

In addition to an in-depth knowledge about *in vitro* antifungal properties of individual LAB, suitability for application is strongly dependent on the environmental parameters that modulate their antifungal activity. Numerous parameters have been considered, including temperature, time of incubation, growth medium, pH and nutritional factors (Batish et al., 1997). In general, a good correlation between bacterial growth and antifungal activity has been reported thus, it can be expected that conditions promoting growth are also favourable to the formation antifungal substances (Batish et al., 1997).

External cereal antinutrients can take the form of filamentous fungal mycotoxins which are products of the secondary metabolism of filamentous micromycetes and are known for their toxic effects (Belakova et al., 2011). Trichothecenes are a group of mycotoxins, which causing a range of acute and chronic symptoms (D'Mello and MacDonald, 1997; D'Mello et al., 1999). Major products of the trichothecene biosynthetic pathway are known as T-2 toxin, deoxynivalenol (DON), diacetoxyscirpenol (DAS), and nivalenol (NIV).

Many reports of mycotoxin (e.g. Trichothecene, Zearalenone, Fumonisine, Ochratoxin, Aflatoxin, Deoxynivaleon) contamination in maize, rice, sorghum, millet, buckwheat, and teff, have been widely reported (Ayalew et al., 2006; Bresler et al., 1995; Reddy et al., 2009; Tanaka et al., 2007). In addition, mycotoxins are generally stable compounds which are not destroyed during most food processing operations (Bullerman and Bianchini, 2007), and may therefore lead to the contamination of finished cereal-based foods. Besides the level of mycotoxigenic contamination originating from raw grains, the accumulation of mycotoxins along with fungal growth has been observed during germination leading to final concentrations exceeding the initial levels (Lancova et al., 2008; Schwarz et al., 1995). Gushing, a negative over-foaming associated with beer, has been correlated to specific fungal compounds i.e. hydrophobins, hydrophobic components of conidiospores or aerial mycelia, as well as to non-specific lipid transfer proteins (ns-LTPs) (Sarlin et al., 2007). The latter are synthesised in grains as a response to fungal infection (Hegrova, 2009; Hippeli and Elstner, 2002; Sarlin, 2005; Sarlin et al., 2007; Stubner et al., 2010).

Dalié et al. (2010) provide a substantial summary on the *in vitro* interaction between LAB and mycotoxins. The authors propose mechanisms for detoxification of these fungal toxins,

illustrating another potential functional aspect of LAB in fermented cereal beverages. For example, the reduction in aflatoxin accumulation in fermentation liquids can be a result of suppressed biosynthesis and/or binding to LAB cell walls and decreased solubilisation of other mycotoxins, i.e. trichothecenes, zearalenone, ochratoxin A and fumonisin B1 and B2, are considered to be solely by LAB binding (Dalié et al., 2010). This appeared to be strongly dependent on the LAB strain used as well as the physiological conditions (Del Prete et al., 2007; El-Nezami, 2002; El-Nezami et al., 1998; El-Nezami et al., 2004; El-Nezami et al., 2002a; El-Nezami et al., 2002b; Fuchs et al., 2008; Niderkorn et al., 2006; Niderkorn et al., 2009; Niderkorn et al., 2007; Pierides et al., 2000; Piotrowska, 2005). It is suggested that carbohydrates and/or protein components such as peptidoglycans are involved in the binding of aflatoxin B1 and fumonisin B1 (Haskard et al., 2001; Lahtinen et al., 2004; Niderkorn et al., 2007). Zearalenone and α-zearalenol binding by LAB is likely due to bacterial cell wall carbohydrates and proteins or only proteins (El-Nezami et al., 2004). Furthermore, the availability of some nutritional factors e.g. protein and amino acids as well as external electron acceptors are considered to play a detrimental role in formation of antifungal compounds (Corsetti et al., 1998). Thus, a possible approach to render cereal-based beverages safe, in a chemical-free, consumer-safe manner, from an antinutrient and mycotoxigenic perspective should involve biopreservation by fermentation with high antifungal, antimycotoxigenic, mycotoxin-binding, proteolytic LAB strains. This indicates the high potential of these bacteria in terms of improving malt quality as well as restricting fungal proliferation in particular of *Fusarium* species.

Reduction of antinutritive factors in cereal based fermented beverages

Cereal contributes over 60% to the world food production providing dietary fibre, proteins, energy, minerals and vitamins required for human nutrition (Charalampopoulos et al., 2002b). However, the nutritional status of cereals is often counteracted by the presence of antinutrient components such as phytates, protease inhibitors, and polyphenols (Reddy and Pierson, 1994). Phytates form complexes with micronutrients such as Ca, Fe, K, Mg, Mn and Zn making them insoluble and thus, unavailable for adsorption in the intestinal tract of humans (Bohn et al., 2008). Protein inhibitors, like trypsin inhibitors, cause growth inhibition by interfering with protein digestion, pancreatic hypertrophy, excessive secretion of pancreatic enzymes, and metabolic disturbance in the utilisation of sulphur amino acids (Reddy and Pierson, 1994). Tannins also form enzyme complexes in the digestive tract adversely affecting the utilisation of proteins and carbohydrates resulting in reduced growth, useable energy, and poor bioavailability of amino acids (Onyango et al., 2005).

Lactic acid fermentation was reported to significantly reduce the phytate content in cereal-based foods (Lopez et al., 2000; Lopez et al., 1983; Svanberg et al., 1993) with a concomitant improvement of mineral solubility (Brune et al., 1992; Lopez et al., 2000). Marklinder et al. (1995) studied the degradation of phytate using various sources of phytases in an oat-based nutrient solution fermented by *L. plantarum* strain 299 V. Among the sources of phytases evaluated, rye sourdough fermented with *L. plantarum* strain 299 V caused a significant degradation of phytate in the oat solution compared with the raw material, reducing the content by 80%. Even though some LAB belonging to species of *Lb. amylovorus*, *Lb. plantarum* (Sreeramulu et al., 1996), *Lb. sanfranciscensis,* (De Angelis et al., 2003) and *Lb. fermentum* (Songré-Ouattara et al., 2009) were reported to produce significant extracellular phytase

activities, Marklinder et al. (1995) suggested that the observed reduction in phytate content by lactic acid fermentation was mainly promoted by endogenous plant phytase activity or a phytate/protein co-precipitation of as a consequence of a fall in pH during lactic fermentation. Thus, LAB provide a favourable pH for the endogenous cereal phytase activity which is in the range of 4.5-5.5 (Greiner and Konietzny, 2006; Svanberg and Lorri, 1997).

Kayode et al. (1993) also reported that LAB and yeast co-fermentation, during *tchoukoutou* African sorghum beer brewing, caused a reduction in the phytate content through degradation to IP5 (inositol-pentakis-phosphate), IP4, etc., and inorganic ortho-phosphate (Pi) that is used by these microorganisms for their growth (Andlid et al., 2004; Kerovuo and Tynkkynen, 2000) and a pH effect on phytase activity is also suggested.

Trypsin inhibitors and tannins are, among other antinutrient compounds, responsible for indigestibility of cereal proteins. Among the cereals; rye, sorghum, pearl millet and barley grains, there are relatively high levels of tannins, trypsin inhibitors and amylase inhibitors (Sharma and Pattabiraman, 1982; Sosulski et al., 1988). LAB fermentation has been shown to lower the levels of proteinase inhibitors in cereal porridges, thus increasing the availability of essential amino acids, such as lysine, leucine, isoleucine, methionine and even tryptophan (Kazanas and Fields, 1981; Nche et al., 1995).

LAB strains, however, differ in their capability to degrade trypsin inhibitors under defined conditions. Holzapfel (1997) reported that when fermenting with *L. plantarum* strain 91 and *Leuconostoc sp*. 106, isolated from Ghanaian fermented foods, an approximate 50% reduction in trypsin inhibitor activity was recorded. Additionally, a kinetic study of *Leuc. mesenteroides* 92 activity showed that a significant decrease in trypsin inhibitor activity was affected, only during

the stationary phase of growth, indicating the importance of the length of the fermentation process.

Sharma and Kapoor (1996) studied LAB (and/or yeast) fermentation after various processing treatments including; grinding, soaking, debranning, dry heat treatment, autoclaving and germination. The fermentations showed good potential to improve the nutritional value of a pearl millet slurry by reducing the antinutrients (phytic acid and amylase inhibitors), thereby enhancing utilisation of the grain nutrients. Mbugua et al. (1992) also report an improvement in the protein digestibility of *uji*, a popular thin porridge beverage made from sorghum flour by LAB and yeast fermentation showing, in parallel, a significant decrease of extractable tannins. The same findings were also reported by Onyango et al., (2005) who reported a significant decrease in tannin content when an extruded *uji* was made with a blend of maize-finger millet after LAB fermentation. As well as in *uji*, due to a strong decrease of the pH, LAB fermentation also improved the sorghum protein digestibility of another liquid porridge called *ting* (Taylor and Taylor, 2002) which is popular in western Africa and Botswana. The acid environment developed during lactic acid fermentation, causes structural changes in the sorghum storage proteins (prolamins and glutenins), making them more susceptible to pepsin attack (Taylor and Taylor, 2002). LAB thus produces a suitable environment to accommodate endogenous cereal enzyme hydrolysis of antinutrients, in addition to their own enzymatic and metabolite contribution as functional cell factories.

Ameliorate nutritional value

LAB fermentation does not just have a preservation function in fermented cereal products. It can also have multiple effects on the nutritional value of food through decreasing anti-nutritional

biomolecules, thus amassing bioavailability of suppressed nutrients, and more directly by hydrolysing carbohydrates and non-digestible oligosaccharides into functional compounds. LAB fermentation impacts the nutritional characteristics of various cereal beverages: *mawè*; LAB (and yeasts) increase digestibility (maize), *tchoukouotou*; LAB and yeasts produce well digestible macro- and micro-nutrients (sorghum), *uji*; LAB improve digestibility (sorghum), *ben-saalga*; LAB improve digestibility (pearl millet), *jnard*; mixed culture improves digestibility (finger millet), etc.

Certain oligosaccharides, such as raffinose, stachyose and verbascose, which are abundant in cereals can cause flatulence, diarrhoea and indigestion (Holzapfel, 1997). These oligosaccharides are often resistant to cooking but can be enzymatically hydrolysed by LAB during fermentation, thus increasing product digestibility (Holzapfel, 1997). Fermented maize products in Ghana showed that *Lb. plantarum* strains could ferment raffinose. Specifically, the cell free extracts of *Leuc. mesenteroides* ssp. *mesenteriodes* DSM 20343 and *Lb. plantarum* 43 reduced the raffinose content by 46-50% and the stachyose content by 76-85% (Holzapfel, 1997). This oligosaccharide hydrolysis improved the nutritional value of the fermented cereal beverage by increasing the sugar content and reducing digestive discomfort.

Uji, a traditional non-alcoholic beverage in East Africa consumed by adults and children, is prepared from the combined fermentation of hammer-milled maize, sorghum, millet and cassava (tuberous root) (Onyango et al., 2004). Traditionally, *uji* processing leads to an improved flavour, however the nutritional value remains poor. Germination and fermentation during processing can cause carbohydrate, enzyme hydrolysis and storage protein mobility thus improving *uji* digestibility, and nutritional attributes (Khetarpaul and Chauhan, 1989).

Yousif et al. (2000) showed that natural fermentation of maize increased total soluble solids and protein content, in particular in the albumin plus globulin fraction, indicating that natural fermentation of maize results in improvement in the nutritional value of the grain. Additionally, maize protein digestibility is elevated through oligosaccharide hydrolysis (Yousif and El Tinay, 2000).

A traditional Nigerian *ogi* study concluded that the high dietary bulk properties of traditional starch-based weaning foods in developing countries are a major constraint when it comes to providing young children with enough food, and can be considered one of the diet-related causes of the high prevalence of malnutrition (Lorri and Svanberg, 1993). Thus, providing a fermented cereal beverage can alleviate this problem through increased fermentate nutrition levels. The energy density of the lactic acid-fermented gruel is about 1.2 kcal/g as compared with 0.4 kcal/g in non-fermented gruel prepared at the same consistency representing a three-fold increase in nutrient density to improve the nutritional status of malnourished infants and children solely through the LAB cell factory delivery of functional biomolecules.

LAB aroma and flavour compound production

Flavour is one of the most important characteristics in the sensory profile and acceptability of cereal foods and beverages. LAB addition to cereal based beverage products can strongly influence their flavour profiles. The changes in aroma and flavour vary considerably depending on the type of starter culture used, the cereal substrate being fermented and other beverage processing steps. In general, LAB contribute to cereal product flavour by producing organic acids through sugar metabolism thus, lowering the pH resulting in an increase in sourness and decreased sweetness (McFeeters, 2004). The decreased pH can also effect endogenous cereal

enzymes involved in flavour compound generation or their precursors (McFeeters, 2004). In addition, LAB produce amino acids and other functional flavour-associated metabolites. Traditional food/beverages made from cereal grains can be bland, lacking in flavour, aroma and body (Charalampopoulos et al., 2002b). LAB cereal fermentations lead to the production of several compounds which contribute to a complex blend of flavours in these products, which are detailed in Table 4.

EPS related to texture development, organoleptic changes and prebiotic nature of cereal fermented beverages

As an extension to the flavour properties of LAB fermented cereals broths, the texture and other organoleptic features, contributed *in situ*, are central to the acceptability of the product. Fermentation also increases the thickening properties of, for example maize *ogi*, likely due to the presence of the LAB derived acids (Banigo and Muller, 1972). Management of LAB peptidase activities also confers control over the texture of various fermented foods and beverages.

In one example, the selective exclusion of some natural occurring LAB and yeasts, and optimisation of the starter culture to ferment the Turkish beverage, *bouza*, showed an improvement in the sensory qualities of this highly viscous traditional cereal beverage (Zorba et al., 2003). Hereby, a starter culture consisting of *S. cerevisiae*, *Leuc. mesenteroides* subsp. *mesenteroides* and *Lc. confusus* was found to positively influence the organoleptic and rheological properties of the end product (Zorba et al., 2003).

Leuc. mesenteroides was of particular interest in another study, which was focused on the investigation of the fermentation microflora of an industrial sample of *kvass*, a non-alcoholic primarily Russian cereal beverage. More than a dozen dextransucrases from *Leuconostoc* spp.

and related genera were characterised on a genetic and biochemical level (van Hijum et al., 2006). These enzymes generally catalyse the synthesis of di- and oligosaccharides in addition to polymer formation with prebiotic potential, including leucrose, panose, isomaltose, and the corresponding higher oligosaccharides (Dols et al., 1997; Rycroft et al., 2001; Seo et al., 2007; Van Loo et al., 1999). Dlusskaya et al. (2008) found that one of the *kvass* isolates, *Leuc. mesenteroides* FUA 3086, harboured a putative dextransucrase gene and formed dextran- and isomalto-oligosaccharides from sucrose and maltose. Moreover, the formation of isomaltotriose by the same strain was observed in model *kvass* fermentation (Dlusskaya et al., 2008). Isomaltotriose and the related higher oligosaccharides are only partially digested in the small intestine, therefore their food applications include use as alternative, low calorie sweeteners and as prebiotics (Van Loo et al., 1999), especially for children, diabetics and weight-conscious consumers. These, and other prebiotics, actively stimulate friendly bifidobacteria in the human intestine, however, in the case of *kvass*, the levels of isomaltotriose are below the reported bifidogenic threshold (Dlusskaya et al., 2008).

Research into the prebiotic and probiotic potential of more traditional cereal fermentates include the African non-alcoholic beverage, *mahewu*. McMaster et al. (2005) tested the suitability of pasteurised commercial *mahewu* as a microencapsulation delivery system for the possible probiotic *Bifidobacterium lactis*. Generic *mahewu* was produced industrially, using *Lb. bulgaricus* var. *delbruecki* or *Lb. brevis* (Edwards, 2003; Schweigart and Fellilngham, 1963) and remained stable for up to 21 days. The researchers also noted significant taste differences for the free and immobilised cells in a 14-day sample and thus recommended the use of fruit-

flavoured *mahewu* for the supply of microencapsulated *Bifidobacterium lactis* as a more suitable form.

Coda et al. (2011) produced and characterised the physical, chemical, functional, and sensory properties of lactic acid fermented non-alcoholic beverage produced from *emmer*. They reported naturally high levels of dietary fibre in the fermented beverage and suggested that viability of probiotic *L. rhamnosus* in the beverage showed its potential as a tool for probiotic metabolite delivery (Coda et al., 2011).

Another spontaneously fermented non-alcoholic beverage traditionally produced in Nigeria from various combinations of millet, wheat and malted rice is called *Kunun-zaki*. Agarry et al. (2010) used controlled fermentation with the LAB dominant in the *kunun-zaki* natural environment; *Lactobacillus* and *Lactococcus* species to positively influence the sensory and nutritional qualities of the beverage. The produced *kunun-zaki* scored higher in all sensory attributes; appearance, aroma, taste, overall acceptability after LAB fermentation than the control, and additionally was higher in mineral content (iron, calcium, magnesium and potassium) (Olasupo et al., 1997), illustrating LAB as a vehicle for the delivery functional biomolecules.

Non-digestible cereal-matrices render themselves suitable as substrates for LAB growth and as prebiotics (Charalampopoulos et al., 2002a, 2003). Furthermore, there is evidence that gastric tolerance of probiotic LAB can be significantly improved by the addition of cereal extracts (Michida et al., 2006). This idea of combining the beneficial properties of cereal derivatives with probiotic starter cultures has brought LAB oat fermentations to the fore of nutritional food research. Oat has a naturally well-balanced nutritional profile (Lockhart, 1986; Webster et al., 2002) and has attracted global attention due to its soluble β-glucan cholesterol lowering effect

(Food and Drug Administration, 1997; Joint Health Claims Initiative Generic Claims, 2004; Salovaara, 2006). Angelov et al. (2006) has developed a functional beverage based on the fermentation of oat derived β-glucan using the probiotic starter *Lb. plantarum* B28. Optimised beverage producing conditions ensured fast fermentation, limited microbial contamination risk (21 day shelf life), high viable cell concentration ensuring a probiotic effect and a functional level of β-glucan (Angelov et al., 2006). In a similar approach, Gupta et al. (2010) used a Box–Behnken optimisation design using probiotic *Lb. plantarum* ATCC 8014 for the development of a fermented drink with almost identical findings to Angelov et al. (2006) and confirmed that β-glucan levels remained unchanged during the fermentation and also during the entire storage period (Angelov et al., 2006; Gupta et al., 2010). These fermented beverages make use of the stability of LAB and their metabolites to add functional properties to the oat drink.

Indeed, commercially available cereal-based functional beverages currently exist in addition to the traditional ones discussed. Proviva® was the first non-dairy probiotic food launched in Sweden by Skåne Dairy in 1994 (Molin, 2001; ProViva AB, 2011). The active component comprises of *Lb. plantarum* 299v fermented oatmeal gruel with malted barley added to enhance liquefaction and the final oat beverage is then mixed with fruit. A second company, B.E. Grainfield have a fermented cereal product called Wholegrain Liquid® which is made from whole organic malt (oats, maize, rice, alfalfa seed, pearl barley, linseed, mung beans, rye grain, wheat, and millet), buckwheat, and filtered water. The liquid is fermented with yeasts and probiotic *Lb.* bacteria (A.G.M. Foods Pty, 2011).

From this research, we can conclude that a presence of high viable cell counts of probiotic LAB provides new opportunities for the development of novel functional cereal-based fermented

beverages, in addition to those traditionally consumed in developing countries. To date, the probiotic food and beverages concept has been emphasised in dairy fermentations, however, the lactose and high cholesterol contents of these products represent two major drawbacks. Additionally, the ability of functional LAB to ferment cereals and synthesise oligosaccharides presents a major opportunity for the development of cereal-based functional beverages to compete with and replace the dairy versions, whilst offering a solution to the expensive inherently difficult chemical production of the these compounds.

LAB as cell factory for nutraceutical compounds

Besides their lactic acid forming capacity, LAB also have the ability to contribute to the production of several important nutraceuticals through fermentation. The term nutraceutical is defined as "any substance that may be considered a food or part of a food and provides medical or health benefits, including the prevention and treatment of disease" (Pszczola, 1992). Cereal fermentation is a well-recognised aid rendering cereal products microbiologically stable and more palatable. Moreover, cereal fermentation represents an important tool for increasing the extractability of bioactive compounds (antioxidants, growth-factors) (Bengmark and Gil, 2006) from various cereal grains or releasing nutraceuticals which are part of the LAB/yeast metabolism. However, the type of raw material (cereal, pseudocereals, and legumes) used is of key importance in the process to ensure optimal release of bioactive compounds for human nutrition.

Among the bioactive compounds, phenolic acid, primarily located on the out layers (aleurone, pericarp) of cereals (Naczk and Shahidi, 2006), can act as antioxidants through a number of different mechanisms. The so-called chain breaking mechanisms, which include hydrogen

donation and radical acceptor (i.e., radical scavenging activity) (Scott, 1985), are the most likely means by which phenolic acids act as antioxidants.

LAB cereal fermentation represents an efficient means to increase the level of phenolic compounds if appropriate fermentation conditions are applied (Đorđević et al., 2010). Additionally, Katina et al. (2007) reported an increase of up to 90% of free phenolic acid content during liquid fermentation of rye bran using an LAB and yeast starter culture.

Further support that fermentation increases antioxidant content of cereal was reported by Đorđević et al., (2010). Liquid LAB fermentation of buckwheat, wheat germ, barley and rye with *L. rhamnosus* increased the total phenolic content and improved the capacity for inhibition of lipid peroxidation in cereals when compared to both the unfermented and yeast (*S. cerevisiae*) fermented counterparts (Đorđević et al., 2010).

Even though most LAB are auxotrophic for several vitamins, it is known that certain *Lactobacillus* strains have the capability to enrich fermented cereal products synthesising water-soluble vitamins such as vitamin C and those included in the B-group (folates, riboflavin and vitamin B12) amongst others. Murdock and Fields, (1984) describe the use of LAB to improve the folate content of fermented cornmeal. They report an increase in the folate level to almost threefold after 4 days of fermentation at 30 °C. Another example of C and B vitamin bioenrichment in fermented foods was reported for *agidi* (stiff gel made from fermented maize dough) (Nout, 1980), *ajon* (alcoholic beverage produced from finger millet) (Mwesigye and Okurut, 1995) and *pito* (fermented alcoholic beverage which is traditionally brewed from sorghum or maize malts) (Orji et al., 2003). Starter cultures consisting of *Lb. plantarum* strains

were shown to produce lysine *in situ* during *ogi* production (Adebawo et al., 2000). This represents another mechanism of LAB-mediated nutritional amelioration of cereal beverages.

Recently, Capozzi et al. (2011) described the biotechnological production of vitamin B2-enriched bread and pasta using a pre-fermentation step started by two riboflavin-overproducing strains of *Lb. plantarum*. The applied approaches resulted in an approximate two to threefold increase in the vitamin B2 content in pasta and bread, respectively.

In conclusion, more research should be done on cereal beverage fermentations to expose LAB bioenrichment of the final products. From research on cereal dough fermentation, it is known that certain LAB strains overproduce vitamins leading to a bioenriched end product. It follows that the same is likely in more liquid cereal systems (beverages, beers, thin porridges) and evidence has been presented from many sources to support this. However, further research is necessary to confirm vitamin production levels, to optimise cereal-based substrates and to find the best producer strains.

LAB anti-allergenic biomolecules

The onset of food allergies continues to rise significantly in world populations, particularly in European infants, leading to reactions ranging from mild irritation to severe anaphylaxis (El-Ghaish et al., 2011). Certain LAB strains present in fermented foods are capable of modulating immune responses to otherwise allergenic compounds (Cross et al., 2001). Clinical evidence for the role of *Lactobacillus* species in fighting allergy responses include; reduced serum levels of inflammatory immunoglobulin-E (IgE), interleukins 4 (IL4) and 5 (IL5), and increased levels of interferon-γ (IFN-γ) upon consumption of fermented products. This results in alleviation of atopic dermatitis, food allergies and intestinal inflammation (Majamaa and Isolauri, 1997). The

mode of action of anti-allergenic LAB is not certain however, plausible approaches include: (i) promotion of deviation from a pro-allergy phenotype; (ii) limit the establishment of an allergic phenotype; (iii) enzymatically hydrolyse food-borne allergens, by altering allergen presentation or cleaving allergenic epitopes, to produce hypoallergenic compounds; (iv) or reduce systemic uptake of allergens through stabilisation of the gut mucosa (Cross et al., 2001; El-Ghaish et al., 2011). Additionally, the LAB Gram-positive cell walls are composed of immunologically active peptidoglycans and lipotechoic acids which aid suppression of a negative immune response (Cross et al., 2001). In cereal technology, particularly GF bread making, gluten detoxification by LAB proteolysis has been implemented (Di Cagno et al., 2008). However, due to the sensitivity of intolerant patients at very low levels of gluten, this method has not been accepted by Coeliac societies. Nonetheless, certainly for people with mild intolerances, LAB gluten detoxification is a viable option deserving further research (Leszczyńska et al., 2009). It is known that LAB can also combat many other human immunological pathologies (Matsuzaki and Chin, 2000). Hence, prevention or restriction of adverse responses to allergenic epitopes, in some segments of the population, could be implemented through controlled LAB strain-specific fermentation of cereal-based beverages. Thus, in addition to the beneficial organic acids, vitamins, prebiotic compounds, anti-microbials, amino acids, and flavour volatiles etc. produced by LAB, their detoxifying and anti-allergenic effects also represent an added dimension to the health status of the final cereal beverage.

Conclusion and future trends

As a consequence of the major economic, social and technological changes, the lifestyle of many consumers is now typified by abundant calorie-rich diet, lower intake of fibre and low physical

activity. This has favoured the onset and spread of several nutrition-related non-communicable diseases (NCDs) such as cardiovascular disease (CVD) and digestive diseases. As a result, nutritional research has shifted from alleviating nutrient deficiencies to nutrition-related NCD prevention, in Western countries. Conversely, in undeveloped countries, nutrition deficiency problems still need to be addressed particularly amongst the young, older and infirm populations. There is a need to naturally produce high nutrition products acceptable to both markets. This is achievable as there is a trend amongst more affluent consumer countries to reject chemical additives, and similarly poorer consumer countries are unable to supply this technology, support mass production or afford the cost. In the last two decades, numerous developments have been realised which aim to improve the functionality of foods through a number of methods. These include; appropriate selection of raw materials, specific physicochemical processing to enhance bioavailability, or through fortification.

However, in many cases food functionality can also be enhanced via biological conversion using LAB. In this review, we have described the mechanisms by which LAB can fulfil the novel role of efficient cell factory for the production of functional biomolecules and food ingredients to enhance the quality of cereal based beverages. In fact, LAB have been demonstrated to be ideal nutraceutical producers offering the opportunity to develop naturally fermented health-orientated products for all markets. LAB fermentation was put forward as a possible approach to render cereal-based foods and beverages safe, in a chemical-free, consumer-friendly manner, from an antinutrient and toxigenic perspective. Additionally, the antiallergenic properties of LAB were discussed as a novel and natural way to economically avoid a number of food allergen-mediated reactions.

Huge market opportunities and potential exist for food manufacturers, retailers and suppliers who can provide the ideal functional beverage adhering to strict consumer needs. This product must address the markets which we have discussed earlier; high-nutrition market (developing world), lifestyle choices (vegetarian, vegan, low-fat, low-salt, low-calorie), NCDs (CVD, high BP, diabetes), green label (Western countries, EU and US directives), etc. The beverage must be available locally at an affordable price and be versatile enough to consume regularly as a breakfast beverage, side-dish, snack or desert as required. Additionally, the product must taste good and be appealing to all age categories.

To fulfil these recommendations, a suitable starter culture must be developed. Such LAB starter cultures would be suitable for the biopreservation of cereal beverages and also have high antifungal, anti-mycotoxigenic, mycotoxin-binding, allergen-detoxifying, proteolytic strains with an ability to ferment cereals, whilst synthesising oligosaccharides, thus presenting a major opportunity for the development of cereal-based functional beverages to compete with and replace the dairy versions. The examples presented in this review demonstrate that LAB are suitable cell factories for the delivery of functional biomolecules and ingredients in the production of fermented cereal beverages.

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Table 1 Common indigenous cereal-based fermented beverages

Beverage	Cereals substrates used	Starter culture(s)*	Countries/ Regions	Reference
Bagni	Millet beverage	Unknown	Caucasus	(Beuchat, 2008)
Bogobe (*Sadza/ Ugali*)	Sorghum porridge beverage	Unknown	Botswana	(Boling and Eisner, 1982)
Bouza	Wheat thick, acidic, yellow alcohol drink	Unknown	Egypt	(Morcos et al., 1973)
Boza	Wheat, millet, maize, rice, barley, oats thick, sweet beverage	***Lactobacillus***, *Saccharomyces cerevisiae*, ***Leuconostoc***, *Pichia*, *Torulaspora*	Turkey, Albania, Romania, Bulgaria	(Hancioğlu and Karapinar, 1997)
Braga	Millet beverage	Unknown	Romania	(Blandino et al., 2003)
Burukutu	Sorghum vinegar-like tasting beer	*S. cerevisiae*, *S. chavelieri*, ***L mesenteroides***, *Candida*, *Acetobacter*	Nigeria, Benin, Ghana	(Faparusi et al., 1973)
Busa	Rice or millet beverage	***Lactobacillus***, *Saccharomyces*	Syria, Egypt, Turkestan	(Blandino et al., 2003)
Busaa	Maize alcohol beverage	***L. helveticus, L. salivarus, L. casei, L. brevis, L. plantarum, L. buchneri***, *S. cerevisiae*, *Penicillium damnosus*	Nigeria, Ghana	(Nout, 1980)
Doro (*hwahwa/ mhamba/ uthwala*)	Finger millet malt colloidial, thick, alcohol drink	Bacteria and yeasts	Zimbabwe	(Madovi, 1981)
Kaffir beer	Kaffir corn beer	**LAB**, yeasts	South Africa	(Van Der Walt, 1956)
Kunun-zaki	Millet/wheat/rice/malted rice non-alcohol fermented cereal beverage	**L. plantarum, L. fermentum, Lc. lactis**	Nigerian	(Agarry et al., 2010)
Kvass	Malt, rye flour, stale rye bread and sucrose based non-alcoholic cereal-based beverage	***L. casei, Lc. mesenteroides*** **spp.** ***mesenteroides, Lc. Mesenteroides spp. dextranicum, L. lactis,*** *S. cerevisiae*	Russia and Eastern Europe	(Dlusskaya et al., 2008)
Mahewu	Sorghum thin beverage	***L. lactis, Lb. delbrueckii, Lb. bulgaricus***	Zimbabwe	(Steinkraus, 1983)
Mangisi	Millet sweet-sour beverage	Unknown	Zimbabwe	
Munkoyo	Kaffir corn, millet or maize with munkoyo root beverage	Unknown	Africa	(Simwamba and Elahi, 1986)

Mutwiwa	Maize liquid porridge	**LAB**, bacteria, mould	Zimbabwe	(Gadaga et al., 1999)
Nasha	Sorghum liquid porridge snack	*Streptococcus,* ***Lactobacillus****, Candida, S. cerevisiae*	Afghanistan, Iran, Sudan	(Graham et al., 1986)
Otika	Sorghum alcoholic beverage	Unknown	Nigeria	
Pito	Sorghum (or maize) sour heavy opaque beer	*Geotrichum canidum,* ***Lactobacillus****, Candida*	Nigeria, Ghana	(Ekundayo, 1969)
Seketech	Maize alcohol drink	*S. cerevisiae, S.chevalieri, S. elegans,* ***L. plantarum****,* ***L. lactis****, B. subtilis, A. niger, A. flavus, Mucor rouxii*	Nigeria	(Blandino et al., 2003)
Takju	Rice and wheat turbid alcohol drink	**LAB**, *S. cerevisiae*	Korea	(Han et al., 1998)
Talla	Sorghum alcohol drink	Unknown	Ethiopia	(Odunfa and Oyewole, 1998)
Tchoukoutou	Opaque sorghum beer	Yeast, **LAB**	Africa	(Kayodé et al., 2007)
Tesgüino	Maize alcohol drink	**Bacteria**, yeasts, moulds	Mexico	
Ting	Sorghum porridge	***L. plantarum, L. casei, L. harbinensis, L. fermentum, L. coryniformis, L. parabuchneri, L. coryniformis, L. reuteri***	Botswana	(Sekwati-Monang and Gänzle, 2011)
Tobwa	Maize beverage	**LAB**	Zimbabwe	(Gadaga et al., 1999)
Uji	Maize, sorghum, finger-millet (cassava) porridge, weaning food, thirst-quenching drink, side-dish	***Lc. mesenteriodes***, ***L. platarum***	Kenya, Uganda and Tanganyika	(Steinkraus, 1995)

* LAB are involved in all fermentations and are in bold font, adapted from (Blandino et al., 2003)

Table 2 Overview of medical conditions for which a gluten/wheat/lactose/dairy free diet is beneficial

Condition	Prevalence (%)	Type of disease/ disorder	References
Coeliac Disease	1-2 % of population	Autoimmune	(Cascella et al., 2011; Fasano, 2010; Hischenhuber et al., 2006)
Wheat allergy	2.5-20 % of allergy clinical population	Autoimmune	(Hischenhuber et al., 2006; Moneret-Vautrin, 2003; Niggemann, 2001; Scott H, 2000)
Gluten sensitive	6-7 % of the population	Autoimmune	(Fasano, 2010)
Lactose Intolerance*	15-100 % population (ethnicity dependant)	Gastrointestinal disorder	(Swagerty et al., 2002; Vesa et al., 2000)
Cows' Milk Protein Allergy or Intolerance	2-3% of infancy population	Immune mediated/ non-immune mediated	(Arne, 2002)
Irritable Bowel Syndrome	2.1-12.1 % of the population	Chronic gastrointestinal disorder	(Mearin, 2001)
Autism	0.2-0.6 %	Neurological/ developmental	(Arvidsson et al., 1997; Baird et al., 2000; Chakrabarti and Fombonne, 2001; Kadesjö et al., 1999; Yeargin-Allsopp et al., 2003)
Dermatitis Herpetiformis	0.01-0.04 %	Bulbous skin disease	(Feldman, 2011; Smith et al., 1992)

* Northern Europeans 2-15%, American Whites 6-22%, Central Europeans 9-23%, Blacks 60-80%, Asians 95-100%

Table 3 Formation of antifungal substances by different LAB

LAB isolate	Nature of compound(s)	Activity spectrum	Reference
Lb. casei		*Aspergillus parasiticus*	(Onilude et al., 2005)
Lb. casei		*Penicillium expansum*	(Florianowicz, 2001)
Lb. casei	Possibly proteinaceous	*Penicillium* spp.	(Gourama and Bullerman, 1997)
Lb. casei var. *rhamnosus*		Broad spectrum	(King et al., 1990)
Lb. casei var. *rhamnosus*	< 1kDa	Broad spectrum	(King et al., 1990)
Lb. casei subsp. *rhamnosus*		*Penicillium* spp.,	(Suzuki et al., 1991)
Lb. casei subsp. *rhamnosus*		*Candida lusitaniae*	(Mäyrä-mäkinen, 1998)
Lb. casei subsp. *pseudoplantarum*	Possibly proteinaceous, <1kDa	*Aspergillus flavus*	(Gourama and Bullerman, 1997)
Streptococcus lactis		*Aspergillus parasiticus*	(Wiseman and Marth, 1981)
Streptococcus lactis		*Aspergillus flavus*	(Coallier-Ascah and Idziak, 1985)
Streptococcus lactis subsp. *diacetilactis*	Possibly proteinaceous	*Aspergillus fumigatus,*	(Batish et al., 1989)
Lb. reuteri	3-HPA (reuterin)	Broad spectrum	(Talarico et al., 1988)
Lb. reuteri	Reuterin		(Chung et al., 1989)
Lb. plantarum		Unspecified mould	(Hill, 1989)
Lb. plantarum,		*Aspergillus* spp.	(Suzuki et al., 1991)
Lb. plantarum		*Saccharomyces cerevisiae*	(Makanjuola et al., 1992)
Lb. plantarum	Cyclic dipeptides, hydroxy fatty acids, 3-phenyl lactic acid, other LMW-compounds	*Fusarium* spp.,*Eurotium* spp., *Penicillium* spp.,*Endomyces fibuliger, Aspergillus niger, Aspergillus*	(Lavermicocca et al., 2000; Makanjuola et al., 1992; Niku-Paavola et al., 1999; Sjögren et al., 2003)

		flavus, Aspergillus fumigatus	
Lb. plantarum	Benzoic acid, methylhydantoin, mevalonolactone, cyclo (Gly-L-Leu)	*Fusarium avenaceum*	(Niku-Paavola et al., 1999)
Lb. plantarum	Phenyllactic acid, 4-hydroxy-phenyllactic acid	Broad spectrum	(Lavermicocca et al., 2000)
Lb. plantarum	3-Phenyllactic acid, cyclo-(Phe-Pro), cyclo-(Phe-OH-Pro)	Broad spectrum	(Ström et al., 2002)
Lb. plantarum	Phenyllactic acid, peptides, Cyclo (Phe-Pro), Cyclo-(Phe-OH-Pro), reuterin, hydroxy fatty acids	Broad spectrum	(Magnusson et al., 2003)
Lb. plantarum	Phenyllactic acid, Cyclic dipeptides	*Aspergillus niger, Fusarium culmorum*	(Dal Bello et al., 2007)
Lb. plantarum	Phenyl-lactate , hydroxy-phenyl-lactate	*Aspergillus candidus, Penicillium nalgiovense*	(Coloretti et al., 2007)
L. plantarum		*Fusarium graminearum*	(Gerez et al., 2009)
Pediococcus acidilactici	Possibly proteinaceous	*Saccharomyces cerevesiae*	(Vandenbergh and Kunka, 1989)
Pediococcus pentosaceus	Phenyllactic acid, peptides, cyclo(Phe-Pro), cyclo-(Phe-OH-Pro), reuterin, hydroxy fatty acids		(Magnusson et al., 2003)
Pediococcus pentosaceus		*Fusarium proliferatum, Fusarium verticillioides*	(Dalié et al., 2010)
Lb. acidophilus		*Aspergillus fumigatus*	(Batish et al., 1997)
Lc. lactis		*Aspergillus parasiticus*	(Luchese and Harrigan, 1990)
Lc. lactis	Proteinaceous	*Aspergillus flavus, Aspergillus parasiticus, Fusarium* spp.	(Roy et al., 1996)
Lc. lactis		*Penicillium expansum*	(Florianowicz, 2001)

Lb. pentosus	Bacteriocin-like protein	*Candida albicans,*	(Okkers et al., 1999)
Lb. paracasei		*Penicillium* spp.	(Schwenninger et al., 2005)
Lb. paracasei subsp. *tolerans*		*Fusarium proliferatum, Fusarium graminearum*	(Hassan and Bullerman, 2008)
Lb. paracasei		*Fusarium proliferatum, Penicillium* spp., *Aspergillus niger*	(Tůma et al., 2007)
Lb. sanfrancisco	Caproic acid, propionic acid, butyric acid, valeric acid	*Fusarium* spp., *Penicillium* spp., *Aspergillus* spp., *Monilia* spp.	(Corsetti et al., 1998)
Lb. delbrueckii subsp. *bulgaricus*		*Penicillium expansum*	(Florianowicz, 2001)
Lb. rhamnosus	Synergistic effect between LAB and sodium acetate	*Penicillium* spp., *Aspergillus* spp., *Fusarium* spp., *Alternaria* spp.	(Stiles et al., 2002)
Lb. coryniformis	Proteinaceous ~5 kDa	Broad spectrum	(Magnusson and Schnürer, 2001)
Lb. curvatus		*Aspergillus parasiticus, Aspergillus flavus*	Hudacek et al. (2007)
Lb. fermentum		*Fusarium proliferatum, Penicillium* spp.	(Tůma et al., 2007)
Weissella paramessenteroides		*Fusarium graminearum, Rhizopus stolonifer, Sclerotium oryzae, Rhizoctonia solani, Botrytis cinerea, Sclerotinia minor*	(Sathe et al., 2007)
Lb. paracollinoides		*Fusarium graminearum, Rhizopus stolonifer, Sclerotium oryzae, Rhizoctonia solani, Botrytis cinerea, Sclerotinia minor*	(Sathe et al., 2007)
Lb. brevis		*Fusarium graminearum Aspergillus niger*	(Gerez et al., 2009)

Lb. brevis	Proteinaceous; <5 kDa, >1 kDa	*Aspergillus niger*, *Rhizopus oryzae*, *Penicillium roqueforti*, *Penicillium camemberti*	(Falguni et al., 2010)
Lb. brevis	Proteinaceous	*Fusarium* spp.	(Mauch et al., 2010)
Lb. citreum		*Aspergillus niger*, *Penicillium roqueforti*, *Endomyces fibuliger*	(Valerio et al., 2009)
Lb. rossiae		*Aspergillus niger*, *Penicillium roqueforti*, *Endomyces fibuliger*	(Valerio et al., 2009)
Weissella cibaria		*Aspergillus niger*, *Penicillium roqueforti*, *Endomyces fibuliger*	(Valerio et al., 2009)
Lb. amylovorus	Carboxylic acids, nucleosides, sodium decanoate, cyclic dipeptides	*Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium culmorum*, *Penicillium expansum*, *Penicillium roqueforti*	(Ryan et al., 2011)

Table 4 Examples of flavour compounds produced by LAB during fermentation of cereal beverages/batters

Beverage	Lactic acid bacteria	Flavour compound(s)	Description	Reference
*Boza**	*W. paramesenteroides*, *Leuc. mesenteroides* subsp. *mesenteroides*, *Lactobacillus* sp., *Lc. lactis* subsp. *lactis*, *Pediococcus pentosaceus*	Lactic acid, amino acids	Enzymatic activity (proteolysis in particular)	(Merih Kinvanc et al., 2011; Prado et al., 2008)
Bushera	*Lb. lactis* subsp. *lactis*, *Leuc. mesenteroides* subsp. *mesenteroides*, *Leuc. mesenteroides* subsp. *dextranicum* and *Lc. citreum*	Lactic acid, acetoin, diacetyl, methyl alcohols	Citrate and pyruvate metabolism, degradation of amino acids	(Gobbetti and Corsetti, 1997; Martínez-Anaya, 1996; Muyanja et al., 2003; Narvhus et al., 1998)
Cereal beverages	LAB	Acetic acid, butyric acid	Organic acid imparting sourness, sharp taste and sweet aftertaste	(Blandino et al., 2003)
*Daqu**	*Lb. acetotolerans*	Organic acids, acetaldehyde, ethyl acetate, ethanol, ethyl hexanoate, ethyl butyrate, isoamyl acetate, isovaleraldehyde.	Maotai-flavour a of combined microbiota fermentation	(Wang et al., 2008)(Zhang et al., 2009)
Emmer beer	*Lb. plantarum* 6E	organic acids, amino acids, volatiles,	Heterofermentative metabolism	(Coda et al., 2011)
*Jui**	Heterofermentative *lactobacilli*	Organic acids	Heterofermentative metabolism	(Kayodé et al., 2007b)
Ogi	LAB	Lactic acid, acetic acid, formic acid, butyric acid: bitterness and sourness	Heterofermentative metabolism	(Banigo and Muller, 1972
*Pito**	*Lactobacillus* sp. primarily *Lb.*	Lactic acid sour taste	Homofermentative metabolism	(Orji et al., 2003)

	plantarum			
Probiotic cereal beverages (oat, wheat, barley and malt)	*Lb. plantarum* NCIMB 8826	Mixed organic acids (oleic acid, linoleic acid, acetic acid, and 5-hydroxy-methylfurfural) α-Keto acids (converted to aroma compounds)	Heterofermentative metabolism and by α-keto acid dehydrogenase and α-keto acid decarboxylase	(Salmeron et al., 2009; Yvon and Rijnen, 2001)
Tchoukoutou beer*	Heterofermentative *lactobacilli*	Phenolic acids	Heterofermentative metabolism	(Kayodé et al., 2007b)
*Togwa**	*Lb. plantarum, Lb. brevis, Lb. fermentum, Lb. cellobiosus, P. pentosaceus, W. confusa*	Free amino acids (may be converted to aromatic acids, aldehydes, alcohols), diacetyl	Formed through proteolysis or metabolic synthesis with various flavours, Buttery flavoured diketone	(Gobbetti et al., 1994; Mugula et al., 2003b) (Blandino et al., 2003; Edema and Sanni, 2008; Mugula et al., 2003a)
Uji	*Lb. plantarum, Lb. fermenti, Lb. cellobiosus, Lb. buchneri, P. acidilactici* and *P. pentosaceus*	Esters with fruity notes, ethanol, higher alcohols and organic acids	Heterofermentative metabolism	(Masha et al., 1998)
Wheat batter (sourdough)	*Lb. reuteri* and *Lb. sanfranciscensis*	Glutamate	Deamidation of glutamine	(Vermeulen et al., 2007)

*Yeasts and/or fungi are also involved in fermentation

