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Fermented beverages with health-promoting potential: past and future perspectives

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ABSTRACT

Fermentation is an ancient form of food preservation, which also improves the nutritional content of foods. In many regions of the world, fermented beverages have become known for their health-promoting attributes. In addition to harnessing traditional beverages for commercial use, there have recently been innovative efforts to develop non-dairy probiotic fermented beverages from a variety of substrates, including soy milk, whey, cereals and vegetable and fruit juices. On the basis of recent developments, it is anticipated that fermented beverages will continue to be a significant component within the functional food market.

Introduction

Societies throughout the world independently discovered the value of fermenting food as a cheap means of preservation, improving nutritional quality and enhancing sensory characteristics. The fermentation of milk, cereals and other substrates to produce beverages with health-promoting properties is indigenous to many regions of Asia, Africa, Europe, the Middle East and South America. Evidence from pottery vessels show that fermented rice, honey and fruit beverages date as far back as 7000 B.C. in China (McGovern *et al*., 2004), and there is evidence of kombucha manufacture dating back to approximately 220 B.C. (Dufresne & Farnworth, 2000), while recent proteomic analysis has shown kefir-like milk to have been fermented some 3500 years ago in Asia (Yang *et al*., 2014). While many such beverages have for quite some time been noted for their putative health-promoting attributes, this interest is now being harnessed by modern biotechnological techniques to develop the next generation of fermented functional beverages.

The global functional beverage market is a growing sector of the food industry as modern health-conscious consumers show an increasing desire for foods that can improve well-being and reduce the risk of disease. Fermented milks, especially yoghurt-style products, are the most popular functional beverages with kefir in Western Europe and North America and ymer in Denmark being good examples. Notably, the global functional food and drink market increased 1.5 fold between 2003 and 2010, and is expected to grow a further 22.8% between 2010 and 2014 to be worth €21.7 billion (Leatherhead, 2011), with other estimates predicting the market will reach €65 billion by the year 2016 (Companiesandmarkets, 2013). Dairy-based produce account for approximately 43% of the functional beverage market, and is mainly comprised of fermented products (Özer & Kirmaci, 2010). It is also intriguing to note that a number of food companies that have been under pressure, due to the poor public perception regarding the 'healthiness' of the foods they produce, are now focusing on developing such functional products.

In this article we review the literature regarding traditional fermented beverages with reputed health benefits, and explore recent trends and developments in this field, as well as areas for future research.

Natural fermented beverages: sources and microbial composition

Naturally fermented milks

The yoghurt and fermented milks market is currently worth €46 billion, with North America, Europe and Asia accounting for 77% of the market. Many communities across the world produce naturally fermented milks with many of these products being of a yoghurt-style consistency. Fermented milk products can be made with milk (or skimmed milk) from various sources, including cow, camel, goat, sheep, yak and even coconut, milk, and can be either pasteurised or unpasteurised. They can be produced through the use of defined starter cultures, back-slopping or allowed to ferment naturally. Although fermented milk beverages are predominantly composed of lactic acid bacteria (LAB), the exact microbial content may vary depending on the source of milk, treatment of the milk (e.g. pasteurisation), use of starters, the nature of the local environmental microbes present, temperatures, hygiene, the type and treatment of containers used and the length of fermentation. Many artisanal fermented milk beverages are produced as a result of back-slopping, whereby a small portion of already-fermented milk is used to begin a new fermentation. In this way, cultures from the LAB naturally present in the raw milk are passed from household to household and between generations. While the consumption of spontaneously fermented milk is common to many different regions, the exact microbial differences between these products have not been ascertained. **Table 1** lists a number of the most popular and best-studied fermented beverages from around the world, along with information with respect to their corresponding microbial compositions. From this, the domination of milk-based beverages fermented by LAB, mainly *Leuconostoc*, lactobacilli and lactococci, is clear. Fermentation in colder climates promotes the growth of mesophilic bacteria such as *Lactococcus* and *Leuconostoc*, whereas beverages produced at higher temperatures usually have greater counts of thermophillic bacteria such as *Lactobacillus* and *Streptococcus.* The contributions of slime-producing species or acetic acid producing species, generally present at low abundance relative to *Lactobacillus* or *Lactococcus* species, vary depending on abundance. There may also be significant numbers of coliforms present, depending on the level of hygiene employed during preparation, with high levels having been noted in some African beverages (Gran, Gadaga, & Narvhus, 2003). The quantity and types of yeasts involved can vary greatly, but *Candida* and *Saccharomyces* are the species most commonly detected.

Of the many fermented milk beverages, kefir, a drink that originated with shepherds in the Caucasian mountains has been a notable success, gaining worldwide popularity, with the market now worth €78.7 million in North America alone (Lifeway, 2014). The microorganisms responsible for the fermentation are actually a symbiotic combination of bacteria and yeast, bound within a polysaccharide matrix, known as kefir 'grains'. Koumiss, sometimes known as airag, is a popular beverage of nomadic cattle breeders in Asia and some regions of Russia. This beverage is similar to kefir, but there is no solid inoculation matrix, and this milk is fermented by back-slopping or by allowing the milk to ferment naturally, and has been reported to contain fewer lactococci. Shubat is a fermented camels milk popular in Asia, also believed to have healing properties (Rahman, Nurgul, Chen Xiaohong, Feng Meiqin, 2009). In Africa, fermented milk beverages are quite popular, where the art of making fermented products is passed down through generations. Examples of such beverages include amasi from Zimbabwe, kivuguto from Rwanda, suusac from Kenya, nyarmie from Ghana and rob and garris from Sudan. Considering that most of these are derived from the spontaneous fermentation of milk by its innate microbiota, it is likely that the fermented milks, although known by different names, are actually quite similar, and can be, in combination, referred to as naturally fermented milk (NFM) (Narvhus & Gadaga, 2003). Nonetheless, accurate categorization remains difficult in the absence of more detailed microbiological and biochemical analyses. Also, in many countries yoghurts are diluted with water to form drinkable fermented milk, such as doogh, ayran, chaas and lassi, with the resulting microbial composition generally being similar to that of yoghurt. The composition and purported health benefits associated with fermented dairy beverages can also be read about in a recent review by Shiby and Mishra (Shiby & Mishra, 2013).

Non-dairy fermented beverages

Another important class of fermented beverages are those made from cereals, which are popular in tropical regions and on the continent of Africa in particular. As with many milk-based products, the natural microbial component is used to ferment grains including maize, millet, barley, oats, rye, wheat, rice or sorghum. The grains are often heated, mashed and sometimes filtered. Back-slopping is again quite common, but the microbial populations responsible for the fermentation of these beverages are not as well characterised.

Boza, consumed in Bulgaria and Turkey, is generated through the fermentation of a variety of cereals including barley, oats, rye, millet, maize, wheat or rice, with the specific composition affecting the viscosity, fermentability and content of the final beverage (Akpinar-Bayizit, Arzu, Lutfiye Yilmaz-Ersan, 2010). The cereal is boiled and filtered, a carbohydrate source is added, and the mixture can be left to ferment independently or with the use of back-slop. Boza has yet to be commercialised and studies have revealed that the microbial population varies. The function of the yeast present, which are only sometimes detected, remains unknown. Of several combinations, it has been suggested that fermentation by *S. cerevisiae*, *Leuconostoc mesenteroides* and *Lactobacillus confusus* produce the most palatable beverage (Zorba, Hancioglu, Genc, Karapinar, & Ova, 2003).

Togwa, a sweet and sour, non-alcoholic beverage, is one of the better studied African cereal beverages. Produced from the flour of maize, sorghum and finger millet and, sometimes, cassava root, the chosen substrates are boiled, cooled and fermented for approximately 12 hours to form a porridge, which is then diluted to drink (Kitabatake, Gimbi, & Oi, 2003). Mahewu is similar in that maize or sorghum meal is fermented with millet or sorghum malt, and is available commercially (Mugochi, Tapiwa, Tony Mutukumira, 2001). Bushera is generally prepared from germinated or non-germinated sorghum grains, and fermented for 1-6 days (Muyanja, Narvhus, Treimo, & Langsrud, 2003). These beverages are often used to wean children, and as a high-energy diet supplement. Koko sour water is the fermented liquid water created in the production of the fermented porridge, koko. This contains a high portion of LAB and is used by locals to treat stomach aches and as a refreshing beverage (Lei & Jakobsen, 2004).

Kvass is a fermented rye bread beverage common in Russia, which has seen much commercial success. The beverage can have a sparkling, sweet or sour, rye bread flavour. Its alcohol content, though usually low, can vary, and has been suggested as a contributor to alcoholism (Jargin, 2009). Amazake is a sweet fermented rice beverage that is the non-alcoholic precursor to sake, produced in Japan. Steamed rice is mixed with rice-*koji* (*Aspergillus*-mycelia and rice) and water, and is heated to 55-60°C for 15-18 hours. Enzymes break down the rice and form glucose content of approximately 20%. Amazake is highly nutritious and is consumed for its purported health benefits (Yamamoto, Nakashima, Yoshikawa, Wada, & Matsugo, 2011). Pozol, which is common to south-eastern Mexico, has quite a different method of production, in that maize grains are heat-treated in an acid solution, ground and shaped into dough balls. These are then wrapped in banana leaves and fermented for 2-7 days, after which they are resuspended in water and consumed as beverages. Pozol is composed of a variety of microorganisms including LAB, non-LAB, yeasts and other fungi (ben Omar & Ampe, 2000).

In addition to milk and cereal-based fermentations, there are also other forms of fermented beverages. One example is kombucha, which is a fermented sweetened tea that was originally popular in China but is now enjoyed worldwide, and is set to be worth €363 million by 2015 in North America (BevNet, 2011). It is fermented by a symbiotic mixture of bacteria (typically acetic acid bacteria, with small quantities of LAB) and yeast, which are embedded within a cellulosic matrix that floats above the fermentate, similar to the mother cultures of vinegar. Due to the high acid content (as low as pH2), the functionality of kombucha is predominantly due to its physiochemical properties (Greenwalt, Steinkraus, & Ledford, 2000). As a result of the tea content, it also contains a number of phenols and vitamins (Dufresne & Farnworth, 2000). Water kefir is similar in concept to milk kefir in that it is fermented by a symbiosis of bacteria and yeast contained within grains. However, these grains are composed of dextran, are translucent and crystal-like in appearance, and are thought to have originated in Mexico where they formed as hard granules fermented from sap on the pads of the *Opuntia* cactus. They ferment sweetened water, to which figs and lemon are traditionally added for additional flavour and nutrients. The composition of water kefir can vary, but is known to contain LAB, including *Lactobacillus*, and *Bifidobacterium* (Laureys & De Vuyst, 2014). Hardaliye is a non-alcoholic, Turkish, fermented beverage made from red grapes, black mustard seeds, cherry leaf and benzoic acid. Ingredients are pressed and fermented for 5-10 days at room temperature. Again, the microbial population has been reported to be predominantly composed of *Lactobacillus* and unknown fungal components, and this beverage is thought to have antioxidant properties (Amoutzopoulos et al., 2013).

Health Benefits

Originally devised as a means of food preservation, over time many beverages, such as kefir and koumiss, became popular due to their reputed abilities to improve gastrointestinal health (Metchnikoff, Elie, 1908; Saijirahu, 2008). However, most of the traditional fermented beverages are poorly studied, with unsubstantiated claims linking them to positive effects on human health. Ideally, any such beverage making health claims should be backed by credible scientific evidence in the form of randomised, controlled and replicated human intervention trials. This form of evidence is rare for these beverages (and particularly so for non-dairy forms), and the generation of such data is an expensive and unappealing prospect for industry, but nonetheless remains a critical area for proof-of-concept and future research. Despite this, however, there is still a perception that many of these beverages are “healthy”, particularly in societies where the beverage is steeped in local tradition, which in turn contributes to their market potential and justifies investing in related research.

For many of the fermented beverages, it is the strong association between the microbial content and improvement of gastrointestinal health that is thought to be responsible for perceived health outcomes. While it is sometimes unclear what functional characteristics traditional beverages confer beyond the basic nutrition of the raw unfermented ingredients, there is evidence that some fermented beverages provide beneficial effects through direct microbial/probiotic action and indirectly via the production of metabolites and breakdown of complex proteins. Nonetheless, natural fermented milks have been shown to have antihypertensive effects, enhance systemic immunity, lower cholesterol and to help lower blood pressure. In recent human trials, they have been shown to aid in the treatment of IBS and to help alleviate constipation (Tabbers *et al*., 2011). Additionally, they have been shown to have modulatory effects on the brain, and demonstrate anti-cancer potential (Kumar *et al*., 2012; Tillisch *et al*., 2013). Of the traditional-style beverages, kefir specifically has been shown to positively impact the gastrointestinal tract, stimulate the immune system, and have anti-inflammatory and anti-carcinogenic effects, albeit not through clinical trials (de Oliveira Leite et al., 2013). Lactic fermented milks often contain compounds not present in regular milk, such as exopolysaccharides, e.g. kefiran in kefir, and natural enrichments, including increased vitamin (e.g. B12 and K2), folate and riboflavin content (Hugenholtz, 2013). Furthermore, fermented dairy products usually possess β-galactosidase activity and a reduced lactose content compared to milk, making them potentially suitable for those suffering from lactose intolerance. Fermented produce can also be a source of bioactive peptides, released through fermentation by proteolytic cultures, and have been linked with many potential health benefits including digestive, endocrine, cardiovascular, immune and nervous system affects.

The occurrence of organic acids, which lower the pH of the beverages, may also confer health benefits. Indeed, the presence of glucuronic acid, one of the primary metabolites in kombucha, is believed to improve detoxification by binding toxin molecules and aiding excretion through the kidneys, and it is this acidic composition that is most associated with the reputed health properties of kombucha, rather than a microbial-gut interaction (Wang *et al*., 2014). Kombucha also contains increased B vitamins and folic acid in addition to a number of healthy components, such as phenols, naturally present in tea (Dufresne & Farnworth, 2000). Acid content, in conjunction with antimicrobials often produced by bacteria, could result in the beverage possessing therapeutic, antimicrobial properties.

Fermented cereals can also contain a high mineral content, and generally have a lower fat percentage than their dairy-based counterparts, but grains are generally lacking in essential amino acids. These forms of beverages can also naturally provide plant-based functional components, such as fibre, vitamins, minerals, flavonoids and phenolic compounds, which can effect oxidative stress, inflammation, hyperglycemia and carcingenesis (Wang, Chung-Yi, Sz-Jie Wu, 2013). As previously mentioned, fermented foods are particularly common in Africa, where palates are accustomed to sour foods. Providing a safe, fermented cereal beverage with reliable probiotic cultures could help reduce diarrhoea and malnutrition caused by contaminated traditional beverages used in weaning children, and help reduce fatalities and improve well-being (Motarjemi, Käferstein, Moy, & Quevedo, 1993).

Despite the need for definitive studies demonstrating direct health benefits on consumers, *in vitro* and animal studies give reason to be optimistic. In many cultures, alleged health benefits are the reason for consumption, and if there are indeed health benefits to be gained from consuming fermented beverages, it is most likely the result of a synergistic effect between substrates, delicate microbial content and microbial end products, the relationship between which should become clearer with further research.

Beyond physiochemical advantages: from microbial content to functionality

Molecular-based microbial characterisation

Despite health claims linked to the microbial composition of fermented beverages, there is a considerable lack of analyses relating to the microorganisms present and the quantities in which they exist in such beverages. In order to address this, it is necessary for the application of unbiased, standardised techniques to assess beverages from different geographical regions, and to reach a consensus on the definition of microorganisms which constitute part of any particular beverage. Since many such beverages are naturally fermented, and thus subject to environmental influences, their microbiota can differ significantly, but the application of reliable technology can help definitively identify a core population (or lack thereof), responsible for characteristic traits of the beverage in question. While some molecular-based, microbial characterisation of these beverages has taken place, most studies have relied on low-throughput approaches, employing techniques such as DGGE, which can only assess 1-2% of a population (Muyzer, de Waal, & Uitterlinden, 1993).

Moving forward, the availability of molecular technologies such as culture-independent, high-throughput, sequencing-based microbial analyses, metabolomics and bioinformatics will prove particularly useful, and will provide a more accurate picture of these populations, surmounting problems associated with relying on phenotypic-based approaches. In-depth molecular studies have the potential to be particularly useful when carrying out analyses across different beverages with a view to attributing specific desirable or non-desirable sensory and organoleptic characteristics with specific microorganisms present (Marsh, O'Sullivan, Hill, Ross, & Cotter, 2013). Such approaches will also ultimately facilitate accurate species identification, leading to novel starter design, and the development of beverages with different and complex flavour profiles. It will also be possible to more effectively monitor the change of proportions of different species throughout fermentation and storage (Cocolin, Alessandria, Dolci, Gorra, & Rantsiou, 2013). Future studies will also shed light on the nature of the symbiosis of such beverages, which is so complex that *in vitro* synthesis of kefir grains has yet to be replicated.

Currently, commercial kefir is produced by defined starters, with probiotic strains added to some products to boost reputed health claims.

Health-promoting microbes

As noted above, it is widely believed that the primary reason for the functionality of these beverages is due to the presence of specific live microorganisms. To the consumer, health claims are more important than nutritional claims (Verbeke, Scholderer, & Lähteenmäki, 2009), so there has and will be a desire to augment the health-promoting potential of these beverages through the addition of certified probiotics. The probiotic market was worth €15.7 billion in 2010, and is expected to increase to €22.6 billion by 2015 (BCCResearch, 2011). The WHO/FAO defines probiotics as "live microorganisms, which when administered in adequate amounts confer a health benefit on the host", and the probiotic sector is the largest component of the functional food market. The physiology of certain strains of lactobacilli and bifidobacteria make them well-suited to both the gastrointestinal and milk environments, and thus lactic acid bacteria and bifidobacteria are the most studied and utilised probiotic organisms. It is generally considered that a minimum of 10^9 cells per daily dose are required for probiotics to be effective (Forssten, Sindelar, & Ouwehand, 2011). Within the EU, the term "probiotic" is now considered a health claim, with strict criteria surrounding its use and resulting in many applications submitted to the European Food Safety Authority (EFSA) being rejected (Guarner *et al.*, 2011). In Europe, boosting numbers of *Lactobacillus* and *Bifidobacterium* in the gut is not deemed to be of sufficient merit to be considered a health benefit; the link must be made to a physiological (e.g. strengthening the immune system or resistance to infections) benefit to the host. Proving such health claims is expensive, and in the midst of unclear definitions and guidelines, industries are currently more likely to develop and market probiotic products in other parts of the world (Katan, 2012). In situations where probiotic strains are added during fermentation, they must not interact antagonistically with starter strains. This becomes less of an issue if strains are added after fermentation is complete, due to the low metabolic rates at refrigerated temperatures. Additionally, microencapsulation technology may aid in the delivery of probiotic strains by protecting them in non-native environments. In one instance, microencapsulation of *Bifidobacterium* successfully increased viable numbers in mahewu, without significantly impacting on flavour, suggesting it could be an effective probiotic delivery system (McMaster, Kokott, Reid, & Abratt, 2005).

A health-related role for the yeast in fermented beverages has yet to be elucidated. The volume of studies reporting significant numbers of yeast in traditional fermented beverages indicates their importance in these fermentations. Yeasts in dairy produce generate desirable aromatic compounds, proteolytic and lipolytic activities and can aid bacterial growth by producing amino acids, vitamins and other metabolites, and contribute to the final composition of the product by producing ethanol and carbon dioxide (Viljoen, 2001). In particular, studies have demonstrated that yeast can exert a positive effect on the abundance of *Lactobacillus* in fermented environments (Gadaga, Mutukumira, & Narvhus, 2001), and this might be a key function in such symbioses, as well as preventing the proliferation of undesirable species. While yeast only comprise <0.1% of the gut microbiota, they are 10 times larger than prokaryotes and can thus impede colonisation of pathogenic bacteria (Czerucka, Piche, & Rampal, 2007). Success has been made in incorporating them in commercial fermented milk products, but excessive gas production during storage can be an issue. Some species of *Saccharomyces* and *Candida* yeasts are common to both fermented beverages and the gut microbiota, such as species, and could be investigated with a view to their contribution to fermentations and optimising health-promoting potential. However, to date, *Saccharomyces boulardii* is the only recognised probiotic yeast.

Rational design of starter cultures

The selection of appropriate starter strains will be key in efforts to accurately reproduce the desirable characteristics of traditional health-promoting beverages for mass production (**Figure 1**). To faithfully reproduce these beverages and traits, microbes should be sourced from the traditional fermented beverages, given that these microbes have adapted over thousands of years to their respective environments, and are more likely to function at the appropriate pH, salt concentration, temperature etc. For instance, amylolytic digestion of starch could be considered desirable for fermented cereal production, and isolates from boza and pozol have been shown to be capable of this metabolic trait. Such populations also have a history of safe human consumption. Rational strain selection to produce the correct balance of flavour, aroma, texture, acidification, bitterness, speed of fermentation, and the optimum quantity of organic acid, vitamins and minerals is essential, as beverages that are too sour or bitter, or contain too much ethanol, will not meet consumers' approval. Over recent years, genetic tools have become available to engineer and select superior starter strains, but legislation currently hinders their industrial use (Hansen, 2002). The inclusion of strains producing antimicrobials, such as bacteriocins, could serve as natural preservatives and help produce a more natural product, while sequential fermentation with yeast, followed by bacteria, could produce a beverage with the desired physiochemical effects, but without biostabilisation issues created by excessive gas production (Kwak, Park, & Kim, 1996).

As stated above, the natural fermentation of beverages involves many different strains of bacteria, and sometimes, yeast. There is an understandable tendency to keep starter formulations simple but, as traditional beverages show, there are often multiple strains involved, including different species or even microorganisms. From a health perspective, multistrain or multispecies probiotic beverages may provide greater beneficial effects than monostrain cultures. Unfortunately, however, there is a lack of studies assessing the effects of combining several natural strains on the physiochemical and sensory characteristics of milk or other functional beverages. Without such information, it is difficult to accurately reproduce the characteristics of the organic beverage with one produced by a defined combination of starters, to match the flavour and properties of the original beverage. This is crucial when marketing beverages to consumers already familiar with the artisanally produced variant of the product, and if wishing to retain any health-promoting characteristics attributed to the original product.

In spite of the wide range of options available when designing novel health-promoting fermented beverages, there will always be an attraction for healthy foods derived from natural processes. Applying the solid inoculation matrices of traditional fermented beverages to new substrates provides a means of generating new beverages while retaining natural microbial populations. For example, kefir grains have been employed to produce whey and cocoa pulp beverages containing potentially health-promoting strains (Londero, Hamet, De Antoni, Garrote, & Abraham, 2012; Puerari, Magalhães, & Schwan, 2012). Similarly, the cellulosic pellicle of kombucha has been successfully used to ferment milk and other substrates (Malbaša *et al*., 2009).

Biotechnology and beverage development

Expanding technological capabilities, especially ingredient exploration and development, has led to increased functional product innovation. The number of new products with functional claims has been growing by approximately 28% per year (Leatherhead, 2011). Consumers' willingness to pay a premium price for fortified products is also a key driver for innovation. While most current functional beverages are aimed at the high-income consumer, there is an argument to be made that those who would benefit most from fermented beverages are from underdeveloped nations, where such beverages could provide a cost-effective means of delivering much-needed nutrition (Van Wyk, Britz, & Myburgh, 2002).

Substrate exploration

The US, Europe and Japan markets account for over 90% of total functional foods, with the majority being functional dairy products. However, non-dairy probiotic delivery has been attracting more attention in recent years, partly due to the success of bio-functional foods and the desire to expand and provide an alternative probiotic choice to conventional dairy-based beverages. Indeed, this market is projected to have an annual growth rate of 15% between 2013 and 2018 (Marketsandmarkets, 2013). Non-dairy probiotic beverages are particularly attractive due to their lack of dairy allergens, low cholesterol content and vegan-friendly status (Prado, Parada, Pandey, & Soccol, 2008). Furthermore, different substrates can provide different combinations of antioxidants, dietary fibre, minerals and vitamins.

To this end, cereal-based beverages could be marketed in response to consumers' awareness of the benefits of high fibre diets. They contain natural prebiotic traits due to the presence of indigestible fibres and the presence of diacetyl acetic acid aromatic compounds make them palatable, and furthermore, could be cheaper to produce. Oats, a major source of beta-glucan which can reduce LDL-cholesterol, are known to function as a prebiotic by boosting bifidobacteria numbers in the gut (Mårtensson *et al*., 2005), and have been investigated with a view to producing synbiotic beverages. Indeed, a fermented oat drink with two *Bifidobacterium* *longum* strains was shown to normalise bowel movements in elderly patients (Pitkala *et al*., 2007). Malt and barley have also been used as beverage substrates (Rathore, Salmerón, & Pandiella, 2012), while Emmer, an ancient European cereal has also shown potential as a functional cereal beverage (Coda, Rizzello, Trani, & Gobbetti, 2011).

There has also been a positive trend towards the consumption soy products, as evident in worldwide soy food sales, which increased from €218 million to almost €2.9 billion between the years 1992 and 2008, and continues to increase (Granato, Branco, Nazzaro, Cruz, & Faria, 2010). Soy-based beverages contain low cholesterol and low saturated fats, are lactose-free, are rich in isoflavones and antioxidants, and have been shown to exert beneficial effects on the host. Soy milks are capable of fermentation by probiotic strains and, when fermented by *Bifidobacterium* and *Lactobacillus*, have been shown to have a positive impact on the ecosystem of the intestinal tract (Cheng *et al*., 2005). Positive consumer attitudes towards soy have encouraged industry to develop probiotic derivatives with several varieties already available commercially (Haelan951® and Jiva™).

The utilisation of waste products to generate functional beverages has seen increased interest, with whey being the most prominent example. Whey is a by-product of the cheese industry, which retains 55% of milk nutrients and contains only 0.36% fat, and has the potential for further use in the human diet. In an effort to add value to whey, numerous studies have investigated its fermentation by lactic acid bacteria (*Streptococcus* and *Lactobacillus*) to produce a lactic probiotic beverage, and probiotic bacteria have already demonstrated good survival in whey (Drgalic, Tratnik, & Bozanic, 2005). Prebiotics have also been successfully incorporated, including oligofructose and inulin, and hydrocolloid thickening agents added to improve viscosity and mouthfeel (Gallardo-Escamilla, Kelly, & Delahunty, 2007).

One of the most exciting developments is the development of fruit juices, which have been shown to have considerable market value and consumer acceptance (Sun-Waterhouse, 2011). Already considered a healthy food product, fruit juices are often fortified with vitamins and minerals, in addition to having a high nutrient and antioxidant content, and represent a new method of nutrient and probiotic delivery. As an increasing number of studies are demonstrating, sugars naturally present in juices can facilitate the growth of cultures with appealing taste profiles. This is true of tomato, pomegranate, pineapple, orange and cashew-apple juice. These microbes can impact on physiochemical aspects, such as increasing the concentrations of flavanones and carotenoids in orange juice, and have shown good survival rates during storage of the beverages. While the final content of such beverages are quite acidic and best suited to fermentation by probiotic *Lactobacillus* species (*L. casei*, *L. acidophilus*, *L. plantarum*, *L. parachesei* and *L. delbrueckii*), the use of microencapsulation technology could aid in the delivery of other viable probiotic microorganisms (Champagne & Fustier, 2007). The enrichment of juices with brewer's yeast autolysate before fermentation positively impacts on the nutritional content of the final beverage, raising the feasibility of co-fermentation by the right combination of bacteria and yeast (Priya, Pushpa, 2013). Examples of commercially available probiotic-containing fruit juices include Biola® and Bioprofit®. Similar microorganisms have also been shown to successfully ferment various vegetable juices including cabbage, beet, pumpkin, courgette and carrot juices supplemented with prebiotics (Martins *et al.*, 2013).

The major challenge with any substrate/culture combination is to overcome the sensory hurdles of sour, acidic fermentates, and produce a palatable beverage that would realistically be consumed regularly to avail of functional benefits. There exists the option of combining fruit or vegetable juices with fermented milks as natural flavourings to overcome undesirable flavours in otherwise promising beverages/products. To this end, the inclusion of sensory panel evaluations provides invaluable information regarding consumer acceptance, especially for non-dairy products which are intrinsically more difficult to sell than their dairy counterparts. The use of direct liquid inoculation systems to include probiotics while avoiding fermentation side-effects has its own problems in ensuring cell viability and stability during storage.

Fermentation parameters

In addition to the importance and ratio of starter selection, as already described, the fermentation of potentially health-promoting beverages needs to be carefully controlled to achieve stability, sensory and safety standards. Changes in the concentration of sugars and other compounds need to be carefully monitored both during and after fermentation, and is particularly important with respect to the production of ethanol and carbon dioxide. Sensitive techniques, including high performance liquid chromatography (HPLC) and gas chromatography

(GC), are now routine for such analyses. Antioxidant levels may also be measured by ferric reducing ability of plasma (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. pH is obviously crucial to the success of a fermentation and can be lowered to a specified level prior to fermentation to encourage enzymatic activity and prevent contamination. The concentration of oxygen in the brewing environment can also be important depending on the homo- or hetero-fermentative nature of the cultures. Metabolic engineering of fermenting microorganisms may eventually be accepted to boost concentrations of desirable compounds in the final products. Other factors to be considered include the choice of substrates, particularly the types and concentrations of carbohydrates, and the treatment of raw ingredients such as cereals, by homogenisation, for example, can allow for more effective metabolism and release of bioactive peptides. The time and intensity of heat during pasteurisation need to be considered. The addition of certain compounds, such as ascorbic acid and NaFeEDTA, can encourage the release by fermentation of bioactives, such as, zinc and iron, in the final beverage. Conversely, the concentration of phytic acid, an inhibitor of mineral absorption, particularly with regards to cereals, could lower the mineral impact of the final beverage, and the inclusion of phytases might be necessary to ensure or augment health claims. The concentration of bulk starch and other factors will impact the consistency of the final drink, providing either a thin and free-flowing product or a thicker beverage with a smoothie-like consistency. Natural antimicrobials, such as bacteriocins, may be included to act as preservatives and prevent the growth of spoilage organisms. As microbes, particularly yeast, continue to grow following storage, packaging must be able to withstand the pressure generated as a consequence of gas production, with either plastic or glass containers. Additionally, viability during storage needs consideration, particularly for cereal-type beverages that would traditionally be stored at room temperature.

Clearly, there a number of parameters and variations that need to be measured, controlled and experimented with to determine the optimum conditions for fermentation, and proven to be consistent following up-scaling. Automated processes such as controlled nutrient availability and stirring can influence efficiency of the fermentation. Ongoing research into community analysis and fermentation biochemistry will inform future decisions regarding the control of processes for these types of fermentations.

Beverage enhancement

There are now a variety of enhancements that can be made to both traditional and novel beverages to boost health claims. Prebiotics, including fructooligosaccharides, inulin and galactooligosaccharides, are often added commercially to fermented milks to promote the growth of favourable bacteria (Huebner, Wehling, & Hutkins, 2007), while investigations of other prebiotics such as oligofructose and polydextrose have also yielded positive results (Oliveira *et al*., 2009). In addition to preventing and treating intestinal-associated diseases, the incorporation of bio-active nutraceuticals such as ω-3 fatty acids, isoflavones and phytosterols in fermented milks also have potential applications (Awaisheh, Haddadin, & Robinson, 2005). Isoflavones are powerful antioxidants, comparable to vitamin E, while plant-derived phytosterols are cholesterol-lowering agents. Addition of such compounds, however, can be complicated as ω-3 fatty acids are sensitive to light, air and heat, and can cause undesirable flavours in the end product, while isoflavones and phytosterols have poor solubility in water, and might be difficult to incorporate into non-fat solutions. ω-3 fatty acids can now be microencapsulated to hide fishy off-notes.

A variety of vitamins and minerals may be added, including vitamins D, E and C, calcium and magnesium, while fortification of fermented milks with iron was shown to improve the growth of preschool children (Silva, Dias, Ferreira, Franceschini, & Costa, 2008). Microorganisms can also provide functional metabolites, which has encouraged the screening of ecological niches, such as marine environments, in addition to the previously referred to gut-derived probiotics, for novel nutraceuticals, the likes of which may eventually be incorporated into functional beverages (Dewapriya & Kim, 2013). Finally, it may be necessary to combat or mask resulting undesirable flavours and aromas arising from the addition of functional ingredients, using flavour enhancers (e.g. fruit flavours or spearmint) and natural or artificial sweeteners.

Conclusions and future prospects for fermented beverages

Most of the beverages described in this review are still in the early stages of commercial development, and require further extensive sensory, physical and chemical characterisation to develop a palatable flavour profile and viable product.

In terms of traditional fermented beverages, there is still a great deal to be understood. First and foremost, there needs to be a consensus with respect to what constitutes the natural microbiota of specific beverages, a description of which are essential for fermentation, and the contribution of each microbe to the final beverage composition. Also important is the characterisation of the relationship between microorganisms, particularly between bacterial and yeast populations. The influence of containers, substrates, metabolites and enhancements on the organoleptic qualities and fermentation kinetics need to be evaluated. Fortunately, technology is advancing such that sensitive techniques can now be used in an increasingly cost-effective manner to provide greater insight.

Critically, there is increasing pressure to identify and confirm proposed health claims for the consumer. The role of traditional beverages in the future of the fermented beverage industry may be to inspire the development of new products (and assess a country's willingness to accept a product), whereby it is easier to develop simple, novel beverages and directly evaluate the functional and sensory properties in controlled fermentations with minimum variables. Indeed, this is a key hurdle in the marketing of such products, especially in light of increasing awareness amongst consumers and the emergence of strict legislation. Considering the costs of development and clinical trials, innovation in the functional food market may need to become a collaborative effort between industry partners and academia (Khan, Grigor, Winger, & Win, 2013). Nonetheless, this is an exciting time for beverage development. Advances in probiotic (including yeast species) discovery and characterisation will advance the possibilities for health claims and starter design. The milk sector has already seen great success in this regard, and as probiotics are intrinsically linked to the health claims of many beverages it is natural to assume this will extend to other varieties of beverages to hit the market, with success already seen with probiotic soy beverages, and exciting developments with juice beverages. This is particularly true as the importance of gut health to our well-being becomes increasingly apparent. As our knowledge and discovery of probiotics increases, so too will the need for alternative means of probiotic delivery. Additionally, as research into the fermentation of waste and by-products products (e.g. whey) continues, there is the potential for a significant environmental impact.

As developed society becomes more health-conscious, particularly in response to the growing obesity epidemic, the market for functional food appears to be in a long-term, sustainable trend (Bigliardi & Galati, 2013), with beverages constituting a substantial share of this market. Aside from marketing to health-conscious (and high-income) consumers, there is evidence that functional beverages could function as a therapeutic product, particularly as a means of delivering nutrition to, and improving the health of, malnourished populations. This medicinal impact may also be augmented by the growing field of nutraceuticals, addition of cholesterol-controlling factors, and in terms of probiotics, the alleviation of intestinal discomfort and aiding in the recovery from antimicrobial treatment. One aspect that cannot be underestimated in the development of beverages is the need to accurately assess the market potential for the product. The obvious hurdle is consumers' willingness to accept an unfamiliar product, and the right combination of starters and substrates, optimum nutrition and flavour development and scientifically-supported health benefits need to be carefully considered. It has been shown that taste, price and base nutritional composition are more important than functional properties (Falguera, Aliguer, & Falguera, 2012). Consumers perceive products that are intrinsically healthy such as yoghurt, fruit juices and cereal as preferable carriers of functional foods (Annunziata & Vecchio, 2011), reflected in the increase in the study of these food types, and which may allow developers to exploit natural mineral and vitamin content of foods and juices already perceived to be healthy.

In conclusion, fermentation is an ancient form of bio-preservation that is common to all regions of the world. With traditional milk-fermented products currently enjoying success in many markets, there is an increasing interest in functional beverages from a scientific, consumer and commercial perspective. There is a movement in the modern consumers' selection of foods that offer health, social and environmental benefits, which has encouraged the food industry to develop new products and market strategies. The functional beverage market is still small and fragmented in most European countries (Siro et al, 2008), but it is expected that this area will see much success in the coming years. Indeed, with the availability and improvements in technology, and consumers' increasing interest in functional foods, the outlook for fermented beverages is more promising than ever.

Table 1 A compilation of various milk, cereal and other fermented beverages popular around the world, with their corresponding microbial populations and substrates

Product	Substrates	Region	Microflora
Amasi	Milk (Cow, Various)	Africa (Zimbabwe)	*Lactococcus* (*L. lactis*), *Lactobacillus*, *Leuconostoc*, *Enterococcus*. Uncharacterised fungal component
Aryan	Milk (Cow, Various)	Turkey	LAB: *Lactobacillus bulgaricus*, *Streptococcus thermophilus*
Garris	Milk (Camel)	Africa (Sudan)	Bacteria: *Lactobacillus* (*Lb*. *parachesei*, *Lb*. *fermentum* and *Lb. plantarum*), *Lactococcus*, *Enterococcus*, *Leuconostoc*. Uncharacterised fungal component
Kefir	Milk (Cow, Various)	Eastern Europe (Caucasian region)	Bacteria: *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Acetobacter*; Yeast: *Naumovozyma*, *Kluyveromyces*, *Kazachstania*
Kivuguto	Milk (Cow)	Africa (Rwanda)	LAB: *Leuconostoc* (*Leu. mesenteroides, Leu. pseudomesenteroides*) and *L. lactis*. Uncharacterised fungal component
Koumiss/Airag	Milk (Horse)	Asia/Russia	LAB: *Lactobacillus*; Yeast: *Kluyveromyces*, *Saccharomyces* and *Kazachstania*
Kumis	Milk (Cow)	South America (Columbia)	Bacteria: *Lb. cremoris*, *L. lactis*, *Enterococcus* (*E. faecalis*, *E. faecium*); Yeast: *Galactomyces geotrichum*, *Pichia kudriavzevii*, *Clavispora lusitaniae*, *Candida tropicalis*
Nyarmie	Milk (Camel)	Africa (Ghana)	LAB: *Leu. mesenteroides*, *Lb. bulgaricus*, *Lb. helveticus*, *Lb. lactis*, *Lactococcus lactis*; Yeast: *Saccharomyces cerevisiae*
Rob	Milk (Unspecified)	Africa (Sudan)	LAB: *Lb. fermentum*, *Lb. acidophilus*, *L. lactis*, *Streptococcus salivarius*; Yeast: *Saccharomyces cerevisiae*, *Candida kefyr*
Suusac	Milk (Unspecified)	Africa (Kenya)	LAB: *Leu. mesenteroides*, *Lactobacillus* (*Lb. plantarum*, *Lb. cruvatus, Lb. salivarius, Lb. Raffinolactis*); Yeast: *Candida krusei*, *Geotrichum penicillatum*, *Rhodotorula mucilaginosa*

Shubat	Milk (Camel)	China	Bacteria: Lactobacillus (*Lb. sakei, Lb. Helveticus, Lb. brevis*) *Enterococcus* (E. *faecium, E. feacalis*), *Leu. lactis and Weissella hellenica*; Yeast: *Kluyveromyces marxianus*, *Kazachstania unisporus*, and *Candida ethanolica*
Amazake	Rice	Japan	Fungi: *Aspergillus* spp
Boza	Various (Barley, Oats, Rye, Millet, Maize, Wheat or Rice)	Balkans (Turkey, Bulgaria)	LAB: *Leuconostoc* (*Leu. paramesenteroides, Leu. sanfranciscensis, Leu. mesenteroides*), *Lactobacillus* (*Lb. plantarum, Lb. acidophilus, Lb. fermentum*); Yeast: *Saccharomyces* (*S. uvarum, S. cerevisiae*), *Pichia fermentans, Candida* spp.
Bushera	Sorghum, Millet flour,	Africa (Uganda)	Bacteria: *Lactobacillus*, *Streptococcus*, *Enterococcus*. Uncharacterised fungal component
Koko Sour Water	Cereal (Pearl Millet)	Africa (Ghana)	Bacteria: *Weissella confusa*, *Lb. fermentum*, *Lb. salivarius*, *Pediococcus* spp. Uncharacterised fungal component
Kvass	Rye bread, rye and barley malt/flour,	Russia	LAB: *Lb. casei*, *Leu. mesenteroides*; Yeast: *Saccharomyces cerevisiae*
Mahewu	Maize, Sorghum/Millet	Africa (Zimbabwe)	Unknown
Pozol	Maize	Mexico (Southeast)	Bacteria: *L. lactis*, *Streptococcus suis*, *Lactobacillus* (*Lb. plantarum*, *Lb. casei*, *Lb. alimentarium*, *Lb. delbruekii*), *Bifidobacterium*, *Enterococcus*. Uncharacterised fungal component
Togwa	Maize flour, Finger Millet Malt,	Africa (Tanzania)	LAB: *Lactobacillus* spp.; Yeast: *Saccharomyces cerevisea*, *Candida* spp.
Hardaliye	Grapes/Mustard Seeds/Cherry Leaf	Turkey	LAB: *Lactobacillus* spp. Uncharacterised fungal component
Kombucha	Tea	China, Worldwide	Bacteria: *Gluconacetobacter (G. xylinus)*, Acetobacter, *Lactobacillus*; Yeast: *Zygosaccharomyces, Candida*, *Hanseniaspora*, *Torulaspora*, *Pichia*, *Dekkera*, *Saccharomyces*
Water Kefir	Water/Sucrose	Mexico, Worldwide	Bacteria: *Lactobacillus (Lb. casei, Lb. hilgardii, Lb. brevis, Lb.*

		plantarum), *L. lactis*, *Leu. mesenteroides*, *Zymomonas*; Yeast: *Dekkera* (*D. anomola, D. bruxellensis*), *Hanseniaspora* (*H. valbyensis, H. vineae*) *Saccharomyces cerevisiae*, *Lachancea fermentati*, *Zygosaccharomyces* (*Z. lentus, Z. florentina*)

Figure 1:

An overview of the interlinked processes and considerations in fermented beverage production and development.

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