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Cereals and millet based probiotic products: A healthy approach for non-dairy consumers: A review

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Abstract
Since the beginning, food is regarded as a source of nourishment that offers sufficient nutrients to satisfy metabolic needs but with increasing concern about the health, life expectancy, and desire of people for improved life quality, has given rise to the concept of functional food which are defined as dietary items that, besides providing nutrients and energy, beneficially modulate one or more targeted functions in the body, by enhancing certain physiological responses and /or by reducing the risk of disease. Probiotics are the good example of functional foods. The dairy based products are important functional food group when inoculated with probiotics but however its consumption is limited due to lactose intolerance and increased cholesterol content. That had gradually led to shifting of demand of the consumers from dairy based products to non -dairy based products, mainly based on cereals, fruits and vegetables. Among the non-dairy based products cereal based products have been widely used due to its easy availability, ability to get fermented, its worldwide consumption and potential source of energy, vitamins, minerals and fiber hence this review provides an insight into the growing trends of cereals and millet based probiotic and its positioning in global market.

Keywords: Cereals, millet, probiotic, approach, non-dairy

Introduction

Over the last few years, the requirement for healthy food has increased on global scale and lead to the diffusion of functional foods which may fulfill nutritional needs and impart advantageous roles on human health. Functional foods can be understood as those food and food components that provide a health benefit beyond basic nutrition", and in particular as a food similar in appearance to, or may be a conventional food that is consumed as a part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic diseases beyond basic nutritional functions (Coda *et al*. 2017) [9].

Probiotics are the good example of functional food. Probiotics are the live microorganisms that are beneficial to host by improving intestinal microflora when administered in adequate amount (Fuller 1989) [15]. Most of the probiotic foods provides nutrient such as fatty acids, vitamins and other important nutrient that increases resistance against the pathogenic microorganism and boost the immunity (FAO/WHO, 2001) [23]. The most common bacteria used as probiotics on commercial basis are *Bifidobacteria* and lactic acid bacteria such as *lactobacilli*, *lactococci*, and *streptococci* (Isolauri, *et al*.2002) [22].

Since from decades milk has been considered as the only food containing all the essential nutrients important for human nutrition the use of dairy based probiotics is widespread.

But nowdays, there is a remarkable shift in the consumers demand from dairy based to plant based products as an alternative for healthier diet, growing trends of vegetarianism, change in the lifestyle, easy availability and cost effective. The major concern related to dairy based probiotics are, lactose intolerance, increased calorie, high fat content, milk protein allergies and hypercholesterolemia (R Manasa *et al*.2020) [28]. Plant based probiotic products exhibits richness in unsaturated fatty acids, antioxidant activity and contains bioactive compounds like phytosterols and isoflavones, which helps to reduce the risk of cardiovascular diseases, cancer, atherosclerosis and diabetes and make it an excellent choice for the consumers (R Manasa *et al*.2020) [28].

However, cereal based probiotics can be a major source of inexpensive calories and other nutrients across the world. They contain plenty of phytochemicals, comprising of phytoestrogens, phenolic compounds, antioxidants, phytic acid and sterols which make them suitable for development of functional foods (Mridula D & Monika Sharma 2014).

Cereals are sources of water soluble fibres (such as Beta glucan and arabinoxylan), oligosaccharides (such as galacto and fructo oligosaccharides) and resistant starch and can readily support the growth of microorganisms, like lactic acid bacteria and thus have the potential to fulfill the concept of prebiotic concept (Shah, 2001). Millets, small seeded drought resistant cereals relatively superior than wheat and rice possess high nutritional and functional properties. Millets like foxtail millet, finger millet and proso millets have low glycemic index and have good probiotic activity to promote gut health and digestion (Arya & Shakya 2021) [2].

Non -dairy probiotic products based on cereals and millets are gaining popularity as a functional food and serves as an alternative for dairy probiotics due to health related risk associated with its consumption. They are also an economical substitute, where cow's milk is expensive or insufficient. The bioactive components they contain and their beneficial effect on the human health make them an attractive option and an area of research for the researchers.

Nutritional significance of millets

Millets are small grained, annual, warm weather cereals belonging to grass family (Kavitha and Kiruthika 2018) [24]. The various types of millets are finger millet, millet, little millet and Sorghum. Millets are termed as "Yesterday's coarse grains and today's nutri-cereals" (Gowda *et al*. 2022) [17]. The majority of the millets are three to five times more nutritious than the most cereals like rice, wheat and maize due to its richness in vitamins, minerals, fibres and protein and are called as superfoods (Ashoka *et al*.2020) [3]. Millets contain about 60-70% carbohydrates, 7-11% proteins, 1.5-5% fat, and 2-7% crude fibre and are also an excellent source of vitamins, minerals, essential fatty acids and phytochemicals which contributes to its antioxidant property. Due to its high dietary fiber content it is capable of promoting multiple health benefits such as improving gastro intestinal health blood lipid profile, and blood glucose clearance. Millets with minimal gluten and low glycemic index are healthy choice for celiac and diabetes.Millets are rich in phytochemicals such as phytosterols, polyphenols, phytocyanins, lignin and phytoestrogens. These are health promoting phytochemicals. The main polyphenols are phenolic acids and tannins (Sarita & Singh 2016) [40]. These phytochemicals act as antioxidant, immunological modulators, detoxifying agents, prevents cardiovascular diseases, type-2 diabetes, and cancer (Gowda *et al*., 2022) [17]. Phenolic compound present in millet inhibits enzyme like alpha-glucosidase, pancreatic amylase and reduce postprandial hyperglycemia by partially inhibiting the enzymatic hydrolysis of complex carbohydrates (Shobana, *et al*. 2009) [42]. Inhibitors like aldose reductase prevents the accumulation of sorbitol and reduce the risk of diabetes induced cataract diseases (Chethan, *et al*. 2008) [8]. Millets are also good sources of magnesium which is capable of reducing the effects of heart attack. They are rich in phyto-chemicals containing phytic acid which is known for lowering cholesterol (Lee, *et al*., 2010) [27]. Millet phenolics prevent LDL oxidation (Shahidi and Chandrasekhar 2013).

Ferulic acid is very strong antioxidant, free radical scavenging and anti-inflammatory. Antioxidants significantly prevent tissue damage and stimulate the wound healing process (Rajasekaran, *et al*.). The chemical reaction between the amino group of proteins and the aldehyde group of reducing sugars, termed as nonenzymatic glycosylation, is a major factor responsible for the complications of diabetes and aging. Millets are rich in antioxidants and phenolics; like phytates, phenols and tannins which can contribute to antioxidant activity important in health, aging, and metabolic syndrome (Hegde *et al*., 2002) [19].

Probiotic product development

Probiotic foods are defined as those that consist of a single or mixed culture of microorganisms that have beneficial effect on the consumer's health by improving their intestinal microbial balance (Fuller, 1989) [15]. According to the International Dairy Federation (International Dairy Federation, 1997) [21], microbial concentration equivalent to 10^6 to 10^7 cfu mL^{-1} is considered to have beneficial effect on the consumer based on a daily intake of 100 mL (Gallo *et al*. 2020) [16]. The majority of probiotics available are dairy-based, however cereals are starting to offer a good option because they avoid issues like lactose intolerance or their effects on hypercholesterolemia (Prado *et al*.2008) [35] and are being researched recently in regards to their possible application in the creation of functional foods. Cereals are cultivated in approximately 73% of the world's harvested land, and put up to over 60% of global food production supplying with dietary fibre, proteins, energy, minerals, and vitamins which are vital for human health. Cereals have the potential to acts as fermentable substrates that promotes the growth of probiotic microorganisms, particularly lactobacilli and bifidobacteria; consist of dietary fibre promoting several beneficial physiological effects; as prebiotics due to their content of specific nondigestible carbohydrates; as encapsulation materials for probiotic to enhance their stability and hence finds application in development of cereals and cereal based functional food (Charalampopoulos *et al*. 2002) [7]. Numerous researches are being conducted to indicate the possibility of lactic fermentation on cereals and the beneficial effect the fermented cereal would impart. The beneficial effect of the cereals and millets enforces the development and designing of new cereal and millet based foods or is added as of the constituents in the food product. Preparation of these kinds of food must take into account a number of important factors, including the processing of cereal grains, their formulation, their capacity for fermentation, and their organoleptic properties, characteristics of the fermented product, etc. The selection of the strain and the substrate's composition are crucial factors to take into account in order to track the final metabolic product (Gallo *et al*. 2020) [16]. Kedia *et al*. 2007 [25] investigated the preparation of a novel cereal-based probiotic food with an appropriate fragrance, flavour, and pH using mixed culture fermentation. The effects of yeast presence on the fermentation of a lactic acid bacteria (LAB), *Lactobacillus reuteri*, were investigated in a medium of 5% (w/v) malt suspension with different inoculations. Three different inoculum ratios between the yeast and the LAB (1:1, 2:1 and 1:2) were experimented. Savas and Akan 2021 prepared oat bran fortified raspberry probiotic drink as oat bran are a valuable source for β-glucan. Freeze-dried raspberries were powdered. The UHT milk was heated to 50 ◦C and 5% (w/v) sugar was added. Then the heated milk was divided into six portion with different formulations after adding oat bran and raspberry, probiotic cultures were inoculated into the mixtures and incubated at 40 ºC. Menezes *et al*. 2018 [29] developed a maize based probiotic beverage using lactic acid bacteria and yeast as a starter culture. Dried maize grain approximately weighing 550 grams were soaked in water for 30 minutes and macereated to obtain flour-like mixture followed by addition of six litres of water and cooked for one hour. Starter culture of *L.paracasei* LBC-81 and yeast strain *Saccharomyces cerevisiae* CCMA 0732 and *S. cerevisiae* CCMA 0731 from caxiri, *Pichia kluyveri* CCMA 0615 from cocoa fermentation, were allowed to ferment for 24 hours at 30ºC. Andres *et al*. 2023 developed a fermented plant based beverages from unused bread flour. Flour was developed using discarded breads which was used as a medium to support the growth of probiotic lactic acid bacteria (LAB) and *Bifidobacterium*. Bread flour was combined with 900 mL of sterile water (20% w/v) for 5 minutes, then pasteurised in a water bath at 70 °C for 5 minutes followed by chilling of mixture in cold water bath for 10 minutes until the temperature reached to 37ºC. Two samples were processed without removal of salt while the other two samples were desalted. 0.0179 mL per 100 g flour of α-amylase (0.021%) and 0.029 mL per 100 g flour of β-glucoamylase (0.033%) were added to half of the desalted and non-desalted blends prior to incubation and mixed for 1 min; control samples were retained without enzymes. Followed by a minute of shaking, the mixture was inoculated with around 107 CFU/g of each starter per essay and incubated for 24 hours at 38± 2ºC in a water bath. In 1L glass bottles containing 180 g of flour and 720 mL of sterile water, the fermentation was carried out. Kittibunchakul *et al*. 2021 [26] produced fermented brown rice milk where fermentation was carried out by *Lactobacillus pentosus* which enhanced the Gamma-aminobutyric acid (GABA) content. Freshly prepared brown rice milk was inoculated with the chosen GABA producing probiotic starter (*Lactobacillus pentosus* 9D3) to achieve a cell count of approximately 10^7 colony forming units per millitere. Fermentation was carried out at 30ºC for 16 hours without being shaken in a sealed container. The strain significantly increased the GABA content in Brown Rice Milk fortified with yeast extract (0-5% w/v), isolated soy protein (0-4% w/v) and pyridoxine hydrochloride (0-200µM). The pH of the fermented brown rice milk was adjusted to 4.5 with 5 N KOH and then called as probiotic GABA drink. The resulting drink had high level of GABA content (14.3 mg/ml), high probiotic count (8.6 log cfu/ml) and phenol compounds and exhibited antioxidant characteristics. Fawzi *et al*. 2022 [13] developed rice based yoghurt and studied the fermenting ability of probiotic lactic acid bacteria of broken rice milk. Three bacterial strains of *Lactiplantibacillus plantarum* ATCC 14917, *Lacticaseibacillus casei* DSM 20011, and *Lactobacillus acidophilus* ATCC20552 and yoghurt was used as a starter culture for fermenting rice milk. For five hours, three cups of cleaned broken rice were soaked in four cups of water. The mixture was blended to a smooth consistency, then passed through double layered cheesecloth for filteration. The prepared rice milk was transferred into jars and cooked for 10 minutes at 95 °C. To increase total solid content skim milk powder was added and heated at 95 ºC for 30 minutes followed by cooling until the temperature reduced to 37 ºC.Then the milk was inoculated with the 10% probiotic cultures and yoghurt and incubated for 8 hours at 37 ºC until decrease in pH value, increase in titrable acidity and coagulation of samples were seen. Value of ash, protein, total solids, phenols, flavonoids and antioxidant activity significantly increased in the fermented milk samples.

Millet based probiotics

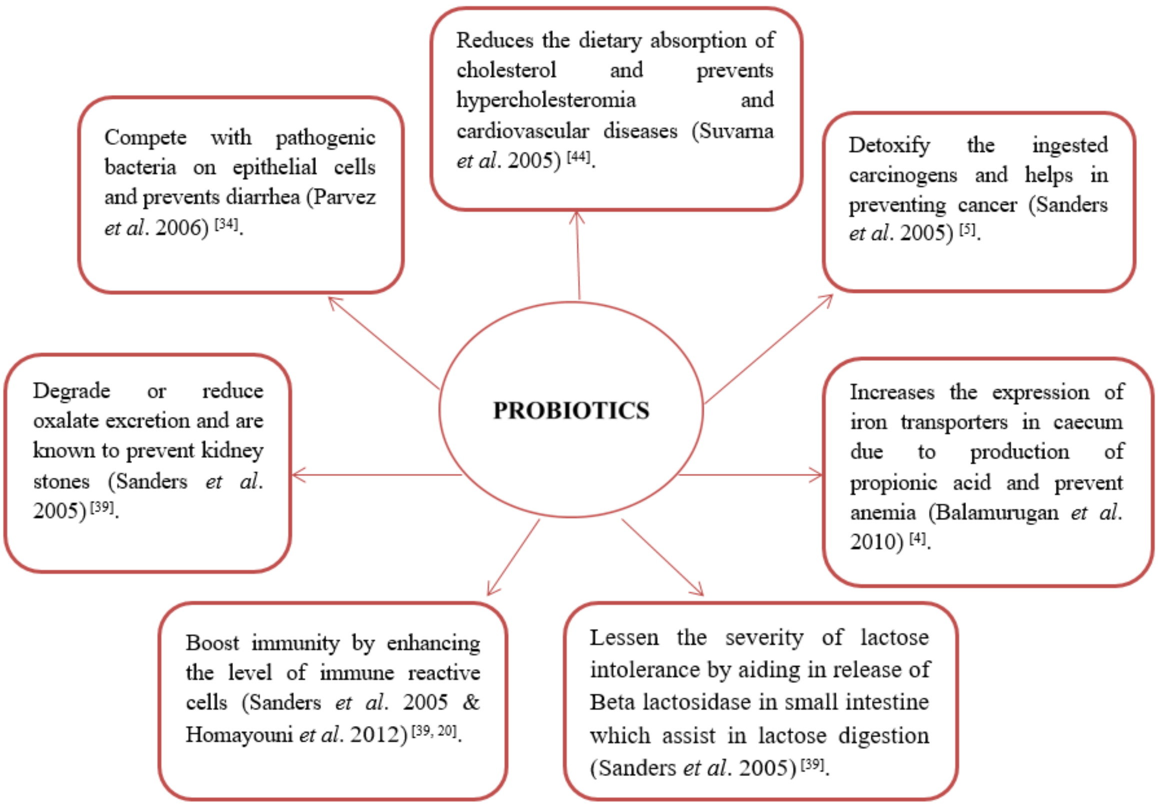
As a low-cost technology, fermentation is frequently used as the primary biotechnology method of food preparation in developing nations like Africa from milk, cereals fruits etc. Fermentation enhances the sensory properties and nutritional value of the foods, decreases toxins and anti-nutritional factors, and preserves the food thereby extending the shelf life of the foods (Misihairabgwi *et al*. 2017) [31]. Hence demand for functional probiotic foods and beverages has grown significantly over the past few years, and this trend is being driven by rising consumer and market demand on a global scale (Real *et al*. 2022, Grand view research 2019) [46, 18]. Among the nutritional probiotic beverages non-dairy functional probiotic beverages have achieved an interesting place in the market. The drawbacks of dairy products, such as their high levels of cholesterol or lactose, trends like plant-based diets that link human health to environmental sustainability, and the widespread prevalence of gluten intolerance and lactose intolerance have created a market for non-dairy products. As an out-turn fruits, legumes and cereal based products are developed (Min *et al*. 2018). Fermentation of cereals with lactic acid bacteria increases the nutritional contents and adds up to health-promoting properties of the final products such as beverages, bread, biscuits and breakfast cereals. Cereal grains mainly contain dietary fibre-phenolic compounds (DF-PC) bound covalently to polysaccharides through ester bonds, on fermentation these ester bonds are broken and release some phenolic acids such as ferulic acid, thus contributing to health benefits(Min *et al*. 2018). Among the cereals, traditional African minor grain crops like sorghum and millets, not only possess benefits in terms of production and stress adaptability but also have an intriguing nutritional profile and imparts positive health benefits on consumer (Real *et al*. 2022) [46]. Therefore, there is an enormous opportunity to investigate the technological potential of its utilization by the food sector to prepare a variety of food products (Kavitha and Kiruthika 2018) [24]. Different millet based traditional preparations are available globally, fermentation of the millets being more common as it enhances the biological value (BV), net protein utilization (NPU), thiamin, riboflavin and niacin contents (Kavitha and Kiruthika 2018 [24], Aliya and Geervani, 1981) [1]. Various fermented millet beverages produced globally are as followed.

Table 1: Micoorganisms result health benefits

Product	Micoorganisms	Result	Health benefits	references
Kodo ko jaanr (beverage)	Yeasts (*Pichia anomala, Saccharomyces cerevisiae, Candida glabrata, Saccharomycopsis fibuligera,* and LAB (*Pediococcus pentosaceus and Lactobacillus bifermentans*)	Fermentation of millet increased the moisture, crude fibre, calcium, magnesium, manganese, iron and phosphorus	Increase in strength of ailing person and Post natal women due to high calorie content	(Thapa and Prakash 2004) [45]
Probiticated millet laddu	*Lactobacillus acidophilus*	Increase in protein content antioxidant and phenolic compounds	Controls cholesterol and blood sugar level and boosts the functioning of liver and kidney	(Rubavathi, 2022) [38]

Yoghurt	*Lactiplantibacillus plantarum*	Increased content of superoxide dismutase and volatile flavor substances	Increased antioxidant effects, reduce hyperurecemia	(Fan *et al*.2022) [11]
Yoghurt	*Lactobacillus rhamnosus* *Streptococcus thermophilus*	Decrease in phytic acid	Increase bioavailability of minerals	(Stefano *et al*.2017) [10]
Chyang (Beverage)	Yeasts *Saccharomyces bayanus,Candida glabrata,Pichia anomala, Saccharomycopsis fibuligera, Saccharomycopsis capsularis, and Pichia burtoni* Lactic acid bacteria, filamentous molds *Mucor circinelloides* and *Rhizopus chinens*	Fermentation improves the taste and, enhances the food value in terms of protein,calcium, fiber, B vitamins,and *In vitro* protein digestibility, and reduces the antinutrients present in food grains	Quenches thirst, gives energy and provide nutrition	(Ray *et al*.2016) [37]
Yoghurt Like beverage	*Weissella confusa* 2LABPT05 *Lactiplantibacillus plantarum* 299v	Increased fibre, dextran threonine, arginine, Gamma Amino Butyric acid and glutamine, enhanced protein digestibility, reduced fat and sugar.	Suitable for lactose or gluten intolerants	(Real *et al*.2017)
Ben saalga (porridge)	*Lactobacillus plantarum* *Lactobacillus fermentum*	Increase folate or vitamin B9 content and improve over all nutritional quality	Mitigates folate deficiency and prevents megaloblastic anaemia and foetal neural tube defects	(Bationo *et al*.2019) [5]
Kambu Koozh (Porridge)	*Lactobacillus fermentum* *Lactobacillus delbrueckii*	Reduced antinutritional factors and increased digestibility of protein and starch, enhanced sugar transformation and bioavailability of minerals	Prevents diarrhoea and constipation	Palaniswamy And Govindswami (2016) [33]

Health benefits from probiotics
Probiotics are the live microorganisms that are beneficial to host by improving its intestinal microbial flora when administered in adequate amounts (Fuller, 1989) [15]. The most common bacteria used as probiotics on commercial basis are *Bifidobacteria* and lactic acid *bacteria* such as *lactobacilli, lactococci, and strepto cocci* (Isolauri, *et al*.2002) [22]. Fermentation by lactic acid bacteria helps in enhancing the nutritional quality of the food. Most of the probiotic foods provides nutrient such as fatty acids, vitamins and other important nutrient that increases resistance against the pathogenic microorganism and boost the immunity (FAO/WHO, 2001) [23]. Apart from this, probiotics have health benefits associated with its consumption and promoting the interest of the researchers towards its wider application. The therapeutic properties of probiotics include anti-pathogenicity, anti- allergic, anti –diabetic, anti - inflammatory, anti-cancer, anti- allergic and also show beneficial effect on the central nervous system. These benefits are regulated through several mechanism which ranges from production of short chain fatty acid to production of bacteriocin, decreasing gut pH, and nutrient competition to stimulation of mucosal barrier function and immunomodulation (Forsythe & Bienenstock 2010) [14].



Conclusion

With the enhancement and improvement of scientific knowledge on the microbial composition and characteristics of traditionally fermented foods, limitation like lactose intolerance, cholesterol content, etc. in dairy products enforces the development of non-dairy probiotic products. Fermentation not only extends the shelf life and organoleptic properties of food but also enhances the bioavailability and bioaccessibility of minerals. Hence fermentation mainly lactic acid is favored to enhance the foods nutraceutical significance because it increases the overall nutritional and organoleptic properties at low cost which is economically feasible. Therefore, there is requirement of robust strategies for the development and formulation of probiotic foods to produce functional foods with improved bioavailability of essential micronutrients, which could contribute to reduction in micronutrient deficiency.

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