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REVIEW

# Germination and probiotic fermentation: a way to enhance nutritional and biochemical properties of cereals and millets

Kirti Kumari[1] · Piyush Kashyap[1] · Prasun Chakrabarti[2]

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**Abstract**
Probiotics have become increasingly popular as consumers demand balanced nutrition and health benefits from their diet. However, lactose intolerance and allergies to milk proteins may make dairy-based probiotics unsuitable for some individuals. Thus, probiotics derived from cereals and millets have shown promise as an alternative to dairy probiotics. Soaking, germination, and fermentation can reduce the anti-nutritional factors present in cereal grains and improve nutrient quality and bioactive compounds. Biochemical properties of probiotics are positively influenced by fermentation and germination. Thus, the current review provides an overview of the effect of fermentation and germination on the biochemical properties of probiotics. Further, probiotics made from non-dairy sources may prevent intestinal infections, improve lactose metabolism, reduce cholesterol, enhance immunity, improve calcium absorption, protein digestion, and synthesize vitamins. Finally, health-conscious consumers seeking non-dairy probiotic options can now choose from a wider variety of low-cost, phytochemically rich probiotics derived from germinated and fermented cereal grains.

**Keywords** Probiotics · Fermentation · Germination · Antioxidant activity · Lactobacillus

## Introduction

Over the last few years, the requirement for healthy food has increased on a global scale and led to the diffusion of functional foods which may fulfil nutritional needs and impart advantageous roles in human health. "Functional foods are those foods and food components that provide advantages for health above and beyond those of basic nutrition." This is particularly true for foods that have physiological benefits as well as lower the dangers of chronic diseases, which may resemble to conventional foods commonly found in a regular diet in addition to serving basic nutritional purposes (Coda et al., 2017). Probiotics are a good example of functional food. Probiotics are live microorganisms that are beneficial to the host by improving intestinal microflora when administered in adequate amounts (Fuller, 1989). The market for functional foods is dominated by probiotic foods, which account for 70% of the market. Statistically, the worldwide probiotic market in 2019 was valued at \$4.62 billion, which is expected to reach \$7.59 billion by 2026. According to numerous clinical researches, it is reported that probiotics show advantages for human health, aiding in the prevention of diseases and disease treatment. Their use reduces asthma symptoms, decreases diarrhoea duration in children, reduces lipid accumulation, and improves intestinal mucosal barrier function (Xu et al., 2022). Most probiotic foods provide nutrients such as fatty acids, vitamins and other important nutrients that increase resistance against the pathogenic microorganism and boost immunity. Commercially available probiotics typically contain bacteria such as Bifidobacterium and Lactobacillus, Lactococci, and Streptococcus (Isolauri et al., 2002).

Traditionally, probiotic foods have been limited to dairy-based, consisting of milk and milk-based fermented products, which include animate members of the Lactic Acid Bacteria (LAB) family. The largest market segment in this industry is

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✉ Piyush Kashyap
p18.kashyap@gmail.com

Kirti Kumari
kirti29008@gmail.com

Prasun Chakrabarti
drprasun.cse@gmail.com

[1] Department of Food Technology and Nutrition, School of Agriculture, Lovely Professional University, Phagwara, Punjab 144411, India

[2] ITM SLS Baroda University, Vadodara, Gujarat 391510, India

dairy-based goods, which are thought to possess a 74% market share for probiotic products (Pericone et al., 2015). The major concern related to dairy-based probiotics is lactose intolerance, increased calorie, high-fat content, milk protein allergies and hypercholesterolemia (Manasa et al., 2020). However, certain communities' stringent for vegans as well as their particular religious beliefs may also restrict the intake of milk-based food products. Thus, there is a remarkable shift in the consumer's demand from dairy-based to plant-based products as an alternative for a healthier diet, growing trends of vegetarianism., change in lifestyle, easy availability and cost-effectiveness. Plant-based probiotic products exhibit a richness in unsaturated fatty acids, and free radical scavenging activity and contain bioactive components like phytosterols and isoflavones, which helps to lessen the threats of cardiovascular diseases, cancer, atherosclerosis and diabetes and make it an excellent choice for consumers (Manasa et al., 2020). Among the plant-based products, cereal has been widely used due to its easy availability, ability to get fermented, its worldwide consumption and potential source of energy, vitamins, minerals and fibre. The widely used cereals are barley, oat, maize, rice, and wheat and millets such as sorghum, finger millet, pearl millet, proso millet, kodo millet, foxtail millet, little millet and fonio. Cereal grains are good substrates and carriers for probiotic fermentation, which can be utilized to create novel functional foods and nutraceuticals in the food business. Approximately 73% of the world's inhabitant grows them, accounting for about half of all food produced worldwide (Di Stefano et al., 2017). Cereals contain a variety of nutrients, including carbohydrates (60–70%), proteins (7–11%), fat (1–5%), crude fiber (2–4%), minerals (0–2.5%), and vitamins (B-vitamins and tocopherols) (Sharma et al., 2022). Millets and cereals contain many phytochemicals and insoluble dietary fibre which are capable of scavenging radicals, as well as profused mineral content and numerous vitamins. Finger millet is considered to be among the richest sources of calcium, containing 300–350 mg per 100 g of grain. (Budhwar et al., 2020). These cereals and millets exhibit the capableness to transfer *Lactobacilli* through the hostile conditions of the Gastrointestinal tract; also, they foster the development of single as well as mixed-culture fermentations of probiotic microorganisms (Sharma et al., 2022). In contrast to the majority of cereals, these millet grains are five times more nutrient-dense as they are an excellent source of all vital nutrients like carbohydrates, vitamins, minerals, fat, gluten-free and rich source of protein and amino acids possessing sulphur (methionine and cysteine) and have healthier fatty acid content, hence, they are also known as superfoods. Bioactive compounds like flavonoids and phenolic acids present in it impart health benefits such as antioxidant and anti-microbial activities. Tannins in finger millet are powerful inhibitors of digestive amylase enzymes and result in lowering glycemic responses. The phenolics present may reduce the risk of kidney damage by reducing protein glycation, the reaction between protein and glucose (Taylor and Kruger 2019).

The nutritional content of cereals and millets is very high, but due to their low protein content, lack of lysine and other essential amino acids, starch availability, presence of certain antinutrients and coarse grain texture (Singh et al., 2015), they have low nutritional as well as sensory qualities compared to dairy products. Cereals' low protein content and quality, swelling of the macronutrient starch upon heating, and poor content and bioavailability of the micronutrients iron and zinc are three significant aspects that limit their nutritional significance (Mouquet et al., 2008; Nout, 1993). To overcome these problems, processes like germination and fermentation have been practised to make cereals nutritionally superior. Germination and fermentation improve protein digestibility in cereals and millet. As fermentation proceeds, antinutrients such as phytates get degraded and insoluble form of protein gets transformed into soluble proteins, this conversion is facilitated by the genesis of proteolytic enzymes by microbiota. The digestibility of carbohydrates is also enhanced by the process of fermentation and sprouting as it leads to the conversion of a complex form of starch into simple soluble sugars and makes it energy dense. The process of germination and fermentation also increases the mineral content by enriching the mineral compound's availability by catabolism of anti-nutritional factors such as saponins and polyphenols, which hinders the bioavailability of minerals. Germination activates the phytase-specific phosphatases enzyme called phytases, which hydrolyze phytate into inositol and orthophosphate thereby releasing minerals (Gowda et al., 2022). It also plays a significant role in the enrichment of dietary fibre content and decreases fat content due to the increased enzymes and fat utilization activities which serves as a source of energy during the germination. During germination, the biosynthetic capability of cereal grains is utilized and different hydrolytic enzymes are synthesized. These reactions in germinating grain cause alterations in the structures of grains and initiates the synthesis of new biomolecules, some of which display increased bioactivity and can boost the nutritional significance and stability of the grains (Kaukovirta-Norja et al., 2004). Based on these considerations, this review paper aims to study the germination effect of cereals and millets on the nutritional and functional properties of probiotic products. Further, it also highlights the various probiotic products developed from germinated cereals and their health benefits.

## Requirement for non-dairy probiotic food

Beneficial microorganisms have been utilized for advancing health benefits through various dairy products. Dairy products have historically been the main and most popular sources of probiotics. However, lactose intolerance, the presence of cholesterol, allergic milk protein (Rasika et al., 2021), economic reasons of various developing countries and the shifting trend towards vegetarianism have led to the urge for non-dairy probiotic products thereby limiting the use of dairy probiotics. Lactose is the foremost, important carbohydrate for promoting the development of the health of newborns (Wahlqvist, 2015). Lactose intolerance is essentially the lack of intestinal brush synthesis of the lactase enzyme, which hydrolyzes lactose into energy-producing absorbable carbohydrates like glucose and galactose. β-Galactosidase released by probiotics in the small intestine helps break down lactose and ease lactose digestion. Using probiotics can thereby lower the risk of lactose intolerance, although the effects will vary depending on the product's cell count and lactose content.

Since plant-based non-dairy food matrices such as cereals, millets, fruits and vegetables, which do not contain cholesterol, but provide a variety of important nutrients such as protein, starches, minerals, fiber, and vitamins, which are all health-promoting factors, have been successfully utilized to manufacture probiotics products with the minimum number of viable probiotics at the time of consumption, which includes searching for non-dairy food options. These sources are easily accessible due to their low costs, phytochemical content, and ability to reduce the severity of cholesterol and lactose intolerance. (Panghal et al., 2018). Owing to this scenario, it is estimated that the market for probiotics will rise from $ 65.9 billion in 2022 to $ 91.1 billion by 2026, with a compound annual growth rate of 8.3%. Among this market share, the non-dairy probiotic products have a good percentage as well as buyers are becoming apprehensive towards the benefits of probiotics, as well as the willingness to purchase premium products containing probiotics. The call for probiotics in fortified foods is expected to remain high. Additionally, in the wake of the COVID-19 pandemic, consumers have shifted their consumption patterns and this has resulted in a change in diet requirements. Instead of junk foods or processed foods, consumers are seeking out products with high nutritional value. Probiotics are in high demand due to the fear of contracting an infection, which has led to healthy lifestyle choices. Considering that everyone is susceptible to virus infection, manufacturers have designed probiotics that are effective for all age groups.

## Mechanism of probiotics activity

To impart various health benefits probiotics exhibit various mechanism which ranges from the production of short-chain fatty acid to the production of bacteriocins thereby decreasing the pH of the gut, nutrient competition to stimulation of mucosal barrier function and immunomodulation. Figure 1 showed the graphical representation of the mechanism of probiotics (Adapted from Bermudez-Brito et al., 2012).

### Enhancing epithelial barrier

The epithelial cells of the intestine are in permanent contact with the lumen of the intestine and the various enteric

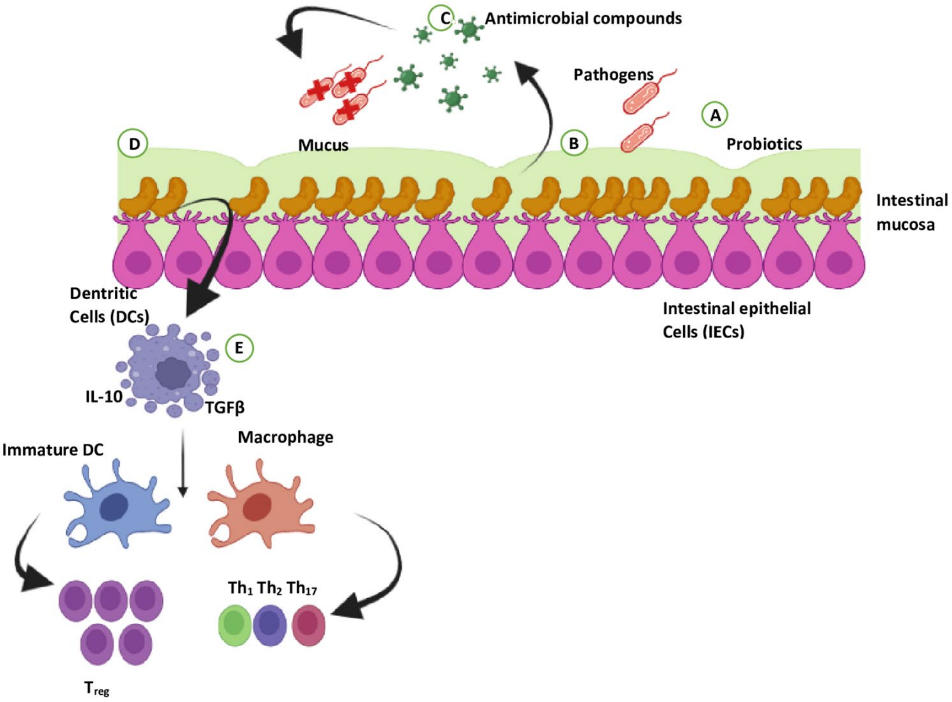

**Fig. 1** Summary of mechanism of probiotics activity (**A**) Enhancement of the epithelial barrier. (**B**) Competitive exclusion of pathogenic microorganisms. (**C**) Inhibition of pathogen adhesion. (**D**) Production of anti-microorganism substances. (**E**) Modulation of the immune system. Adapted from Bermudez-Brito et al. (2012)

flora. The intestine acts as a barrier and is a source of major defence. The mucous layer, antimicrobial peptides, secretory IgA, and the epithelial junction adhesion complex make up the intestinal barrier's defence system (Ohland and Macnaughton, 2010). The performance of the intestinal barrier is aided by probiotics this is done by increasing the expression of genes related to tight junction signalling, which improves the integrity of the intestinal barrier (Fig. 1) (Anderson et al., 2010). For instance, in a T84 cell barrier model, lactobacilli are known to alter the regulation of numerous genes encoding adherence junction proteins, including E-cadherin and β-catenin. Moreover, lactobacilli in the intestinal cell variably influence the amount of protein kinase C (PKC) isoforms, such as PKC, and the phosphorylation of adhesion junction proteins, positively influencing the barrier function of the epithelium (Hummel et al., 2012).

## Improved intestinal mucosal adhesion

Adherence to the mucosa of the intestine is considered to be a crucial probiotic property for invasion and also plays an important role in the interaction between probiotic strains and the host. The interaction of lactic acid bacteria (LABs) with intestinal epithelial cells (IECs) and mucus is mediated by a variety of surface determinants. The primary component of mucous, mucin, which is a complex glycoprotein combination secreted by intestinal epithelial cells (IECs), hinders the adherence of harmful microorganisms. Other components of mucous gel are lipids, free proteins, immunoglobulins and salts. Adhesion to intestinal mucosa is promoted, through lactobacilli protein adhesins that mediate attachment to the mucous layer (Buck et al., 2005). The most prevalent type of bacterial adhesin produced by *Lactobacillus reuteri*, is mucus-binding protein (MUB) which targets mucus. In lactobacilli, these proteins are primarily secreted and associated on the surface of the cell, either connected to the membrane via lipids or incorporated within the cell wall. These proteins might aid in the colonisation of the human intestine by allowing cells' extracellular matrix to degrade or by promoting intimate interaction with the epithelium. For example, the binding of *L. reuteri* and *L. fermentum* to mucus is governed by the protein MapA (mucous adhesion-promoting protein). Enteropathogenic *E. coli* adhesion is inhibited by probiotics such *L. plantarum* induced MUC2 and MUC3 mucins (Bermudez-Brito et al., 2012).

## Competitive exclusion of pathogen

The term "competitive exclusion" refers to a situation in which one species of bacteria competes more fiercely than another for receptor sites in the intestinal tract and excludes or inhibits the growth of the relatively weaker microorganisms through a variety of mechanisms, including creating an unfavourable microenvironment, removing available receptor sites of bacteria through steric hindrance at enterocyte pathogen receptors, competitive depletion of vital nutrients, selective metabolites or by producing and secreting antimicrobial substances including organic acids such as acetic acid and lactic acid (Bermudez-Brito et al., 2012).

## Synthesis of antimicrobial substances

The formation of antimicrobial compounds such as organic acid and bacteriocins contributes to the health benefit that is imparted by probiotics. The presence of organic acids, especially acetic acid and lactic acid, has been demonstrated to exhibit significant inhibition of Gram-negative bacteria and are considered important compounds for the control of harmful microorganisms. (Bermudez-Brito et al., 2012). Upon entering a bacterial cell, the undissociated organic acid dissociates inside its cytoplasm, which causes a gradual reduction in the intracellular pH or an accumulation of the ionized organic acid within the cell kills the pathogen. Many Lactic Acid Bacteria synthesise antibacterial peptides, such as bacteriocins and small antimicrobial proteins (AMPs). Bacteriocin-mediated killing involves destructing target cells through the formation of pores and/or preventing the production of cell walls. Nisin, for instance, produced by Lactobacillus coccus inhibits spore-forming bacilli from synthesizing their cell wall by forming a complex with lipid II, the ultimate precursor to their cell walls. Later, the complex aggregates and incorporates peptides into the membrane of the bacteria to form a pore (Bierbaum and Sahl, 2009). Some unique antibacterial compounds such as bacteriocin, and bifidocin B, produced by *B. bifidum* NCFB 1454 are active against Gram-positive bacteria. A strong inhibitory effect of two *Bifidobacterium* strains against several pathogenic bacteria, including *Salmonella enterica ser. typhimurium* SL1344 and E. coli C1845 have been reported and the reason explained for the inhibition is the production of a potential low molecular weight (LMW) lipophilic compounds. In addition, an LMW protein known as BIF, synthesized by *B. longum* BL1928, shows its activeness against Gram-negative bacteria. They do not directly inhibit or kill, but show inhibitory action on *E. coli* and prevent it from adhering to human epithelial cell lines (Bermudez-Brito et al., 2012).

## Immunomodulation

Probiotic bacteria are known for regulating the immune system as they can interact with dendritic cells (DCs), cells of the epithelium, and other blood components like monocytes/macrophages and lymphocytes. The immune system is composed of innate and adaptive systems. The latter relies on lymphocytes called B and T cells, which are tuned to recognize particular antigens. The former, however, react to common molecular patterns shared by most pathogens known as pathogen-associated molecular patterns (PAMPs) (Gomez et al., 2010). Pattern recognition receptors (PPRs), when attaches to PAMPs, initiate the initial response to pathogens. The most researched Pattern recognition receptors are Toll-like receptors (TLRs) (Bermudez-Brito et al., 2012). These are transmembrane proteins that detect microbial compounds and trigger an immune response by releasing substances recognized as chemokines and cytokines. They are found on both immune (dendritic cells, macrophages, and natural killer cells) and non-immune cells (epithelial and endothelial cells). As TLR4 is broadly dispersed on the surface of enterocytes, bacteria in the intestinal lumen can pierce the mucus barrier. TLR4 is widely distributed on the surface of enterocytes and is accessible to bacteria in the gut lumen that can pierce the mucus barrier. One of the many TLR4 ligands is lipopolysaccharide (LPS), present in the cell membrane of Gram-negative bacteria. On binding of lipopolysaccharide to TLR4 the myeloid differentiation primary response gene 88 (MyD88) gets activated resulting in the release of kinases that enables nuclear factor kappa-light-chain enhancer of activated B cells (NFκB)-related proteins leading to their translocation to the nucleus. NFß-B complex causes gene transcription that produces proteins implicated in the immunological, inflammatory, and stress responses and on activation begins producing proinflammatory cytokines, particularly interleukin (IL)1β, IL6, and tumour necrosis factor-alpha (TNFα). To counter effect this several strains of probiotics such as *Lactobacillus* and *Proprioni bacterium* are known to produce a surface layer protein (SLP) that inhibits the in vitro generation of pro-inflammatory cytokines (Halloran and Underwood 2019). For instance, the *L. acidophilus* NCFM produces an SLP that reduces the inflammation caused by LPS by preventing NF-B p65 from entering the nucleus and reducing TNFα, IL1β, and reactive oxygen species (Wang et al., 2018).

## Biochemical changes during germination and probiotic fermentation

A malting process involves steps such as soaking, controlled germination, and drying, which increase enzyme activity and transform grains into malt (Amadou et al., 2011). A germination process, by definition, includes the absorption of water by dormant seeds, followed by the elongation of their radicles that extend through the surrounding structures. A sprouting seed undergoes enzymatic activity that triggers the breakdown of proteins, carbohydrates, and lipids into simpler forms, as well as activating proteases that are involved in protein degradation, which results in increased bioavailability of nutrients (Sruthi and Rao, 2021). Further, activation of these hydrolytic enzymes leads to the breakdown of starch, non-starch polysaccharides and proteins in barley (Rimsten et al., 2003), wheat (Yang et al., 2001), oats (Mikola et al., 2001), and rice (Manna et al., 1995), which leads to the accumulation of oligosaccharides and amino acids. The process of germination also initiates a coordinated metabolic activity that leads to the net conversion of oil into sugars by mobilizing triacylglycerols from oil bodies within the grain. This occurs due to the breakdown of liberated free fatty acids (FFA) through the processes of β-oxidation and the glyoxylate cycles. Nutrient availability of coarse cereals can be improved by malting/germination (Hejazi et al., 2016). The product's properties such as structure, bioactivity, flavour, stability and digestibility were also extensively affected due to germination (Singh et al., 2015). For example, in barley, finger millet, oat and rye decomposition of polymers with high molecular weight results in the production of biofunctional substances and the improvement of organoleptic qualities due to a softening of texture and an increase in flavour (Sruthi and Rao, 2021). Furthermore, the phytate, tannin, and oxalate contents of the seeds were reduced by 40%, 16.12%, and 49.1%, respectively, during germination. Pradeep and Sreerama (2015) found that millet flour obtained from the germinated barnyard, foxtail, and proso millets had the highest levels of phenolic content and the highest antioxidant activity. During germination, aglycones could have been released from glucosides by activated glucosidases or phenols may have been biosynthesised, which could have produced higher levels of phenolics in germinated millets.

Probiotic fermentation is a process of growing and metabolizing beneficial microorganisms in food or beverages. It is one of the most widely used fermentation processes in the food industry and has recently attracted the attention of many researchers seeking to maximize its benefits in bioprocessing raw foods. It involves gram-positive rod bacteria that ferment carbohydrate substrates into lactic acid, resulting in the production of lactic acid by anaerobic, but aerotolerant, nonsporulating, and catalase-negative bacteria. The primary result of lactic acid fermentation is the reduction of pH following lactic acid production. Nevertheless, the production of alternative metabolic byproducts, including hydrogen peroxide, carbon dioxide, diacetyl, low-molecular-weight antimicrobial substances, and bacteriocins, induces diverse modifications (Khosroshahi and Razavi, 2023). The microorganisms that are added to the fermentation process produce enzymes (like amylases, proteases, lipases, and others) that are responsible for breaking down complex carbohydrates, proteins, and fats into simpler ones. Due to this transformation, complex compounds become smaller molecules that are easier for the human body to digest and absorb. Fermentation also alters the biochemical composition of the food matrix. For example, microorganisms metabolize sugars to produce organic acids such as lactic acid and acetic acid. These organic acids provide the fermented product with a tangy flavor and extend its shelf life. During fermentation, additional compounds such as vitamins, amino acids, and bioactive peptides can be generated, further augmenting the nutritional content and functional attributes of the end product.

## Effect of germination and probiotic fermentation on characteristics of probiotics

### pH, acidity and growth profile of probiotic bacteria

Germinating and malting cause numerous biochemical reactions that impact the product's structure, flavor, stability, bioactivity and digestibility (Singh et al., 2015). These biochemical changes offer a more favourable environment which supports proper growth and development of probiotic bacteria and increased cell counts were found in food products derived from germinated cereals than those made from non-germinated cereals. Hydrolysis of germinated flours that have undergone germination might be responsible for the higher cell count since it provides a convenient environment for the optimum growth and development of microorganisms (Budhwar et al., 2020). Table 1 represents the effect of germination and fermentation on the properties of probiotics. Fermented food mixtures made from germinated barley flour exhibited significantly higher acidophilus growth (8.88 log CFU/g) than non-germinated mixtures (7.75 log CFU/g) (Arora et al., 2010). Chavan et al. (2018) used a combination of non-germinated and germinated barley, finger millet and moth bean (2.5:1.5:1) for the preparation of four probiotic drinks i.e. Distilled water, soya milk, almond milk and coconut milk probiotic drink. Germinated millet probiotic drink with coconut was found to contain the highest cell (9.47–11.07 cfu/ml) compared to non-germinated (10.03–11.04 cfu/ml). Similarly higher acidity was observed in probiotics developed from germinated cereals and millets. Hydrolysis of starch into sugars and subsequent conversion of lactic acid may contribute to the decrease in pH during germination (Chavan et al., 2018). Higher count of lactobacilli and Bifidobacterium was achieved in germinated brown rice during shelf-life determination which indicates its suitability as an effective probiotic carrier (Pino et al., 2022). Following probiotic fermentation, the amount of crude protein and crude fibre in the food mixture significantly declined. However, Germinated cereal grains and millets are also known to be nutritionally more diverse following fermentation by increasing the amount of tryptophan, lysine, and methionine in them. Several hydrolytic enzymes are released by bacteria during the fermentation process, which breaks down complex proteins into simpler ones. Elkhalifa and Bernhardt (2010) studied that the nitrogen solubility index increased substantially in germinated sorghum because the concentration of proteases increased. This could be explained by the continuous conversion of accumulated protein to amino acids and small peptides. Moreover, this resulted in a steady rise in the protein digestibility of germinated sorghum grains, making partially hydrolyzed reserve proteins more easily absorbed by pepsin and enhancing the overall nutritional value of these grains. Liu et al. (2022) also studied that probiotic fermented beverages prepared from germinated barley and rice mixture have higher nutritional value and better sensory properties in contrast with ungerminated.

### Anti-nutritional compounds

The addition of probiotics to food or dairy products occurs via supplementation and fermentation. As an ancient and affordable method of food preservation, fermentation enhances the nutritional content of raw sources by enriching sensory attributes and by improving physiological characteristics (Rakhmanova et al., 2018). However, the excessive concentration of inhibitors and antinutrients impedes the bioavailability of cereals. Likewise, the function of amylolysis and proteolysis is also known to be impaired by tannins and phytic acids (Peyer et al., 2016). Several methods either alone or in combination with other methods are known to reduce the antinutritional factors. Anti-nutrients such as phytic acid, tannins, and polyphenols in cereals are complex with proteins and limit their digestion. Phytic acid carries a negative charge and attracts cations such as calcium, magnesium, zinc and iron that carry positive charges forming complexes with them thereby reducing their bioavailability (Budhwar et al., 2020). Tannin phenolic compounds exhibit the property of precipitating protein by the formation of transient and irreversible tannin protein complexes between the hydroxyl group of tannins and the carbonyl group of proteins which further reduces the bioavailability of proteins and leads to vital amino acids depletion. Germination and Fermentation are vital and well-known methods which significantly diminish the antinutrient concentration (Fig. 2) and result in an increment of the total nutritive value of coarse cereals and other food grains (Table 1). The process of in vivo biotransformation improves hydrolytic enzyme activity, contributing to a higher level of vitamin, carbohydrate, and mineral absorption (Hejazi et al., 2016). These processes lead to a reduction of phytic acid due to the production of phytases specific phosphatases enzyme called phytases, which hydrolyse phytate into inositol and orthophosphate and liberate minerals. Hence, enhancing the mineral content (Yousaf et al., 2021). A major drop in various antinutrients such as phytic acid and tannins was noticed when millet was exposed to fermentation for a duration of 12 and 24 h (Budhwar et al., 2020). Reduction in

**Table 1** Effect of germination and fermentation on properties on cereals or millets probiotics

| Cereals or millets used | Product | Germination condition | Microorganism involved | Outcome | References |
|---|---|---|---|---|---|
| Wheat, Barley,<br>Pearl millet, and green gram with and without soymilk | Probiotic drink | Seed to water ratio (1:2)<br>Soaking: 8 h at 30 °C<br>Green gram sprouting for 24 h and wheat barley and millet for 36 h | *L.acidophilus* | Increased probiotic count | Mridula and Sharma (2015) |
| Barley, Ragi<br>Moth bean | Probiotic drink | Soaking of cereal in water in the ratio (1:2)<br>Germination at 30 °C and 95%RH<br>Barley, Ragi, Moth bean germination for 48 h, 36 h and 24 h respectively | *L.acidophilus* | Increased probiotic count<br>Higher antioxidant capacity and total phenolic content<br>Improved overall acceptability and functional property of the beverage | Chavan et al. (2018) |
| Wheat | Beverage and baking flour | Soaking at 26 ± 2 °C for 6 h,<br>Germination 20 ± 2 °C and RH at 80 ± 3%,<br>Sprouted wheat removed after germination after 12 & 24 h | *L. acidophilus* | Short-term sprouting of 12 h can yield sprouted wheat flours with end-use functionality without compromising the properties of the dough | Peñaranda et al. (2021) |
| Barley | Probiotic drink | Germination for 24 h at 10 °C | *L. acidophilus*<br>*L. plantarnum* | Media containing malt promoted the growth of LAB, and significant amounts of lactic acid were produced (0.5–3.5 g/L)<br>High cell concentration of 7.9 and 8.5 $\log_{10}$CFU/ml aftr 6 h fermentation<br>Enhanced organoleptic and functional properties of drink | Rathore et al. (2012) |
| barley | Probiotic<br>Food mixture | Seed to water ratio (1:5)<br>Soaked for 24 h at 37 °C with frequent spraying of water after 24 h | *L.acidophilus* (NCDC-16) | Higher growth of Lactobacillus<br>Reduction in protein content<br>Higher amount of niacin and thiamine<br>Increase in reducing and non-reducing sugar,<br>Decrease in starch content and soluble and insoluble dietary fibre | Arora et al. (2010) |
| Pearl millet | Probiotic food mixture | Seed to water ratio (1:5)<br>Soaked for 24 h at 37 °C with frequent spraying of water after 24 h | *L.acidophilus* (NCDC-16) | Improved content of thiamine, niacin, total lysine, protein fractions, sugar, soluble dietary fibres<br>Increased in vitro availability of Ca, Fe and Zn | Arora et al. (2011) |
| Brown rice | Yoghurt | Soaking in distilled water for 24 h at 28 °C and germinated for 48 h and 96 h | Lactic acid bacteria | High phenolic content and increased GABA | Cáceres et al. (2019) |
| Sorghum | Probiotic food mixture | Seed to water ratio of (1:5)<br>Soaking for 12 h<br>Germination for 24 h at 37 °C with frequent spraying of water | *L.acidophilus* | Lower dietary fibres<br>Increase in thiamine, riboflavin and niacin | Jood et al. (2012) |
| Oats | Oat based substrates | Soaked for 12 h<br>Germination at 28 ± 2 °C for 24 h | *L. rhamnosus* HN001 | Improved nutritional characteristics<br>Promotes growth of probiotic<br>Embrace flavor characteristics | Herrera-Ponce et al. (2014) |

**Table 1** (continued)

| Cereals or millets used | Product | Germination condition | Microorganism involved | Outcome | References |
|---|---|---|---|---|---|
| Oats | Fermented beverage | Germination at 18 °C for 4 days | *L. plantarum* | Enhanced content of protein, essential amino acids (methionine, cysteine and phenyl aniline) riboflavin, polyunsaturated fatty acids, phenolic compounds, GABA and antioxidant activity | Aparicio-García et al. (2021) |

tannin content is discerned due to the production of tannase enzymes during microbial fermentation. Tannase activity degrades tannin content. A decrease in tannin content from 6.16 to 0.36, 0.53, and 0.16% was observed when sorghum was fermented by *L. bulgaricus, L. casei and L. brevis* respectively (Gunawan et al., 2022). Lactic acid fermentation of different cereals and millets helps to reduce the concentration of phytic acid and tannins, which in turn improve the availability of protein starch and minerals. Flour from germinated seed when blended with a small quantity of lactic starter culture and undergoing fermentation was reported to have improved dietary bulk properties (Ilango and Antony, 2021). A declining trend in phytic acid by about 66% was seen when single culture of *L. casei* was used as fermenting bacteria while a reduction of phytic acid by 64% was reported when fermented by *L. plantarum*. While phytic acid was eliminated when the combined form of both these cultures was utilized for food mixture fermentation (Sindhu and Khetarpaul, 2001). In another research where the impact of germination and fermentation when developing pearl millet-based probiotic was examined a significant improvement was noted in the content of niacin, thiamine, protein fraction, soluble dietary fiber, sugars and in vitro availability of calcium, zinc and iron in food mixture (Sihag et al., 2015).

## Phenolic content and antioxidant activity

Different components of millet and cereal grains have biological action. These substances are referred to as phytochemicals or bioactive molecules. Based on their synthetic pathway these bioactive molecules are classified as nitrogen-containing compounds, terpenes and phenolics. Among these, phenolic compounds are the most diverse class of phytochemicals. These consist of flavonoids, phenolic acids, alkyl resorcinol and coumarins which impart oxidative stability besides providing colour, flavor, taste and texture. They are present in bran and possess nutraceutical properties. Phenolic acids are categorized as hydrocinnamic acid, present in millets mostly in the insoluble-bound phenolic fraction and hydroxybenzoic acids. Food's ability to function as an antioxidant is closely correlated with the presence of phenolic chemicals. The antioxidant ability of cereal grains is signified by their ability to reduce, ability to suppress reactive oxygen species and the scavenging of free radicals (Budhwar et al., 2020). However, these phytochemicals are mostly found in bound form and are less bioavailable than the free form but bioprocessing methods such as fermentation and germination are being adapted to bring improvement in the nutritional value of plant food which subsequently leads to increased antioxidant properties. Results demonstrated that germination enhances the nutritional value of the bioactive molecules liberated during the hydrolysis process, as aided by endogenous enzymes,

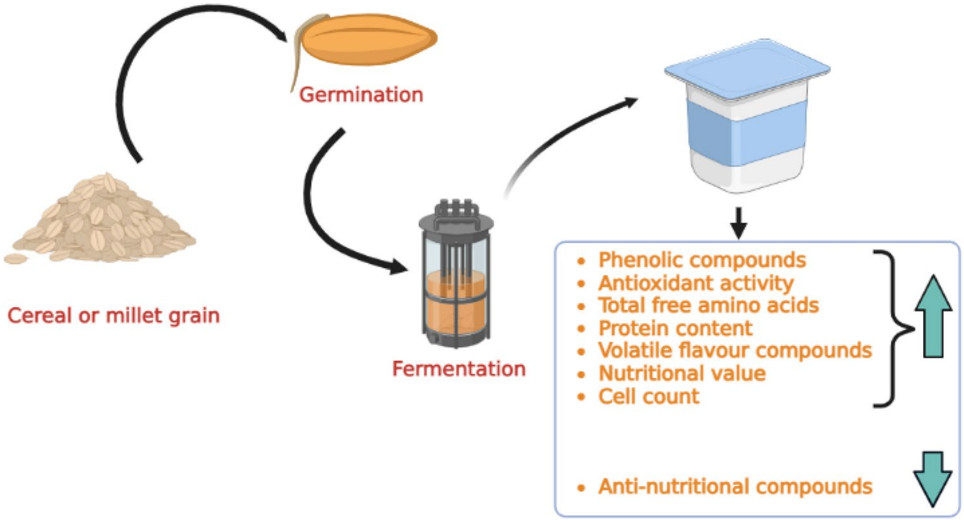

**Fig. 2** Effect of probiotic fermentation on germinated grains properties

which are activated during the process of degradation of reserves necessary for respiration and for the production of new cell components for embryonic development (Fig. 2) (Paucar-Menacho et al., 2022). Through several studies, it has been concluded that the germination process enhances the quantity of solvent-extracted compounds containing phenols (Hassan et al., 2006). The reason attributed to the increment is the enhanced activity of polyphenol oxidase (PPO). The increase in polyphenols during seed germination is attributed to the solubilization of condensed tannins during water soaking and their migration to the outer layer during germination (Hassan et al., 2006).

As observed finger millet on germination showed a percentage increase in total phenolic content (TPC), total flavonoid content (TFC), 1,1-diphenyl-2-picryl-hydrazil radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP),2,2-Azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) to 16.67%, 13.62%, 8.98%, 13.11% and 51.94% respectively. The reason stated for the increment was due to the activation of an enzyme that promoted the formation of phenolic compounds. Increment in phenolic compound positively influenced the antioxidant activities (Azeez et al., 2022). Similarly, total phenols and antioxidant activity increased for germinated brown rice. The increase in the free forms of phenols could be due to cell wall breakdown. The cell wall of rice grains contains polysaccharides that are coupled to insoluble phenolic chemicals, which make up the cell wall. Induced saccharolytic enzymes break down the end during germination, increasing the overall amount of phenolic and flavonoid and boosting antioxidant activity. (Nissar et al., 2017). A significant increase in antioxidant activity from 48.30 to 51.13% was noted for the germinated finger millet of different durations (Abioye et al., 2018).

Microorganisms modify plant constituents by releasing the chemically bound compound during fermentation, thereby bringing about the enrichment of phytochemicals contents with increased bioavailability and bioactivity (Yeo and Ewe, 2015). An increase in the bioactive compounds in cereals during fermentation can also be attributed due to the breakdown of their cell wall (Budhwar et al., 2020). As reported by Dordevic et al. (2010) enzymes from cereals for example, proteases, amylases and xylanases produced during microbial fermentation modify the composition of grains and release attached phenol before extraction thereby leading to the structural disintegration of cereal cell walls/generation of bioactive compounds. The fermentation of cereal grains with *Lactobacillus rhamnosus* produced a cereal product with enhanced antioxidant properties (Fig. 2). The effect of fermentation on the total phenolics, anthocyanin levels, and antioxidative activity of fermented black beans was reported by Lee et al. (2018). According to the investigation made augmentation of bioactive components after fermentation was noted, this was brought on by the catalytic action of -glucosidase, which triggered the release of lipophilic aglycones from iso-flavone glucosides like genistin. Different types of enzymes namely glycoside hydrolase, cellulose or xylan degrading enzymes, and esterase, are produced during the fermentation of cereals by fungi, which are known to soften kernel structure, disintegrate cell walls of cereals, and therefore release stored esterified insoluble-bound nutrients (Cai et al., 2012) causing hydrolysis of the β-glucosidic bonds of varied phenolic compounds that occur primarily in conjugation with one or more sugar residues attached to hydroxyl groups hence, increasing the available amount of free polyphenols (Kadiri, 2017). As studied the total phenolic content and antioxidant activities measured by diphenyl-2-picryl-hydrazyl radical scavenging activity, ferric ion-reducing antioxidant power, and lipid peroxidation inhibition ability increased when a varied number of cereals, including barley, were fermented by *L. rhamnosus* for 24 h (Sharma et al., 2022). Moreover, fermented brown finger millet also showed a remarkable increment in the concentration of total phenol and antioxidant activity. (Sharma et al., 2022).

## Health benefits of probiotic fermented products

Foods containing probiotic microorganisms with scientifically proven health claims have huge potential for improving the health of the hosts. A probiotic microorganism can withstand the harsh conditions in the host's gastrointestinal tract and colonize the epithelium, creating a stable microflora that regulates the host's immune system, thus determining their effects on the host's health (Mishra et al., 2022). The metabolites produced during the probiotic fermentation of cereals and millets are responsible for the health implications which may include antioxidant properties, anti-diabetic properties, anti-inflammatory properties, and anti-cancer properties (Aravind et al., 2021). A famous fermented rice dessert, Khoaw-Maak, has high antioxidant activity and reduces inflammation and heart disease. Moreover, it is also capable of inhibiting tyrosinase (which produces melanin) and matrix metalloproteinases 2 (MMP-2) which dissolve collagen matrix, thereby reducing cancer and skin ageing (Monosroi et al., 2011). In a study, the probiotic beverage produced from germinated pearl millet and liquid barley malt extract using *L. acidophilus* showed effective control of Shigella-induced pathogenicity in mice. The probiotic beverage reduced the spread of the pathogen throughout the body and increased IgA secretion in the intestinal fluid (Ganguly et al., 2019). Kambo Kooz, a probiotic fermented pearl millet porridge was found to produce glutamate decarboxylase, cholesterol-reducing and DPPH scavenging activity (Palaniswamy and Govindawamy, 2016). White polished rice generally causes thiamine deficiency. However, the LAB fermented rice tape-ketan (black glutinous rice fermented product) has been identified to produce thiamine (Sharma et al., 2020). Similarly, another probiotic fermented rice product Koji/Red yeast rice assists in improving blood circulation, digestion, and neurotoxicity prevention (Mishra et al., 2022). Nagpal et al. (2012) studied the presence of LAB in Kunu (a probiotic fermented cereal product) has the potential to serve as biotherapy for Type 2 diabetes. These LAB, such as Bifidobacteria and *Lactobacillus acidophilus*, are capable of modulating the gut microflora and reducing insulin resistance in human subjects. In another study, the potential anti-diabetic effects of oat extract fermented with *L. plantarum* strains were examined using a streptozotocin-induced diabetic rat model. As a result of this study, fermented oats were found to have significant antidiabetic and hypolipidemic effects. The production of γ-aminobutyric acid GABA helps in attenuating the blood glucose level in rats (Algonaiman et al., 2022). Fan et al. (2022) found that *Lactiplantibacillus plantarum* fermented cereal-based probiotic yoghurt showed 63.01 to 146.79% higher superoxide dismutase (SOD) content than yoghurt commonly available in the market. The higher SOD content may provide this yoghurt with anti-ageing and anti-inflammatory properties.

## Cereals and millets based probiotic food products

Cereals and pseudocereals are valuable sources of micronutrients, dietary protein, carbohydrates, vitamins, minerals and fibre worldwide and are a significant source of energy and form an important part of a balanced diet. However several factors such as deficiency of amino acids such as lysine, reduced availability of starch, and presence of anti-nutritional factors like tannins, phytic acids and polyphenols can sometimes make cereal products nutritionally little inferior when compared with milk and milk-based product. But unit operations such as fermentation and germination have been practised for the intensification of its nutrient content in cereals. Countless benefits such as preservation of food, increased food safety, add to taste and aroma and acceptability, the addition of variety in the diet, improved nutritional significance, and decreased anti-nutritional components are associated with fermentation. While germination leads to modifications in structure and production of new compounds having high bioactivity resulting in enhanced nutritional value and grain stability (Singh et al., 2015). In recent years grains undergoing germination are being used as an ingredient in various food product development because of their intriguing nutritional value, appealing technological properties and sensory attributes. The germination conditions are important for ensuring the sprout's quality and functionality as food ingredients. Various studies are conducted for the development of wholesome foods due to the interest of the consumers in adopting healthy diets at present and as cereals have the potential to be the substrate for the probiotic they are being utilized for various probiotic food product development.

According to the studies made probiotic drinks are developed using various sprouted cereals such as wheat, barley, green gram and pearl millet separately with oat as a stabilizer and sugar using *Lactobacillus acidophilus.* Soymilk and distilled water constituting the liquid portion showed an increase in the probiotic count with an increase in the grain flour (Mridula and Sharma, 2015). The probiotic drink was made from barley, ragi and moth bean using both germinated and un-germinated seeds. The drink resulting from the germinated seeds showed a higher value of Trolox equivalent antioxidant capacity and total phenolic content as compared to those developed from ungerminated seeds (Chavan et al., 2018). Some of the traditional probiotics produced from cereals and legumes involve the fermentation process as a unit operation by using lactic acid bacteria and other probiotic bacteria are Bushera, Mahewu, Boza, Pozol, Ogi, Uji, Kenkey, Togwa, Kefir, Velli, Tempeh, Probiotic oat flour, Ricera and Koozh.

### Bushera

Bushera a common traditional beverage of Uganda is a probiotic product prepared from sorghum and flour of millet where probiotic microorganisms like *L. brevis, Enterococcus, Streptococcus, Lactococcus,* and *Lactobacillus* were used. Bushera is commonly consumed as a traditional beverage. The advantages associated with its consumption are enhancement of insusceptibility, and energized body (Panghal et al., 2018).

### Mahewu

Mahewu is a probiotic product that originated in African countries by using a mixture of grains, including maize, sorghum, millet malt, and wheat flour and probiotic *L. lactis* carrying out the fermentation process. Flavour enhancement is carried out by the addition of fruit flavourings at the end of fermentation. Mahewu has high levels of phytochemicals and starch content (Panghal et al., 2018).

### Togwa

Togwa a probiotic product having its origin in Japan and China is developed by germination of cereal grain for 3 to 6 h and further fermentation of multigrain like sorghum, finger millet flour and maize. Fermentation is carried out by *Lactobacillus, Streptococcus* and *L.planetarium*. Togwa has a high concentration of starch and phytonutrient value (Panghal et al., 2018).

### Kefir soy

Kefir soy is a carbonated beverage that originated in Greek developed from the fermentation of soya beans carried out by lactic acid bacteria like *L. brevis, L. mesenteroides, L. helviticus* and *L.kefir* and yeast-like *Kluyveromyces marxianus, Kluyveromyces lactis*. Functional properties of Kefir are antimicrobial, anticarcinogenic properties, Cholesterol-lowering effects, improving gastrointestinal system, and improving immune systems (Guzel-Seydim et al., 2011).

### Velli

Velli is a probiotic product developed by involving the use of oat brans. Fermentation is carried out by using probiotic bacteria like *B. bifidum*, and *L. acidophilus.* Fruits are added to enhance the flavor.

### Tempeh

Tempeh is produced by coagulating soymilk by bacteria *L. rhamnosus, and Bifidobacterium.* This product has a significantly larger number of probiotic bacteria in it. It is rich in vitamin B12 which is mostly found in animal products.

### Boza

Boza is a traditional drink prepared by fermentation and is primarily consumed in Turkey, Romania Bulgaria and many other countries by fermentation of cereals like maize, wheat or rice or semolina as well as involving millets or flour beverages. Fermentation is carried out by lactic acid bacteria and other probiotic microbes like *L. caprophilus, L. plantarum, L. acidophilus, Leuconostoc mesenteroides, S. uvarum.* Boza has beneficial nutritional properties helps in the improvement of gastrointestinal health, boosts the immune system, and reduces cholesterol levels (Cosme et al., 2022).

### Pozol

Pozol is a slightly acidic beverage produced from the fermentation of maize sourdough with *L. lactis.* It has bactericidal and bacteriolytic activities against pathogens (Cosme et al., 2022).

### Ogi

Ogi is a fermented maize product based on probiotic cereals, commonly consumed in western parts of Nigeria and West Africa. It is prepared by fermenting the paste of wet-milled and sieved-soaked sorghum or maize. Bacteria involved in fermentation are *L. acidophilus, L. plantarum, L. brevis and L. fermentum. Yeast like S.cerevisiae, R. graminis, C. krusei, C. tropicalis, G. candidum and G. fermentum* are predominant. It has therapeutic benefits and aids in the prevention and treatment of maladies including lactose intolerance, dysentery, and diarrhoea as well as maltase and sucrose deficiency (Panghal et al., 2018).

### Uji

Uji developed in Kenya is a probiotic food made from maize or sorghum or both in varied proportion combinations.

Fermentation is carried out by *L. plantarum, L. fermentum, L.cellobiosus, L. buchneri, Pediococcus nacidilactice, P. penosaceuus, L. rhamnosus* and *S. thermophillus.* Uji can be served as infant foods along with mother's milk (Panghal et al., 2018).

### Kenkey

Kenkey, a probiotic drink consumed mainly in Africa is prepared by using maize, millet and sorghum. The fermentation process is carried out by *L. plantarum, L. fermentum, L. brevis, L. reuteri, P. pentosaceus*. Predominant yeast during fermentation is *C. krusei and S.cerevisiae*. It can be consumed as a prepared meal.

### Koozh

Koozh is a fermented probiotic food product prepared from cereals such as rice and millet flour that originated in South India. Fermentation is carried out by probiotic microbes like *W. paramesenteroids, L. plantarum* and *L. fermentum.*

The purpose of this review is to explore the adequacy of coarse cereals and millets as viable delivery vehicles for probiotic-based fermentation to develop a functional probiotic product based on cereals. Using probiotic bacteria in non-dairy products is a challenging and crucial step for the research and commercialization of healthy beverages. However, germination and fermentation of cereals and millets could be beneficial because during these processes many bioactive compounds can be produced, hazardous microorganisms can be suppressed, food safety is improved, and texture is improved. Therefore, they have significant growth potential and may be explored by developing new ingredients, reengineering processes and products for the food industry. Also, we conclude that the combination of germinating and fermenting an indigenous cereal-based food blend provides a potential application for improving and improving the nutritional quality of the product. The consumption of such fermented food blends has both nutritional and therapeutic benefits, as well as being safe for human consumption. Apart from these benefits, fermented cereal products must be accepted by consumers, who must be convinced of their benefits for them to remain viable and successful. Therefore, the traditional positive attitude associated with germinated and fermented foods mustn't be compromised by these technological developments.

## Declarations

**Conflict of interest** The authors declare that there are no conflicts of interest.

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