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2022

Sharma , R , Mokhtari , S , Jafari , S M & Sharma , S 2022 , ' Barley-based probiotic food mixture : health effects and future prospects ' , Critical Reviews in Food Science and Nutrition , vol. 62 , no. 29 . https://doi.org/10.1080/10408398.2021.1921692

http://hdl.handle.net/10138/353471
https://doi.org/10.1080/10408398.2021.1921692

Barley-based probiotic food mixture; health effects and future prospects

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Abstract

Consumers around the globe are increasingly aware of the relation between nutrition and health. In this sense, food products that can improve gastrointestinal health such as probiotics, prebiotics and synbiotics are the most important segment within functional foods. Cereals are the potential substrates for probiotic products as they contain nutrients easily assimilated by probiotics and serve as the transporters of Lactobacilli through the severe conditions of gastrointestinal tract. Barley is one of the important substrates for the probiotic formulation because of its high phenolic compounds, β-glucans and tocols. The purpose of this review is to examine recent information regarding barley-based probiotic foods with a specific focus on the potential benefits of barley as a substrate for probiotic microorganisms in the development of dairy and non-dairy based food products, and to study the effects of food matrices containing barley β-glucans on the growth and features of Lactobacillus strains after fermentation.

Keywords: Probiotics; Lactic acid bacteria; Barley; Functional foods

1. Introduction

The word “probiotic” comes from the Greek word “προ-βίος” that means “for life”. Probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (Fenster et al., 2019). Normally, the intestinal flora is in a constant state of flux but the balance between them is disturbed by junk food, alcohol, antibiotics, stress, aging and digestive disorders (Amara and Shibl, 2015). Based on the amount of ingested food along with the effect of storage on probiotic viability, it was suggested that a daily intake of 10^8–10^9CFU/g probiotic bacteria could survive the upper gastrointestinal tract (GIT) to exert their positive physiological functions in the human body (Turkmen et al., 2019).

Probiotics are an important concept for healthcare in the 21st century. The global probiotics market should reach $69.1 billion by 2024 from $48.4 billion in 2019 at a compound annual growth rate (CAGR) of 7.4% (BCC, 2020). In the global probiotic market, the European market is the largest and fastest growing with an average annual growth rate of around 20% with more use of probiotics in food and medicine. The health benefits of probiotics and rising awareness among consumers are expected to drive the industry growth over the next few years (Zelinska et al., 2018). Microorganisms recognized as probiotics, mainly members of the Lactobacillus and Bifidobacterium genera, are increasingly being used in food preparations and for the development of novel functional foods (Nakkarach and Withayagiat, 2018).

The label “functional food” was introduced in 1980 in Japan, which was the first country that stated a specific regulatory approval process for functional foods, known as Foods for Specified Health Use (FOSHU). Several critical factors have been recognized as the key factors leading to the emergence of functional foods: health deterioration due to busy lifestyles, consumption of convenience foods and insufficient exercise, increased incidence of self-medication, increased awareness of the link between diet and health, and a crowded and competitive food market (Begum et al., 2017). In recent years, consumers have become more aware towards the relationship between food and health, which has led to an explosion of interest in functional foods (Periconne et al., 2015). Well-known examples of functional foods are those containing or prepared with bioactive compounds, such as dietary fibers, oligosaccharides, and active and friendly bacteria that promote the equilibrium of intestinal bacterial strains. In addition to the well-established functional ingredients, such as vitamins, minerals, and micronutrients, probiotics belong to an emerging generation of active ingredients, which includes prebiotics, phytonutrients and lipids (Adefegha, 2018). The market of functional foods is characterized by an increasing trend, and some researchers reported that probiotic foods represent 60-70% of functional foods (Perricone et al., 2015). Probiotic foods are classically confined to traditionally dairy-based, comprising milk and its fermented products containing live organisms of the Lactic Acid Bacteria (LAB) family. Dairy based products are the main segment of this sector, and it is estimated that they account for 74% of the probiotic products market share (Periconne et al., 2015). However, lactose intolerance, milk protein allergy, high levels of saturated fatty acids and cholesterol content associated with dairy products, tend to enforce the recent shift to the non-dairy probiotic foods (Enujiugha and Badejo, 2017). Furthermore, cultural (strict vegans) as well as specific religious believes among certain communities may also limit the consumption of dairy foods. In such situations, non-dairy probiotic carrier foods are convenient mode of probiotic deliveries (e.g, in tablet forms) (Randheera et al., 2017).

Non-dairy matrices (legumes, cereals, pseudocereals, fruits, and vegetables) represent potential carriers of probiotics, prebiotics, and bioactive compounds (Salmeron et al., 2015; Valero-Cases et al., 2020) owing to the growing trend on the market for vegetarian foods, together with the high percentage of lactose intolerant people and the presence of cholesterol in dairy products. Hence, there are nutritional reasons for testing lactic acid fermentation as a potential process for production of fermented juices from fruits and vegetables. During storage of fermented drinks, the low pH, nutrient depletion and accumulation of lactic acid is a challenge for the survival of probiotic bacteria being difficult to keep the right microbial doses at the time of consumption. In addition, the studies have reported a viability count greater than minimum recommended (10^6 -10^7 CFU/mL) during the storage making these matrices as an alternative source to dairy probiotics, that can also be consumed by people who are intolerant or allergic to milk proteins, those who are hypercholesterolemic, or those who are vegetarian, among others (Valero-Cases and Frutos, 2017; Valero-cases et al., 2020; Mantzourani et al., 2020). The studies also proved that the fermentation of these matrices can improve the shelf life and their safety due to the organic acids generated during the fermentation period, which further improve digestibility, nutritional and functional composition. In addition, dairy products are generally stored at temperatures close to 5°C, so probiotic cell viability is probably guaranteed during product shelf life. Storage at room temperature, which is common for many types of non-dairy products can create a great challenge for probiotic viability (Vinderola et al., 2017). A schematic illustration on the advantages and disadvantages of dairy and non-dairy probiotic foods are given in Figure 1.

Fig. 1

Cereals are the potential viable substrates as they hold nutrients easily assimilated by probiotics. Formulation of beverages with cereals is the promising next class of food matrices to serve as the carriers of probiotic bacteria (Salmeron et al., 2015). They have the capability to transport Lactobacilli through the severe conditions of GIT; also, they stimulate the growth of single and mixed-culture fermentations of probiotic microorganisms (Markowiak et al., 2017). Cereal grains can be used as suitable fermentable substrates/carriers for probiotics to produce new functional products in foods and nutraceuticals in the food industry. They contribute over half of the global food produced, and they are grown in over 73% of the world population (Stefano et al., 2017). Cereals are comprised of carbohydrates (60–70%), proteins (7–11%), fat (1.5–5%), crude fiber (2–7%), minerals (1.0- 2.5%), and vitamins (B-vitamins and tocopherols) (Koehler et al., 2014; Gull et al., 2016). Barley (*Hordeum vulgare* L.) represents 12% of the cereal grains and ranks fourth after wheat, rice, and maize (Schulte et al., 2009). Approximately, 65% of cultivated barley is used for animal feed and 33% for malting, whereas only 2% is used directly for human consumption (Idehen et al., 2017). Recently, interest in barley as a food grain is reviving due to heightened consumer awareness of good nutrition and increased interest in foods and food ingredients rich in dietary fibre (Izydorczyk and Dexter, 2008). It has also gained popularity due to the functional properties of its bioactive compounds.

The purpose of this review article is to examine recent information regarding barley-based probiotics foods with specific focus on the potential benefits of barley as a substrate for probiotics microorganisms in the development of functional food products. The review will also highlight the great nutritional value and health benefits of barley supporting probiotics beverages.

2. An overview of the composition of barley

Barley grain is clean, bright yellow-white, plump, thin hulled, medium-hard, uniform in size, and is generally suitable for food uses and preferred for pearling (Sharma and Kothari, 2017). Grain hardness is an important characteristic of barley because it determines the pearling and subsequent end-use quality of barley. Whole barley grain consists of about 65-68% starch, 10-17% protein, 4-9% β-glucan, 2-3% free lipids, and 1.5-2.5% minerals (Gupta et al., 2010). Total dietary fibre of barley ranges from 11% to 34% and soluble dietary fibre from 3% to 20% (Fastnaught, 2001). Hordeins are the most abundant proteins (40% to 50%) found in a barley grain (Osman et al., 2002). In addition to hordeins, other proteins have been identified, including albumins, glutelins (globulins), friabilin, enzymes, and serpins (Osman et al., 2003; Boren et al., 2004). Figure 2 shows the main components of barley grain.

Fig. 2

Barley presents many bioactive compounds and natural antioxidants. Barley is one of the best sources of tocols among cereals due to a high concentration and favourable distribution of all eight biologically active vitamers (Moreau et al., 2007) that are known to reduce serum low density lipoproteins (LDLs) through their antioxidant action (Gupta et al., 2010). Many of the natural antioxidants present in barley exhibit a wide range of biological effects including antibacterial, antiviral, anti-inflammatory, anti-allergic and antithrombotic effects, and may also be involved in vasodilatory actions (Chandrasekara and Shahidi, 2018). Compared to other grains, the amount of arabinoxylans, another antioxidant present in barley, is similar to that in wheat (5.8%), but higher than in oats (2.7% to 3.5%), sorghum (1.8%), or rice (2.6%) (Izydorczyk and Biliaderis, 2007). Polyphenols comprise a prominent proportion of antioxidants in barley including anthocyanins, flavonols, phenolic acids, catechins and more than 50 types of proanthocyanidins (Friedrich et al., 2000). The major phytochemicals in barley includes phenolic acids, flavonoids, lignans, vitamin E (tocols), sterols, and folates (Idehen et al., 2017). β-glucan is also the predominant soluble fiber found in barley and has been shown to reduce serum cholesterol and improve post-prandial insulin and glucose responses in healthy and diabetic adults (Tosh, 2013).

3. Uses of barley

Whole barley grain is mostly used for animal feed, whereas de-hulled barley grain items are mostly used for human consumption. Barley flakes, grits, and flour are all commercially available wholegrain products (OECD, 2019). Cooked pearled barley is used in the production of miso, barley tea, and rice extender. Bread, flat breads (pitas, tortillas, and chapatis), cakes, muffins, cookies, noodles, and extruded snack foods can all be made with barley flour (Ullrich, 2010). Barley starch is used in conjunction with barley malt to make beer and also has applications such as sweetening and binding agent in the food industry. Malt extract is a source of soluble sugars, protein, and amylase in the dough, and promotes yeast development, which is used to make breakfast cereals, fermented and non-fermented bakery products (e.g., crackers, cookies, and muffins) with improved texture and volume (Tricase et al., 2018).

The current utilization of barley grain is for bioethanol production in the United States and the European Union when the cheapest starch sources, such as corn or wheat, are unavailable or there is a surplus of barley production (Nghiem et al., 2017). However, the use of barley residues or residual barley by-products as bio-energy sources is being investigated and as a result, hydrothermal liquefaction technology may be useful in obtaining bio-oil for use in transportation or the energy sector to generate heat and/or electricity (Zhu et al., 2015). Furthermore, the high concentration of phenolics, vitamin E and β-glucan, sterols, fatty acids, and bioactive peptides in barley grain and distillery and brewery by-products makes barley a possible source to be used in the pharmaceutical and cosmetic industries. Lactic acid, xylitol, and microbial enzyme are also barley products that are useful in a variety of industries (Nigam, 2017).

4. Barley-based probiotic foods

Barley is rich in β-glucans, a functional bioactive ingredient which comprises a group of β- D-glucose polysaccharides found in the cell walls of cereals, yeasts, bacteria, and fungi, with different properties depending on the source (Gangopadhyay et al., 2015). Food and Drug Administration (FDA) has approved β-glucan (3 g/day) to qualify for the coronary heart disease (CHD) claims (FDA, 2005). Fortification of foods with β-glucan is of great interest including pasta, tea, muffins, bread, yogurt and beverages (Ahmed et al., 2017). Foods containing probiotics are frequent on the market, and it could be argued that co-ingestion of probiotic strains could affect health outcomes rising from gut fermentation of indigestible carbohydrate substrates (Nilsson et al., 2016). The dairy and non-dairy probiotics food products with barley as a substrate are discussed in the following sections.

4.1. Dairy-based barley probiotics

Barley has been reported to be a great supplementation for dairy probiotic foods since it is naturally healthy, readily available and relatively inexpensive (Newman and Newman, 2006). Ahuja (2015) developed a barley milk-based probiotic beverage with *Lactobacillus plantarum* culture for 12 h at 37 ˚C and reported an approximate 8.59 logCFU/mL of probiotic count and 0.14g/100g of β-glucan. Gupta et al. (1992) attempted preparation of barley butter milk-based traditional beverage popularly using curd starter called as rabadi at different time-temperature combinations (30, 35 and 40 ˚C for 6, 12, 18, 24 and 48 h). The beverage was reported to have overall acceptability score in a range of 6.35-8.36 on the basis of 9-point hedonic scale and this also depends on time and temperature of incubation. Barley flour rabadi fermented at 35 ˚C for 18 h had the highest overall acceptability (Gupta et al., 1992). Ganguly and Sabikhi (2012) also developed a composite dairy-cereal substrate consisting of whey skim milk, germinated pearl millet flour and liquid barley extract which was fermented by *Lactobacillus acidophilus* NCDC 13, (National Collection of Dairy Cultures). A high count of 13.22 log CFU/mL was reported in the substrate with 4% inoculum level and 8 h incubation at 37 ˚C. In another study by Ganguly et al. (2014) the phytic acid, polyphenol contents and phytate phosphorous were reported to be reduced by 80, 47.2, 76.5% with concomitant increase by 69 and 64% in the bioavailability of Ca and Fe, respectively. The protein and starch digestibility of the mixture were reported to increase from 45.4 and 43.4% to 62.4 and 57.8% respectively. Table 1 summarizes the variety of dairy-based barley probiotics.

Table 1

4.2. Non-dairy-based barley probiotics

Non-dairy based probiotic foods are finding their way into our routine life one by one. This group of probiotic beverages are not new, and many non-dairy preparations of cereals such as wheat, maize and barley have been traditionally made for centuries in many parts of the world. Cereals have complex nutrient composition and are being consumed on a daily-basis all over the world as one of the staple foods. Many of cereals have been recognized as origin of some strains of probiotics, whereas microorganisms used as probiotics are mostly of human or animal origin (Kumar et al., 2015). Numerous fermented dairy products using probiotic microorganisms have been prepared so far, but much less work has been done on the development of probiotic fermented products based on cereals (Enujiugha and Badejo, 2017). Table 2 summarizes the variety of non-dairy based barley probiotic products worldwide.

Table 2

A non-dairy fermented probiotic drink based on germinated and non-germinated seeds of barley and legume (finger millet and moth bean) was developed by Chavan et al. (2018). The drink mixtures were added to distilled water and milks like soy, almond and coconut in different concentrations and inoculated with *Lactobacilli acidophilus.* According to them, fermentation improved the overall acceptability and functional properties of beverage during fermentation. Changes in the pH, acidity, bacterial count, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and polyphenol content were increased as the concentration of drink mixture increased in milk and distilled water. Mridula and Sharma (2015) developed a non-dairy probiotic drink using a mixture of sprouted cereals including barley, wheat, pearl millet and green gram separately with oat, stabilizer portion. Acidity and pH in different probiotic samples ranged from 0.45 to 1.02% and 4.11 to 4.49, respectively. Probiotic count ranged from 10.36 to 11.17 log CFU/mL in barley-based probiotics, respectively with increasing level of grain flour (Mridula and Sharma, 2015).

Single and mixed cereals (barley and malt) based probiotic beverages containing *Lactobacillus plantarum* and *Lactobacillus acidophilus* in the range of 7.9 and 8.5 log CFU/mL also have been developed by Rathore et al. (2012) and proved malt to be the best substrate (as single and mixed media) for LAB growth with significant amounts of lactic acid were produced (0.5-3.5 g/L). This development concludes that the functional and organoleptic properties of cereal-based probiotic drinks could be considerably modified by changing the substrate or inoculum concentration (Rathore et al., 2012). Moreover, Helland et al. (2004) estimated the growth and metabolism of *Lactobacillus reuteri*, *Lactobacillus acidophilus* (LA5 and 1748) and *Lactobacillus rhamnosus* GG in maize porridge with added malted barley. The results showed most strains reached the maximum cell count of 7.2-8.2 log CFU/g after 12 h fermentation, with a pH below 4.0. High amounts of diacetyl and acetoin were detected in porridge when inoculated with *Lactobacillus rhamnosus* GG. The inoculated cell concentration was shown to be particularly important during the first hours of the fermentation period, showing a delayed production of most metabolites in porridge inoculated with approximately 6 log CFU/g (Helland et al., 2004).

5. Fermented barley as a probiotic functional food

Probiotic microorganisms are delivered into food or dairy products via supplementation and fermentation. Fermentation is an ancient and inexpensive food preservation method as it improves the nutritional value of raw products by enhancement of sensory characteristics, and improving functional qualities (Rakhmanova et al., 2018). Anti-nutrients such as phytic acid, tannins, and polyphenols when present in cereals can bind to proteins and lead to a reduction in digestibility.

Fermentation by LAB showed a reduction in phytic acids and tannins content, therefore enhancing the protein availability and digestion (Salari et al., 2015). Fermentation also provides optimum pH for enzymatic degradation of phytate which may increase the amount of soluble iron, zinc and calcium (Blandino et al., 2003). The gut microbiota comprises mostly anaerobic bacteria that need fermentative substrates to obtain metabolic energy for their growth and activity (Jalli-Firoozinezhad et al., 2019; Arena et al., 2014). Numerous fermented dairy products using probiotic microorganisms have been prepared so far, much less work has been done on the development of probiotic fermented products based on cereals (Enujiugha and Badejo, 2017).

Fermentation of cereals increase the shelf-life, digestibility and bioavailability of many nutrients such as B-group vitamins, minerals such as phosphorous, iron and zinc due to the action of microbial enzymes such as phytases and/or organic acids produced during fermentation of cereals (Kumar et al., 2015; Keşkekoğlu and Üren, 2013). During fermentation, the grain constituents are modified by the action of both endogenous and bacterial enzymes, including esterases, xylanases and phenoloxidases, thereby affecting their structure, bioactivity and bioavailability. Cereal-based LAB fermentation has been shown to increase the levels of nutrients including folates, soluble dietary fiber and total content of phenolic compounds in cereals, and to improve the protein digestibility and short chain fatty acid production in vitro (Anson et al., 2009). It has also been reported that the antioxidants in buckwheat, wheat germ, barley and rye increased after the fermentation with *Lactobacillus rhamnosus* and *Saccharomyces cerevisiae* (Đorđevicet al., 2010). Improvement in cell growth of probiotic bacteria in fermented barley beverage was reported by Salari et al. (2015) where they study characteristics of synbiotic beverages based on barley and malt flours fermented by *Lactobacillus delbrueckii* and *Lactobacillus paracasei* strains. They found the highest microbial growth (9.7 log CFU/mL) in malt medium after 15 h of fermentation. Many studies on probiotics formulated with barley cereal as a substrate is showing that the LAB count is increasing after the addition of barley extract or any form of it. Coda et al. (2012) used cereal (rice, barley, emmer and oat), "concentrated red grape must" and soy flours for making vegetable yogurt-like beverages. Two selected strains of *Lactobacillus plantarum* were used for lactic acid fermentation and were inoculated at a cell density of approximately 7 log CFU/g. The starters remained viable at 8.4 log CFU/g throughout storage (Coda et al., 2012).

Moreover, malt-based beverages fermented with *Lactobacillus delbrueckii* were reported to be the best sample due to the highest cell viability (1.2×10^{6} CFU/mL) after 4 weeks under cold-storage (Salari et al., 2015). Apart from the contribution of fermentation to improving survival of probiotics in cereal foods, cereal extracts also showed a capacity to increase the tolerance of probiotic bacteria to harsh conditions. For instance; cereal extracts from malt, barley and wheat significantly improved the acid tolerance of three Lactobacilli (*Lactobacillus plantarum, Lactobacillus acidophilus* and *Lactobacillus reuteri*) to gastric acid (Charalampopoulos et al., 2003). This could be due to the total sugar, reducing sugar, soluble sugars, and free amino nitrogen content of cereal extracts, which all contribute to the breakdown of starch and proteins resulting in higher cell viability. Since food formulations with pH ranging from 3.5 to 4.5 and high buffering capacity will increase the pH of the gastric tract, the buffering capacity and pH of the carrier medium are important factors that improve the probiotic strain's stability. In this analysis, however, the effect of buffering power on cell viability was minimized. Michida et al. (2006) also compared the influence of malt and barley extracts on the survival of *Lactobacillus plantarum* in gastric and bile acids, and found the higher content of sugars in the malt extract enabled these bacteria to tolerate the acid conditions better than the barley extract (Michida et al., 2006).

Improvement of bioavailability in barley beverages by fermentation has also been reported. Hole (2012), conducted a study to enhance the bioavailability of the dietary phenolic acids in flours from whole grain barley following fermentation with LAB strains, *Lactobacillus johnsonii* LA1, *Lactobacillus reuteri* SD2112 and *Lactobacillus acidophilus* LA-5. Their results exhibited high feruloyl esterase activity with an increase of free phenolic acids from 2.55 to 69.91 μg g^{-1} Dry Matter (DM) in whole grain barley. In particular, they observed that ferulic acid content in barley was 81.9% higher than in non-fermented substrates after fermentation (Hole, 2012). Arora et al.

(2010) also examined the effect of germination and probiotic fermentation on nutrient composition of barley-based food mixtures and observed that when germinated autoclaved barley mixture was fermented with probiotic (*Lactobacillus acidophilus*) it caused an enhancement of thiamine (14%), niacin (11%) and lysine (34%). The cell count also was found to be significantly higher in the fermented food mixture formulated from germinated flour (8.88 log CFU/g) as compared to the non- germinated barley-based food mixture (7.75 log CFU/g) (Arora et al., 2010). Similarly, the production of sixty volatile compounds were identified by Salmeron et al. (2009) using the probiotic strain, *Lactobacillus plantarum* NCIMB 8826 (National Collection of Industrial and Marine Bacteria), in cereal-based media (oat, wheat, barley and malt). The aroma profile was significantly changed by *Lactobacillus plantarum* and the most abundant volatiles detected in barley was acetic acid (Salmeron et al., 2009). Moreover, the β-glucans present in cereals including barley have been reported to be highly fermentable by the intestinal microbiota in the caecum and colon; consequently, enhancing both growth rate and lactic acid production of microbes isolated from the human intestine (Kedia et al., 2008).

Fermentation of barley has been also shown to reduce anti-nutrients. In the study of Sindhu and Khetarpaul (2001), single culture fermentation or sequential culture fermentation (by Lactobacilli and yeast) of an indigenously developed mixture containing barley flour, milk co-precipitate, sprouted green gram paste and tomato pulp was reported to drastically decrease the levels of anti-nutrients such as phytic acid, polyphenols, trypsin inhibitor activity, while improved the *in vitro* digestibilities of starch and protein (Sindhu and Khetarpaul, 2001). An increase in gamma aminobutyric acid content was also reported when germination and sourdough fermentation of barley flours by strains of *Lactobacillus plantarum*, *Lactobacillus rossiae* and *Lactobacillus sanfranciscensis* were used (Montemurro et al., 2018). Đorđevicet al. (2010) showed that fermentation of several cereals including barley by *Lactobacillus rhamnosus* for 24 h increased 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 (Đorđevicet al., 2010).

6. Physiological effects of barley probiotics

The pivotal role of nutrition for maintaining a good state of health is a well-accepted notion. A correct diet can have preventive and curative effects on the diseases and disorders of various origins, including obesity, phlogosis, immune dysfunctions, cancer and the detrimental consequences of aging (Jhonston et al., 2017). Cereals (wheat, barley, corn, rice) and pseudocereals (buckwheat, amaranth) are known to be important sources of bioactive peptides with anticancer, anti-inflammatory, antioxidant and cardiovascular protective properties (Laurent-Babotand Guyot, 2017). Fermentation of cereal and pseudo-cereal flours with sourdough LAB was shown to successfully increase the concentration of the anti-cancer peptide lunasin known for its anticancer activities (Hernandez-Ledesma et al., 2013; Rizzello et al., 2012). There are two types of dietary fibers: soluble fiber including pectin, fructo-oligosaccharides and oat β-glucan; and insoluble fiber including cellulose (Sima et al., 2018). After soluble fiber consumption, a delay occurs in the intestinal absorption of glucose and lipids and inhibition of absorption and reabsorption of cholesterol and bile acids accompanied by increased excretion of bile acids. The reduced absorption may be caused by the high viscosity of β-glucan solutions, which increases the viscosity of the intestinal contents (Ames et al., 2008).

According to the FDA (2006), the recommended level of β-glucan in functional drinks should be 0.75 g, which results in 3 g per day in four servings. Barley is a great source of β-glucan; functional drinks made from barley also present β-glucan in substantial quantities. Use of barley as a suitable substrate for the growth of probiotic microorganisms improves functionality of colonic strains due to presence of non-digestible components such as β-glucan, arabinoxylan, galacto- and fructo-oligosaccharides, and soluble dietary fibre of barley grain, as well as enhancing the bioavailability of LAB (Charalampopulous et al., 2002a; Elsanhoty et al., 2009). Barley also contributes to decrease cholesterol absorption, lowering blood glucose levels and improved gut microbial balance

(Wang et al., 2016). Daily intake levels of 0.75 g barley β-glucan for 30 days has demonstrated a bifidogenic effect in older healthy volunteers (Mitsou et al., 2010). β-glucan may affect the colonic mucosa as well as mucosal and systemic immunity, including mucosal repair in chronic inflammation and the reduction of pro-inflammatory cytokines (Hung and Suzuki, 2016). Supplementation with isolated barley β-glucans of different molecular weights had small effects on cardiovascular disease markers. Molecular weight of the barley fiber altered the body weight with the high-MW fiber significantly decreasing body weight (Smith et al., 2008). Figure 3 summarizes the physiological effects upon consumption of barley probiotics.

Fig. 3

Animals and human models have been used to evaluate the effects of probiotics on serum cholesterol levels over the years. Many studies have used rats, mice, hamsters, guinea pigs and pigs as models due to their similarities with humans in terms of cholesterol and bile acid metabolism, plasma lipoprotein distribution, and regulation of hepatic cholesterol enzymes. These animals also share an almost similar digestive anatomy and physiology, nutrient requirements, bioavailability and absorption and metabolic processes with humans, making them useful experimental models for research applications (Ooi and Liong, 2010). Table 3 represents the *in-vivo* and *in-vitro* studies on physiological effects of barley probiotics.

Table 3

Ganguly et al. (2019) studied the effect of whey-pearl millet-barley based probiotic beverage on *Shigella* induced pathogenicity in murine model. Probiotic beverage prepared from whey-skim milk (60:40, v/v), germinated pearl millet flour (4.73%, w/v) and liquid barley malt extract (3.27%, w/v) with *Lactobacillus acidophilus* NCDC 13 was found effective in controlling *Shigella*-induced pathogenicity in mice model by reducing translocation of pathogen in various organs and increased secretion of IgA level in intestinal fluid (Ganguly et al., 2019). Similarly, Hypocholesterolaemic effect of probiotic yogurt enriched with barley β-glucan in rats fed on a high-cholesterol diet was examined. Four treatments of yogurt were formulated, where the first and second treatments was produced from skim milk and without the addition of β-glucan and fermented by yogurts starter. The third and fourth treatments was produced from skim milk with and without the addition of 0.75% β-glucan and fermented by *Bifidobacterium lactis* plus *Lactobacillus acidophilus*. The results indicated that yogurt containing probiotic bacteria and β-glucan was more effective in lowering of plasma and liver cholesterol levels than other treatments (Ahmed et al., 2017).

Oro-gastrointestinal (OGI) tract is the tract from the mouth to the anus and includes all the organs of the digestive system; tolerance of probiotics to this tract is essential for cell survival, intestinal passage, and further colonization of the colon (Damodharan et al., 2019). The ability of a probiotic to survive through the GIT system depends mainly on their acid and bile tolerance. During GIT passage, the strains are required to tolerate the presence of pepsin and the low pH of the stomach, the presence of enzymes in the duodenum, and the antimicrobial activity of bile salts (Milllette et al., 2013). Arena et al. (2014) reported the effects of food matrices containing barley β-glucans on growth and probiotic features of four Lactobacillus strains. They observed that the food matrices, containing β-glucans, enhanced the OGI stress tolerance by probiotic strains. Although survival in the OGI transit was substantially unaffected by the presence of β-glucans in the carrier matrix, the effect of β-glucans-containing food on bacterial adhesion onto enterocyte-like cells was analysed and a positive influence on probiotic-enterocyte interaction was observed. The matrices also improved the growth rate of the tested bacteria in unstressed conditions (Arena et al., 2014).

Zhang et al. (2019) investigated the effect of fermented barley extracts with *Lactobacillus plantarum* dy-1 for modulating glucose consumption in HepG2 cells (a human liver cancer cell line) via miR-212 regulation. Moreover, the contribution of miR-212 to the occurrence of palmitate-reduced glucose consumption (insulin resistance) was studied. They reported that fermented barley extract and phenolic acids with significant effects on glucose consumption may have a potential role in the prevention of obesity (Zhang et al., 2019). Zhang et al. (2016) investigated the effect of supplementary *Lactobacillus plantarum* dy-1 fermented barley on obesity in high-fat diet (HFD) induced obese rats. They reported a lower rate of increase in body weight and percentage of body fat and a reversal of HFD-induced glucose intolerance, with ameliorated hyperinsulinemia, decreased levels of triglycerides and total cholesterol, and inhibited concentration of interleukin (IL)-1β, IL-6 and tumor necrosis factor-α (Zhang et al., 2016). Same group also reported in their next study that oral administration of an aqueous extract of fermented barley with *Lactobacillus plantarum* dy-1 significantly prevented body weight gain and fat mass increase, and improved lipid profiles and glucose tolerance in high fat diet-induced obese male rats. In contrast, an aqueous extract of fermented barley with *Saccharomyces cerevisiae* had no significant anti-obesity effects. This report indicates the role of probiotic strain in the final functional properties of the food. They also reported that phenolic acids (mainly vanillic acid and ferulic acid) and β-glucan in fermented barley with *Lactobacillus plantarum* dy-1 were responsible for the lipid accumulating actions and may be considered primary anti-obesity mediators. The data indicated the potential of fermented barley in future strategies for functional supplements against obesity and obesity-related diseases (Zhang et al., 2017).

7. Challenges with barley probiotic food products

The presence of a husk that is difficult to remove and the lack of gluten protein in barley limits its use in leavened bakery products. Barley grain contains considerable amounts of polyphenol oxidase (Sharma and Kothari, 2016). Polyphenol oxidase reacts with phenolic compounds to produce *o*-quinones, which further react with other phenolic compounds or amino acids causing discoloration in various foods made from barley (Lagassé et al., 2006). On the other hand, probiotic bacteria experience several challenges during food processing and storage due to various factors such as acid-base changes, oxidative stress, temperature and molecular entrapments (Trujillo-de Santiago et al., 2012). There is also after-consumption stress of acid and bile in the upper GIT inhibiting the viability of probiotics. Probiotics, therefore, must exhibit high survivability in food products during storage and through the upper GIT. The packaging materials used and the storage conditions under which the products are kept are also important for the quality of products containing probiotic bacteria (Saarela et al., 2000). Survival of Lactobacillus strain at -18°C was poor, showing a decrease of 1 log units in cell count. At -35°C, however, its viability improved, with a decreased cell count to 0 to less than 1 log unit. They were fully stable at -45 °C with no losses.

Storage at room temperature, which is common for several types of non-dairy products such as cereal products and drinks, can create an overwhelming challenge for probiotic stability (Saxelin et al., 1999). It is reported that the survival of *Lactobacillus helveticus* in barley-based fermented milk products decreased during storage at room for 120 days. The decrease in total LAB counts ranged from 6.586 log CFU/mL on 0 days to 5.753 log CFU/mL on 120 days of storage (El-Aidie et al., 2017). Survivability problem in probiotic products can be solved by using encapsulation technology, which provides great potential to protect beneficial bacteria and compounds from undesirable effects of environmental conditions, thus retaining the structural integrity until the time of consumption or administration (Mokhtari et al., 2017a; Pourjafar et al., 2018; Abdolhosseinzadeh et al., 2018; Qi et al., 2020; Misra et al., 2021; Malmo et al., 2021). Encapsulation of probiotic bacteria enhances their survival both in the food during the storage time and in the adverse conditions of the GIT (Mokhtari et al., 2017b; Oberoi et al., 2019; Pourjafar et al., 2020; Zhao et al., 2020; Yao et al., 2020; Zhang et al., 2021). There is a need for the controlled delivery of probiotics and/or bioactive compounds in barley based probiotic products, and few details are available on the performances of these systems in the GIT. Foods used for distribution of probiotics are usually fermented foods, which are produced by a microbial fermentation in which fermentable carbohydrates are transformed into ethanol and/or organic acids mainly acetic, lactic and propionic acids. In fermented probiotic products, it is important that the probiotic culture used contributes to good sensory properties during storage time. Therefore, it is quite common to use probiotic bacteria mixed together with other types of bacteria suited for the fermentation of the specific product (Eningjuha and Badejo, 2017).

8. Conclusion and future aspects

The last decade has witnessed a considerable change in consumer demands for food product. The growing interest of developing healthy and natural foods drives consumer towards a healthy lifestyle and natural diet. Barley as a substrate have a great potential to develop novel probiotic foods that promote the gastrointestinal health, reduce the risk of chronic diseases such as obesity, cardiovascular disease, type 2 diabetes and some cancers. Barley fermentation with specific probiotic strains can lead to significant increase of bio available compounds, and the strain used determines the kind and quantity of the compound to be improved. Addition of barley into the other probiotic beverages also improves cell viability of the probiotic bacteria. Nonetheless, due to the sensitivity of probiotics to the environmental conditions such as those during food manufacture/processing as well as the condition in gastrointestinal, it is a challenge to develop barley probiotic products with desirable shelf life that can maintain both organoleptic properties of the food and viability of the probiotic cells. To formulate a successful barley-based probiotic foods, cell viability, survival and targeted release in the intestine is the important aspects to take into consideration for the bioavailability. Therefore, protection of probiotics using encapsulation technologies such as extrusion, spray drying, coacervation and internal gelation can be recommended in the future studies on barley functional foods.

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Table 1: Variety of dairy-based probiotic products containing barley

Product	**Microorganism**	**Microbial cell count in the product**	**Temperature/ incubation time**	**Obtained results**	**Country**	**Reference**
Fermented Milk	*Lactobacillus helveticus*	6.586 CFU/mL	42˚C/- ND	The fermented milk improved the nutrient value, shelf life and decreased the production cost for the end products	Egypt	El-Aidie et al. 2017
Yogurt	*Bifidobacteriumbifidum*Bb-12	9.42 CFU/g	37 °C/23 h, 42°C/3 h	The survival of *Bifidobacteriumbifidum*was within biotherapeutic level (> 7 log CFU/g) as a result of the prebiotic effect of barley	Turkey	Ozcan and Kurtuldu (2014)
Milk	*Lactobacillus rhamnosus* GR-1	16.9×10^{8} CFU/mL	37.5°C/ 24 h.	The probiotic milk supported the growth of *Lactobacillusrhamnosus*GR-1 at viable levels (10^{8} CFU/mL) during the first 14 days of storage	Canada	Maselli and Hekmat (2016)
Yogurt	*Lactobacillus acidophilus*	7.42-7.77 $\log_{10}$ CFU/mL	42°C / 4 h	Incorporation of barley bran in low-fat yogurt containing *Lactobacillus acidophilus* significantly affected viable probiotic bacteria in comparison with control group	Iran	Hasani et al. 2017
Yoghurt	*Lactobacillus rhamnosus* GR-1	10.4×10^{8} CFU/mL	38 °C / 24 h.	Yoghurt did not have any negative effect on the growth and survival probiotics and had the potential as a vehicle to deliver *Lactobacillusrhamnosus* GR-1 to consumers	Canada	Soltani et al. 2018
Yoghurt	*Bifidobacteriumanimalis* ssp. lactis (Bb-12TM)	ND	42 ˚C /48 h	Supplementation of yogurt with selected prebiotics improved viability and stability of *probiotics* in yogurt during 4-wk cold storage. The barley β-glucan addition	Australia	Vasiljevic et al. 2007

				suppressed proteolytic activity		
Milk	*Lactobacillusplantarum* NCDC344 (Lp344)	8.59 log CFU/mL	37 ˚C/ ND	The optimised drink rated 7.80 on a 9-point hedonic scale, and 0.144 g/100 g of β- glucan	India	Ahuja et al. 2017
Milk	*Lactobacillusparacasei subsp. paracasei B117*	7.93-8.92 log CFU/mL	40 °C/ ND	*Lactobacillus paracasei* showed good compatibility with the yogurt starter culture and the addition of β -glucan enhanced the viability of the probiotic strain in the fermented products throughout cold storage (4 ˚C)	Greece	Lazaridou et al. 2014
Low-fat yoghurt	*Bifidobacteriumlactis Bb-12; Lactobacillus acidophilus LA-5*	9×10^7 CFU/mL	37 ˚C / 24h.	Addition of barley β-glucans improved the formation of flavors in yoghurt. The substitution of fat with β-glucans enhanced sensory attributes of yoghurt, wherein β-glucans -enriched samples recorded high score and acceptability	Egypt	Elsanhoty and Ramdan (2018)
Yoghurt	*Lactobacillus acidophilus, Bifidobacteriumlactis* Bb12	10^7 CFU/g	40 °C/ ND	Values of carbohydrate, volatile fatty acids, unsaturated fatty acids, antioxidant activity were higher in milk supplemented with barley flour; addition of vanilla (0.1%) or cocoa powder (0.5%) improved the sensory properties	Egypt	Ismail et al. 2018

ND: Not defined

Table 2: Non-dairy probiotic products containing barley

Product	**Microorganism**	**Microbial cell count/**	**Temperature/ incubation time**	**Obtained results**	**Country**	**Reference**
Beverage	*Lactobacillus rhamnosus*GG	6.68–7.58 log CFU/g	37°C / 10 h	Barley flour fermented in water produced the highest probiotic culture density for *Lactobacillus rhamnosus*GG when compared to other cereal-grain flours	Slovakia	Kocková and Valik (2014)
Beverage	*Lactobacillus plantarum* NCIMB8826, *Lactobacillus acidophilus* NCIMB 8821	7.9 - 8.5 Log_{10} CFU/mL	30 ˚C / 28 h	LAB growth was enhanced in media containing malt and (0.5-3.5 g/L) of lactic acid were produced	UK	Rathore et al. 2012
Beverage	*Bifidobacteriumadolescentis NCIMB 702204, B. infantis NCIMB 702205,B. breve NCIMB 702257, B. longum NCIMB 702259*	8.73-9 $\log_{10}$ CFU/mL	37˚C/ 24–36 h	The results showed an increase in bacterial population between 1.5 and 2.0 log10 cycleswith a maximum growth rate of approximately 0.2 per hour	UK	Rozada-Sa´nchez et al. 2008
Beverage	*Lactobacillus paracasei, Lactobacillus delbrueckii*	1.2×10^6 CFU/mL	37 °C /24 h	Significant decrease in pH value to 4.25 and a considerable increase in titratable acidity level to 2.96 g/100 g lactic acid were obtained by initial 6h fermentation of *Lactobacillus paracasei* on malt medium	Iran	Salari et al. 2015
Probiotic drink	*Lactobacillus acidophilus* *Bifidobacteriumlactis* Bb12; *Bifidobacteriumlactis* Bb12	8.1- 8.60 log CFU/mL	37 ˚C / 48 h	Acidity, pH, probiotic count, level of antioxidants and polyphenols increased as the concentration of drink mixture increased. Germinated probiotic drink had higher	India	Chavan et al. 2018

				values of Total phenolic content		
Beverage	*Lactobacillus acidophilus* NCIMB 8821;*Lactobacillus plantarum* NCIMB 8826; *Lactobacillus reuteri* NCIMB 11951	7.73 ± 0.08-8.20 ± 0.07 CFU/mL	37 °C/ 20 h	The beverage formulated with *Lactobacillus plantarum* and malt substrate exhibited greater acceptance and it encompassed the highest concentration of acetaldehyde	UK	Salmeron et al. 2015
Porridge	*Lactobacillus reuteri* SD 2112; , *Lactobacillus acidophilus* LA5; *Lactobacillus acidophilus* NCDO 1748; *Lactobacillus rhamnosus* GG (ATCC 53103)	7.2 – 8.2 log CFU/g	37 °C / 24 h	Small amounts of diacetyl, were detected in porridge inoculated with *Lactobacillus acidophilus* 1748 and *Lactobacillus acidophilus* LA5	Norway	Helland et al. 2004
Food Mixture	*Lactobacillus acidophilus*	8.88 CFU/g	37 ˚C / 12 h	Improvement in reducing sugar, thiamine, niacin, lysine and soluble dietary fibre contents of barley based food mixtures	India	Arora et al. 2010
Probiotic drink	*Lactobacillus acidophilus*	9.10 - 11.32 log CFU/mL	37 ˚C / 8h	Acidity (in terms of lactic acid) and pH in probiotic drink samples ranged from 0.45 to 1.02% and 4.11 to 4.49	India	Mridula and Sharma (2015)

Table 3: *In Vivo* and *in Vitro* studies on the physiological effects of barley-based probiotics

Types of disease or disorder	**Product**	**Probiotic strains**	**Probiotic outcome/ results**	**Subject**	**Country**	**Dose level**	**References**
Coronary heart disease	Indigenous food mixture	*Saccharomyces boulardii*; *Lactobacillus casei*	Serum cholesterol and LDL cholesterol concentrations	Mice	India	NA	Sindhu and Khetarpaul (2003)
Hypocholesterolemic impact	Yoghurt	*Streptococcus salivarius* subsp. *Thermophiles*; *Lactobacilliusdulbrueekii* sub sp. *Bulgaricus*	Lowering of plasma and liver cholesterol levels	Rats	Egypt	NA	Ahmed et al. 2017
Metabolic syndrome (MetS) related diseases (obesity and type 2 diabetes)	Probiotic yoghurt (Activia); Probiotic tablet: (Probiomax) Probiotic tablet: (Probimage)	*Bifidobacteriumanimalis* DN-173 010; *Lactobacillus reuteri*DSM 17938; *Lactobacillus plantarum* 299v	Postprandial glycemic regulation and increased plasma concentrations of gut hormones important to metabolic regulation and appetite control	Mice	Sweden	200 ml/day; 20 ×10^9 CFU/day 10×10^9 CFU/day 0.1×10^9 CFU/day	Nilsson et al. 2016
Gastrointestinal diseases	ND	*Lactobacillus reuteri*	Strain showed great resistance to GIT conditions, including strong adherence to HT-29 cells and inhibitory activity against *E. coli*, *Shigellaflexneri*, *Salmonella paratyphi* β, and *S. aureus*.	Mice	China	_	Chen et al. 2018

Inflammatory bowel disease, ulcerative colitis and Crohn disease	ND	*Clostridium butyricum*	Suppresses the Dextran Sulfate Sodium-induced Experimental Colitis	Rats	Japan	NA	Araki et al. 2000
Shigelosis and infectious diarrhoea	Beverage	*Lactobacillus acidophilus* NCDC 13	Reduction in *Shigella* induced pathogenicity	Murine	India	5g/animal /day	Ganguly et al. 2019
Intestinal diseases	ND	*Lactobacillus rhamnosus* MA27/6B; *L. acidophilus* MA27/6R	Ability to survive feed processing and intestinal tract conditions; they have no antibiotic resistance-linked plasmids, adhere to Caco-2 cells and have a wide anti-microbial spectrum	Swiss mice	France	NA	Bernardeau et al. 2002
Intestinal diseases	Food mixture	*Lactobacillus acidophilus*	Alleviation of kidney and liver lesions caused by *E. coli* infection	Mice	India	NA	Jood et al. 2012
Inflammation of the colon	ND	*Lactobacillus rhamnosus* 271; *Lactobacillus paracasei* 87002; *Lactobacillus plantarum* HEAL 9 and 19; *Bifidobacterium infantis* CURE 21	Decrease in the caecal and portal levels of acetic acid, amino acids (glycine, proline, asparagine and phenylalanine) in the portal blood of rats also increased	Rats	Sweden	0.82 g/day	Zhong and Nyman (2014)

NA: Not applicable; ND: Not defined

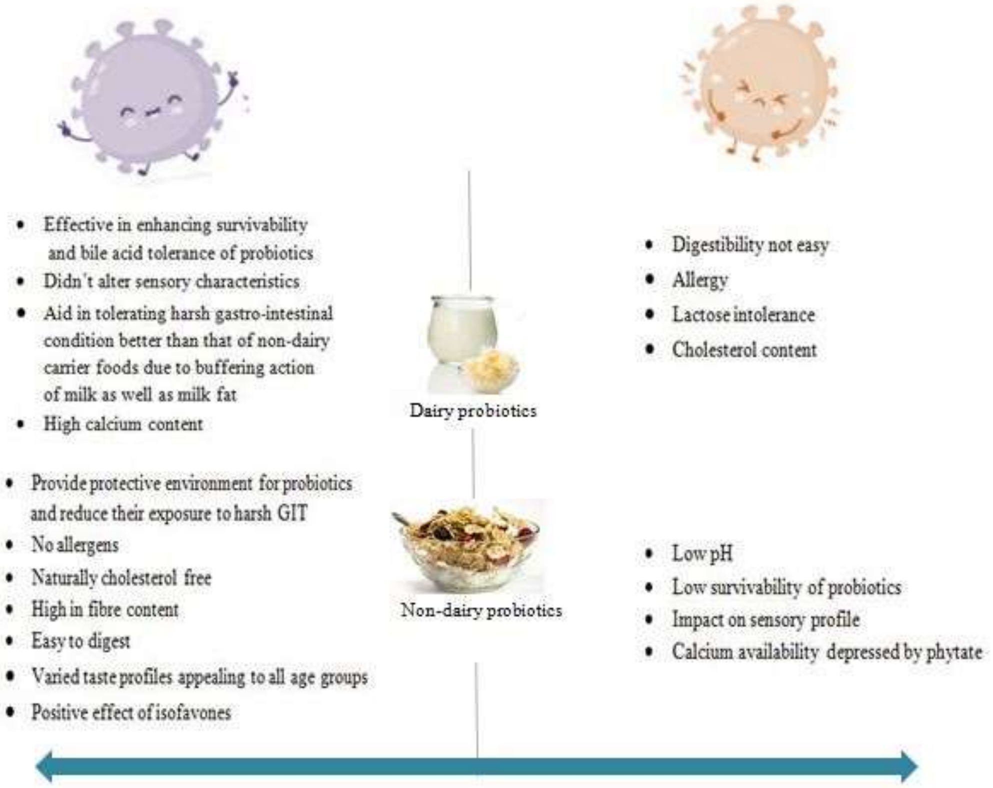
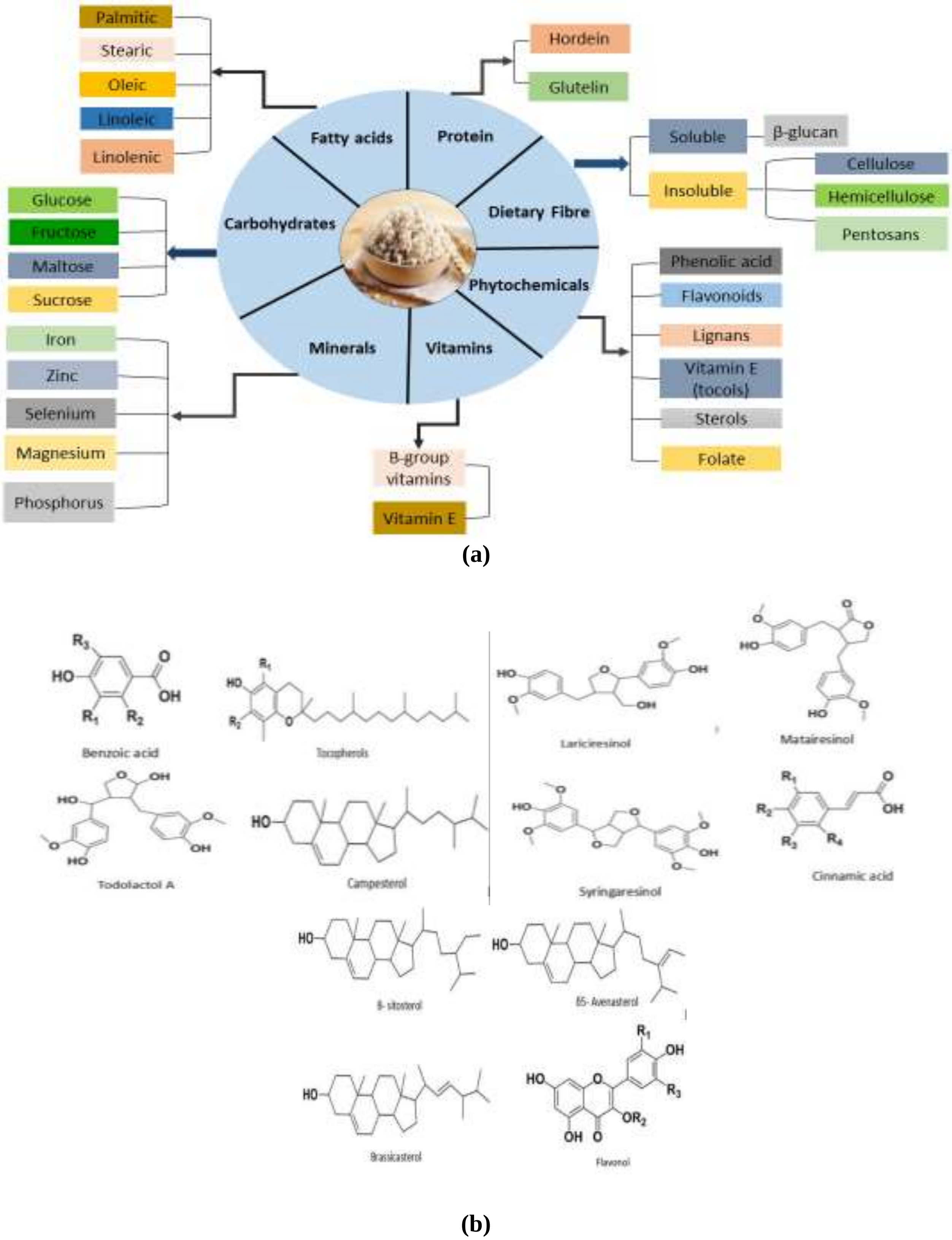


Figure 1. Comparative advantages and disadvantages of dairy and non-dairy probiotic foods



(a)

(b)

Figure 2. Representation of (a) nutritional profile, and (b) bioactive compounds of barley

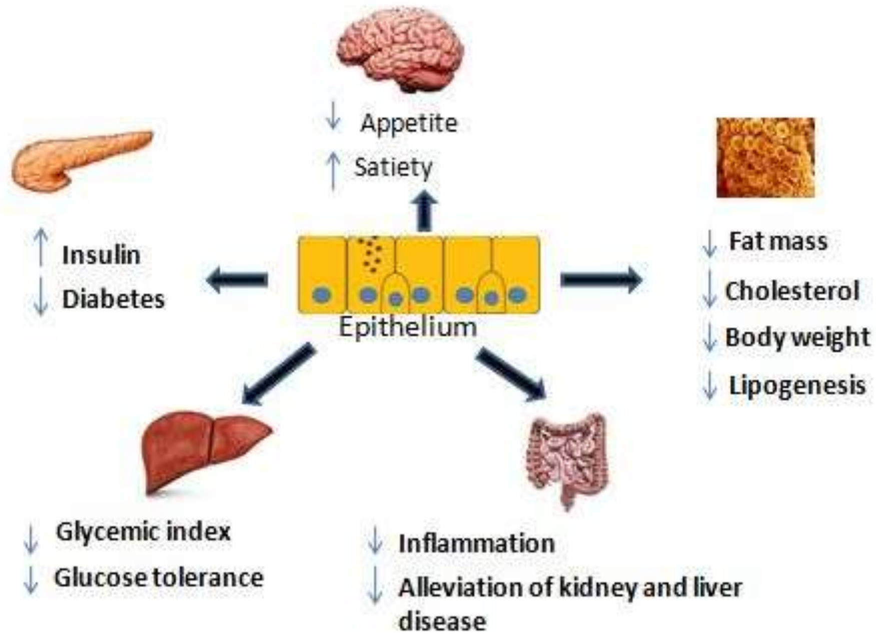


Figure 3. Modulation of intestinal microbiota and physiological effects upon consumption of barley-based probiotic food mixture