

# Role of Probiotic Bacteria on Bioavailability **10** of Functional Ingredients Under Fermentation Process

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#### Abstract

Consumer attention to consume healthier foods has been significantly encouraged the food industry to formulate new products within the area of so-called functional foods. Functional foods are defined as whole foods, enriched, enhanced, and fortified foods or dietary compounds that in addition to traditional nutrient contents possess healthy and physiological benefits. Food products containing probiotics compromise the majority of functional food market worldwide. This chapter focuses on the bioactive compounds produced in different probiotic fermented food matrices and investigates how these metabolites and fermentation conditions affect the bioavailability of different compounds in foods.

## Keywords

Probiotic · Functional foods · Bioactive compounds · Postbiotics · Dairy products

## 10.1 Introduction

Probiotics are defined as live microorganisms which when ingested in adequate numbers (at least  $10^6_{-}10^7$  CFU/ml) impart health benefits to the host and include mainly *Lactobacillus* and *Bifidobacterium* genera but some other bacteria and yeast species are also considered as probiotics (de Melo Pereira et al. 2018; George Kerry et al. 2018; Meira et al. 2015; Morton 2015) (Table 10.1). Probiotics have

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| Category and      |   |
|-------------------|---|
| genus             | Species   |
| Bacteria          |   |
| Lactobacillus     | Lb. acidophilus, Lb. amylovorus, Lb. brevis, Lb. casei, Lb. curvatus,       |
|                   | Lb. crispatus, Lb. delbrueckii subsp. bulgaricus, Lb. fermentum,            |
|                   | Lb. helveticus, Lb. gasseri, Lb. johnsonii, Lb. reuteri, Lb. rhamnosus,     |
|                   | Lb. salivarius, Lb. paracasei, Lb. plantarum                                |
| Bifidobacterium   | B. adolescentis, B. animalis, B. bifidum, B. lactis, B. breve, B. infantis, |
|                   | B. longum, B. thermophilum, B. essensis, B. laterosporus                    |
| Streptococcus     | S. cremoris, S. diacetylactis, S. intermedius, S. salivarius                |
| Propionibacterium | P. freudenreichii, P. freudenreichii subsp. shermanii, P. jensenii          |
| Enterococcus      | E. faecalis, E. faecium   |
| Lactococcus       | L. lactis subsp. cremoris, L. lactis subsp. lactis                          |
| Other bacteria    | Pediococcus acidilactici, Leuconostoc mesenteroides, Bacillus cereus,       |
|                   | Clostridium butyricum, Escherichia coli Nissle 1917                         |
| Yeast             | Kluyveromyces lactis, Saccharomyces boulardii, Saccharomyces cerevisiae     |

 Table 10.1
 List of some important probiotic microorganisms (Morton 2015)

anticarcinogenic antimutagenic activities and are able to suppress cholesterol level and blood pressure. They improve digestive system function, epithelial homeostasis, nutrient uptake, intestinal barrier function, immune modulation, and antagonism action against pathogens (Liptáková et al. 2017; Marhaida et al. 2015).

Traditionally, the effectiveness of probiotics was assumed to be related to cell viability. Apart from probiotic cells, bacterial products may have similar benefits to the host. These products are characterized as postbiotics which have biological activity in the host cell (George Kerry et al. 2018; Wegh et al. 2019). Postbiotics are generally regarded as functional fermentation products and include a wide range of metabolites such as bacteriocins, enzymes, vitamins, amino acids, oligosaccharides, exopolysaccharides, short-chain fatty acids, and immunomodulatory compounds (George Kerry et al. 2018; Zielińska and Kolożyn-Krajewska 2018).

In other words, the functionality of probiotics in fermented foods is accomplished in different ways which eventually affect the nutritional quality of foods which include: 1) increase of nutrient density, mostly due to a decrease of sugar content, 2) hydrolysis of polymers from the raw material and bioactive compounds content, 3) biosynthesis of bioactive molecules, 4) degradation of toxic or anti-nutritional factors, and 5) synthesis of promoters for absorption and uptake (Septembre-Malaterre et al. 2018; Tamang et al. 2016).

## 10.2 Probiotic Fermentation of Foods

Food fermentation is considered as one of the oldest ways of food processing and preservation. Fermentation results in the enhancement of the flavor and nutritional quality of food and extending its shelf life (Beena Divya et al. 2012). Fermentation is microbe-driven process in which the low value substrates are converted to addedvalue products (Hussain et al. 2016; Sadh et al. 2018). According to scientific data, both nutritive and non-nutritive components are in fermented foods which could potentially implement specific target functions in the body relevant to well-being and health of the consumers (Tamang et al. 2016). Probiotic bacteria as functional microorganisms, in fermentation process, convert the chemical constituents of raw materials of plant/animal sources leading to the enhancement of the bioavailability of nutrients, enrichment of sensory quality of the food, improvement of food safety, degradation of toxic components and anti-nutritive factors, production of antioxidant and antimicrobial compounds, stimulation of the probiotic functions, and fortification with some health-promoting bioactive compounds (Homayoonfal et al. 2018; Mousavi and Mousavi 2019; Rollán et al. 2019). In fact, the probiotic microorganisms promote beneficial effects in a host which are due to the production of bioactive compounds (Indira et al. 2019).

These bioactive compounds play an important role in bio-preservation of fermented food products including dairy, fish, seaweeds, microalgae, beverages, and fruits and vegetables (Mousavi and Mousavi 2019). Additionally, they show antimicrobial activities against food pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus* and *Enterococcus faecalis*. In addition to their antimicrobial properties, these metabolites can be aromatic which can influence the sensory and organoleptic features of food products . Some peptides with health benefits are also produced as bioactive compound in fermentation of and prevent diseases associated with metabolic syndrome (Indira et al. 2019; Ojha and Tiwari 2016) (Table 10.1).

## 10.3 Production and Modification of Bioactive Compounds Over Probiotic Fermentation

Bioactive compounds as result of probiotic fermentation have two major sources. The first source is direct synthesis of the compound by the probiotic such as bacteriocins, exopolysaccharides (EPS), or enzymes and they can be found in either supplements or foods. The second source of bioactive is a compound that only appears as a result of the modification of the food matrix itself by the probiotic culture fermentation (Champagne et al. 2018). The following section will discuss the bioactive compounds produced during probiotic fermentation and their effect on food bioavailability.

#### 10.3.1 Bioactive Peptides

Bioactive peptides are short sequences of amino acids generally consisting from 2 and 20 amino acids. Such sequences stay intact and inactive when present in the parental protein, but can be released after protein hydrolysis during gastrointestinal digestion (GID), *in vitro* enzymatic hydrolysis, or microbial fermentation. These peptides have biological activities that may influence human health in addition to basic human nutrition (Erdmann et al. 2008). Cardioprotective functions, modulation of immune system, anti-atherosclerosis, antioxidant, mental health, and general well-being functions are associated with bioactive peptides (Ojha and Tiwari 2016; Septembre-Malaterre et al. 2018). According to various researches, it is concluded that microbial fermentation could be regarded as an appropriate approach improving protein bioavailability and digestibility in different food products (Chi and Cho 2016; Hur et al. 2014; Limon et al. 2015; Wu et al. 2015).

#### 10.3.1.1 Dairy Products

Milk-proteins and associated bioactive peptides released during microbial or enzymatic fermentation of milk offer a broad spectrum of new functional properties, for instance antihypertensive, antimicrobial, antioxidative, immunomodulatory, opioid, and mineral-binding properties (Beermann and Hartung 2013).

Calcium casein phosphopeptides (CCP) are phosphorylated bioactive peptides derived from calcium-sensitive caseins ( $\alpha$ s1,  $\alpha$ s2, and  $\beta$  caseins). These peptides are inactive fragments entrapped in the sequence of precursor protein, and exhibit biological action after its release during the passage through the gastrointestinal tract. In addition, they are also produced in vitro by the action of specific enzymes during fermentation of a number of dairy-based products such as cheese, yogurts, and fermented milks (Ledesma-Martínez et al. 2019; Mohanty et al. 2016). The main activities of CCP include anticancer, body fat reduction, prevention of cardiovascular diseases through the reduction of atherosclerosis lesions and levels of cholesterol and triacylglycerides, anti-inflammatory, and antioxidant. A great number of studies approved the role of CCP on calcium, iron, and zinc (Ledesma-Martínez et al. 2019).

The effect of peptidases activity of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophiles* on milk proteins resulted in the production of antimicrobial and hypotensive peptides. These small biological peptides can be used as food supplements to improve the health-promoting qualities of liquid and semisolid dairy foods prepared by the yogurt fermentation process (Paul and Somkuti 2009).

Investigations revealed that probiotic LAB such as *Lactobacillus helveticus* produces bioactive peptide like, proline-containing peptides isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP) which may induce greater availability of calcium (Dubey and Patel 2018). The study on the level of level of calcium, magnesium, phosphorus, and zinc absorption in a series of fermented goat and cow milk showed that the bioavailability of minerals was significantly higher compared with non-fermented milks (Bergillos-Meca et al. 2013).

Oxidative damage caused by various free radicals which are by-products of physiological reactions within human body can be protected by antioxidants. It has

been found that yogurt and fermented milks have a higher antioxidant activity than milk. In fermented milks, bioactive peptides are released following the proteolysis of milk proteins, especially lactalbumin, lactoglobulin, and casein (Melini et al. 2019). Yogurt produced with camel milk by fermentation with *Lactobacillus rhamnosus* strain PTCC 1637 has a higher antioxidant activity than cow milk, because of the higher proline content in camel milk caseins. The presence and position of the amino acids tryptophan, tyrosine, and methionine in the peptides are claimed responsible for the antioxidant activity of fermented milks as well.

#### 10.3.1.2 Fruits, Vegetables, Legumes, and Grains

Various studies showed that probiotic fermentation of nondairy foods including vegetables, fruits, legumes, and grains could enhance the level of protein, peptides, and amino acid in these products (Septembre-Malaterre et al. 2018). Bioactive peptides have been mainly studied from milk or whey hydrolysis during lactic fermentation. However, different studies on fermented soybeans, grapes, and cereal flours also showed a significant increase in their bioactive contents (Septembre-Malaterre et al. 2018). Probiotic LAB are naturally present in legume grains; they have also been traditionally used for legume fermentation. Evidences showed that fermentation of legumes with *Lactobacillus* genera can encourage the production of bioactive compounds, improving health benefits beyond basic nutrition. Fermentation of cowpeas with *Lactobacillus plantarum* resulted in the modification of phenolic compounds and improvement of antioxidant activity (Dueñas et al. 2005; Limon et al. 2015).

*L. plantarum* B1-6 has been studied for its potential proteolysis effect on mung bean protein during fermentation. Electrophoresis profiles revealed that *L. plantarum* B1-6 degraded Mung bean proteins with the hydrolysis percentages between 49 and 64%. In addition, reverse phase high-performance liquid chromatography (RP-HPLC) analysis showed that larger/more hydrophobic peptide contents decrease the amount of smaller/more hydrophilic peptides has substantially augmented after fermentation (Wu et al. 2015). In addition, the degradation of gluten could render the final product to be suitable for celiac consumers (Heredia-Sandoval et al. 2016; Houben et al. 2012; Poutanen et al. 2009; Verni et al. 2019). Cereals are in general good sources of proteins. The proportions of essential amino acids and their digestibility mainly determine protein nutritional quality. Peptidase enzymes produced by LAB convert peptides to amino acids. Specific products of these enzymes are responsible for the aroma and taste of final products (Pessione and Cirrincione 2016; Verni et al. 2019).

Solid-state fermentation of whole soybeans by *Lactobacillus plantarum P-8* mixed with *B. subtilis natto* also resulted in an intensive protein degradation and generation of hydrophilic peptides during fermentation (Pessione and Cirrincione 2016; Zhang et al. 2014).

Various scientific reports stated that hydrolyzed peptides produced by probiotics during fermentation can act as antioxidants (Coda et al. 2012; Raveschot et al. 2018; Taha et al. 2017). Rapeseed proteins are hydrolyzed to amino acids and peptides by proteases produced by probiotic *Bacillus subtilis* during fermentation (Rong et al.

2012). Hydrolysis of peptide bonds enhances the levels of free amino and carboxyl groups, resulting in increased solubility. This enhanced solubility may improve the antioxidant activity of the peptide (Karami and Akbari-Adergani 2019; Sohaib et al. 2017). Low-molecular-weight peptides have been reported to exhibit better radical-scavenging activities than their high-molecular-weight counterparts (Xie et al. 2008). Thus, increasing the low-molecular-weight peptides by enzymatic hydrolysis may influence the antioxidative activity during fermentation. Metal-chelating amino acid residues, such as methionine, glutamic acid, glutamine, lysine or arginine, within the sequences of these peptides contributed to the superior Fe<sup>2+</sup>-chelating ability of the antioxidant peptides (Hur et al. 2014).

According to different studies, proteolytic activity of probiotic LAB could enhance the level of bioactive peptides in fermented cereals. Antihypertensive properties are attributed to these bioactive peptides. In addition, thanks to the production of flavoring free amino acids and other amino acid derivatives during fermentation which convey tastiness to fermented cereals such as bread, it is possible to decrease salt content in the final product (Melini et al. 2019).

#### 10.3.1.3 Fish

Large quantity of liquid and solid waste generated by fish industry can be regarded as a potential resource for valuable products. Due to their high protein contents, fish waste could be used as a suitable medium for culturing probiotic bacteria. Fermentation of fish waste can partially degrade the protein contents, which could help the absorption from the gut and influence its bioactive properties through the production of bioactive peptides (Venegas-Ortega et al. 2019).

## 10.4 Digestible Saccharides

## 10.4.1 Fruits and Vegetables

Fruits and vegetables are a rich source of sugars. During fermentation of fruits and vegetables, monosaccharide are significantly consumed by probiotic bacteria specially LAB species. However, with the help of glucosidases and glycosyl hydrolases produced from the cells, hydrolysis of polysaccharides occurs, which release monomers of sugars. Release of monomers contributes to the increase in nutrient density of the fermented products (Ojha and Tiwari 2016).

## 10.4.2 Cereals

*Lactobacillus* species are the predominant organisms involved in the fermentation of cereal-based foods and beverages in African countries (Richard and Jooste 2012). A multiple of researches showed that cereal fermentation is considered as a significant potential in improvement and design of the nutritional quality and health effects of foods and ingredients (Rollán et al. 2019). Cereal grains are primarily a source of

carbohydrates, and thus a good source of energy. However, a high proportion of starch in cereals is in the form of amylopectin, which is not completed digested and absorbed in the small intestine. Digestible polysaccharides are produced as a result of probiotic lactic acid fermentation of cereal, which are more accessible to gut microbiota. (Liptáková et al. 2017).

## 10.5 Exopolysaccharides (EPS)

EPS are secondary metabolites with long chain of homo or hetreo-polysaccharides containing repeated units of sugars or sugar derivatives. These polysaccharides are produced outside of the cell. Depending on the carbon source, LAB belonging to the genera *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus,* and *Weissella* are able of producing a variety of EPS (Zeidan et al. 2017).

## 10.5.1 Dairy

In dairy-based foods, extracellular polysaccharides (EPS) are produced from ropy probiotic cultures (*Streptococci, lactobacilli, and lactococci* strains) (Prasanna et al. 2012). These EPS could improve physicochemical and rheological properties of foods. In addition, they may also protect cells to against phage attack, desiccation, and osmotic stress, thus behaving as prebiotics and improve immunity to fight against pathogenic organisms (Ruas-Madiedo et al. 2002). In addition, blood cholesterol-lowering, immunostimulatory, antitumoral, and antiulcer activity have been also attributed to EPS produced in fermented probiotic dairy products (Madhuri and Prabhakar 2014; Shao et al. 2014).

## 10.6 Galacto-Olygosaccharides (GOS)

GOS are non-digestible carbohydrates and comprise a chain of galactose units usually with a terminal glucose unit. They are derived from lactose by the action of  $\beta$ -galactosidase enzyme in a trans-galactosylation reaction that occurs simultaneously with the hydrolysis. These bioactive compounds can be synthesized by probiotic microorganisms in fermented products during processing (Otieno 2010). In the case of use of probiotics as enzyme sources for GOS synthesis, they could provide the double advantage as probiotics as well as in prebiotic. GOS are fermented by the beneficial gut microflora of the large intestine resulting in the inhibition of pathogenic and putrefactive bacteria growth. Therefore, the level of toxic metabolites is significantly decreased which could prevent diarrhea, constipation relief, and lactose tolerance. Also, metabolism of GOS results in the production of short-chain fatty acids which could assist in increased calcium and magnesium absorption, control of serum lipid and cholesterol level, and reduction of cancer risk (Davani-Davari et al. 2019).

## 10.6.1 Dairy

Milk sugar (lactose) is a component of dairy by-products especially from whey which is half consumed by human and animals and the remaining is generally discarded. Various reports used different probiotic strains specially *Lactobacillus* and *Bifidobacterium* species to produce GOS from milk, cheese, whey, and yogurt which can be used as a suitable substrate for GOS synthesis. Therefore, fermented dairy-based foods could be considered as the main carrier of GOS (Lappa et al. 2019; Sabater et al. 2018; Song et al. 2013). There are many parameters affecting the synthesis of these compounds such as  $\beta$ -galactosidase enzyme source and concentration, type and counts of microorganisms, concentration of substrate (lactose), composition of food matrix, conditions of fermentation and storage, and time/ temperature of hydrolysis/transgalactosylation (Morton 2015).

## 10.7 Conjugated Linoleic Acid (CLA)

CLA is a collective term used to describe a heterogeneous mixture of positional and geometric isomers of octadecadienoic acid or linoleic acid (c9,c12-C18:2) in which double bonds are conjugated (cis-, trans-, or mixed configurations). Biological and biochemical roles attributed to CLA include anticancer, body fat reduction, prevention of cardiovascular diseases through the reduction of atherosclerosis lesions and levels of cholesterol and triacylglycerides, anti-inflammatory and antioxidant. Linoleate isomerase (LAI) enzyme is responsible of CLA synthesis, which is bond to the cell membrane of microorganisms. CLAs exert various health benefits and their effectiveness depends on CLA isomer form. Studies demonstrated that trans-9, trans-11 C18:2 has a much higher inhibitory and antiproliferative effect on the growth of the human colon and breast cancer cells, than cis9, trans-11 CLA isomer (Beppu et al. 2007; El Roz et al. 2013; Park 2009).

In contrast, the results of other studies showed that cis-9, trans-11 CLA has extra beneficial effects, such as anti-inflammatory and antiatherogenic effects (Tricon et al. 2006). However, the mixture of the two CLA isomers (cis-9, trans-11 and trans-9, trans-11 CLA) had a synergistic anti-proliferation effect on a human colorectal carcinoma cell line (Zhong et al. 2012).

## 10.7.1 Meat Products

CLA is a compound found mainly in the meat of ruminants that is recently the subject of many researches due its health-promoting properties, i.e., antiatherogenic, cancer inhibition, anti-diabetic, obesity lowering, and improved immunity (Mulvihill 2002). In a detoxification mechanism, some probiotic bacteria of *Lactobacillus* and *Bifidobacterium* types are able to change fatty acid profile in meat sausages by converting polyunsaturated fatty acids into CLA through isomerization, hydrogenation, and dehydration (Galgano et al. 2015).

## 10.7.2 Dairy

In some countries, liquid milk, powdered milk, fermented milk, yogurt, and cheese enriched in CLA are marketed. On the other hand, the known fact that several strains of bacteria possess the ability to synthesize CLA in vitro in the presence of precursor substrate raised the possibility for increasing the production of CLA *in situ* during manufacture of fermented dairy foods. The co-culture of *L. rhamnosus* and yogurt starter in the presence of hydrolyzed soy oil as the lipid source showed that CLA contents significantly increased in the final fermented (Xu et al. 2005). A study performed by Ribeiro et al. (2017) showed that *Lactobacillus plantarum* isolated from Pico cheese exhibited probiotic properties and presented the highest production of both cis-9, trans-11 and trans-9, trans-11 CLA isomers, exhibiting a great potential for application in health-promoting food product.

## 10.8 Short-Chain Fatty Acids (SCFA)

SCFA such as such as butyrate, acetate, propionate, and lactate are secondary metabolites released from the hydrolysis of food fiber and non-digestible carbohydrates in gut by probiotic bacteria and are used as a source of energy for colon cells. In humans, 10% of the daily caloric requirement is from short-chain fatty acids produced in large intestine. Among all short-chain fatty acids, 60–70% of the energy is from butyrate produced in colonocytes. SCFAs, particularly butyrate, have a therapeutic effect in various diseases such as inflammatory bowel disease, antibiotic-associated diarrhea, colon cancer, and heart diseases (Indira et al. 2019; Septembre-Malaterre et al. 2018).

According to different researches, the increase of Ca bioavailability by probiotics would definitely satisfy the bone health. The mechanism behind the increase in Ca bioavailability and ensure the bone health is that the probiotics produce short-chain fatty acids, which increase the solubility of available calcium. Simultaneously, the level of the para-thyroid hormone level (increased PTH level causes the Bone resorption by stimulating the osteoclasts) decreases and minimizes the bone loss (Dubey and Patel 2018).

## 10.9 Vitamins

Vitamins play an important role in regulating the intestinal metabolism and absorption of minerals. Calcium absorption is enhanced in the presence of Folate and vitamin C, D, and K (Kiela and Ghishan 2016). Probiotics are associated with the synthesis of vitamins and increase the metabolism and absorption of available calcium (Parvaneh et al. 2014; Whisner and Castillo 2018). Therefore, food fermentation with probiotic bacteria could result in an increased vitamin content of the final product (Richard and Jooste 2012). Probiotic LAB are able of producing B vitamins

including niacin (B3), panthothenic acid (B5), folic acid (B9), and also vitamins B1, B2, B6, and B12 (Capozzi et al. 2012; Septembre-Malaterre et al. 2018).

## 10.9.1 Fruits and Vegetables

Vitamin B12 deficiencies in plant based-diet forced researchers to investigate potential ways to fortify plant-based foods with vitamin B12 (Chamlagain 2016; Melini et al. 2019) Cereal-based products such as Ogi, Mageu, and Kenkey, which are considered as traditional fermented products in Africa, have been reported to have an improved B-vitamin content. Beside probiotic LAB benefits in the enrichment of foods with vitamins, they may lower production costs by eliminating the need to add synthetic vitamins (Rollán et al. 2019). A study performed by Varmanen et al. (2016) showed that *L. reuteri* can be used for vitamin B12 fortification in soy-yogurt.

## 10.9.2 Dairy

Folate, as an essential vitamin, plays an important role in human life for the synthesis of nucleotides, vitamins, and some amino acids. However, this vitamin could not be synthesized by human and have to be taken by daily diet. Dairy products, especially yogurt, are an appropriate choice for bio-fortification of folate as they contain folatebinding protein which improves folate bioavailability. It is reported that the use of folate-producing probiotic bacteria in combination with *S. thermophilus* and/or *L. bulgaricus* provides the largest increase in folate during the fermentation process of probiotic yogurt compared to original milk and conventional fermented milk (Rad et al. 2016). The level of vitamin B12 is significant in dairy products. This vitamin is necessary for the maintenance of the nervous system and the formation of blood cells. Fermentation by probiotic bacteria could increase its content up to 10-folds (Melini et al. 2019).

## 10.10 Enzymes: Anti-Nutrient Degradation

Food fermentation is considered as an important part in food detoxification. Probiotics LAB are able to metabolize anti-nutrient compounds including phytates, trypsin inhibitors, saponins, tannins, cyanogens, or phenolic compounds in foods. This effect can be associated with modification of minerals bioavailability (Septembre-Malaterre et al. 2018).

## 10.10.1 Phytates

According to clinical investigations, it has been found that vegetarians may suffer from nutritional deficiencies and, specially, they have an impaired absorption of



Fig. 10.1 Effect of fermentation on minerals, phytochemicals, and proteins bioavailability of foods

trace minerals, such as zinc, iron, and calcium, proteins, vitamin  $B_{12}$ , and folate (Bergillos-Meca et al. 2013; Masum Akond et al. 2011; Popova and Mihaylova 2019; Rekha and Vijayalakshmi 2010). This malabsorption syndrome may cause severe health-threatening diseases ranging from anemia to neurological disorders and immune deficiency (Hunt 2003). It is postulated that this intestinal malabsorption of minerals is due to the high content of phytate in cereals, nuts, legumes, and oilseeds. Furthermore, it accounts from 60% to 90% of total phosphorus content in cereals and is, therefore, the major storage compound for phosphorus (Gupta et al. 2015). Phytate is able of chelating nutritionally important cations such as Ca<sup>2+</sup>, Mg<sup>2+</sup>, Fe<sup>2+</sup>, and Zn<sup>2+</sup>, thus decreasing the dietary bioavailability of these nutrients.

Intestinal microfloras, especially LAB, are an important source of phytase with high activity. The consequence of phytate hydrolysis by LAB in gut is the release of phosphate, other metal ions and proteins through the degradation of complexes formed by phytate (Dubey and Patel 2018; Famularo et al. 2005) (Fig. 10.1). Various studies have approved an improvement in mineral bioavailability by different probiotic microorganisms used in the fermentation process (Bergillos-Meca et al. 2013). Daily diet enriched with probiotic lactic acid bacteria could minimize phytate or phytic acid in plants. The fermentation of bran with probiotic LAB could provide optimal pH conditions for enzymatic degradation of anti-nutritional factors induced by the degradation of phytate (up to 90%). This results in better bioavailability of minerals (Lopez et al. 2001; Rollán et al. 2019). According to researches, Ca absorption is related to pH in the colon (Diaz de Barboza et al. 2015; Rekha and Vijayalakshmi 2010). Calcium is a divalent cation which salt form is available in food. The soluble and ionized form of Ca is absorbed. Phytate and oxalate in a diet form insoluble salts with calcium and inhibit the calcium absorption (Dubey and Patel 2018). Fermentation of soymilk with five strains of probiotic lactic-acid bacteria (L. acidophilus B4496, L. bulgaricus CFR 2028, L. casei B1922,

*L. plantarum* B4495, and *L. fermentum* B4655) with the yeast *Saccharomyces boulardii* made Ca more soluble (Parvaneh et al. 2014; Ramsubeik et al. 2014; Rekha and Vijayalakshmi 2010). In a study performed by Lorusso et al. (2017), evaluations showed that the minerals bioavailability in quinoa-based pasta flour fermented by selected LAB with phytase activity substantially augmented. A similar study (Rizzello et al. 2016) reported that phytase activity of quinoa sourdough has increased 2.75 times after fermentation with autochthonous LAB (*L. plantarum T6B10* and *L. rossiae T0A16*).

## 10.10.2 Phenolic Compounds

Phenolic compounds as secondary metabolites produced by plants are widely used as dietary supplements and have numerous biological and pharmacological effects such as anticancer, antioxidative, antiviral, anti-inflammatory, and antiatherogenic activities (de Souza et al. 2019; Hur et al. 2014; Rollán et al. 2019). Many phenolic compounds occur in food as esters, glycoconjugates, or polymers, which are not directly bioavailable (Rossi et al. 2013). According to estimations,, as little as 5-10% of total ingested phenolic compounds can be absorbed in the small intestine, whereas 90-95% reach the colon because of insufficient gastric residence time, low permeability or solubility in the intestine (de Souza et al. 2019). The evidences showed that the gut microbiota are major responsible of polyphenols biotransformation into more biologically active components (de Souza et al. 2019; Pereira-Caro et al. 2018). Enzymatic activity of intestinal bacteria able to catabolize phenolics could results in the production of various compounds with different bioavailability and biological functions to their parent compounds (Dudonné et al. 2015) As oligoand polysaccharides bounded to phenolic compounds are the major carbon sources for saccharolytic fermentative bacteria, in the first step of phenolic degradation, aglycones are released from glycol-conjugated forms of polyphenols by microbial enzymes including glycosidases, glucuronidases, and sulfatases (Rossi et al. 2013). These aglycones are further degraded through several functional groups cleavages reactions (dehydroxylation, demethylation, and decarboxylation) and ring-fission. Therefore, the produced microbial metabolites are absorbed from the colon and are also subjected to liver metabolism, resulting in their conjugated derivatives. This intensive microbial metabolism ultimately reduces the structural diversity of native phenolic compounds to a limited number of smaller phenolic acids and derivatives of phenylpropionic and phenyl acetic acids metabolites. Biological activities of phenolic compounds have mostly been attributed to their microbial metabolites, present in higher quantities in circulation than the native compounds (Marín et al. 2015).

Modulating the activity of gut microbiota by the incorporation of appropriate probiotics into daily diet can enhance bioavailability and/or biological activity of these phenolic compounds. In a study performed by Rekha and Vijayalakshmi (2010), investigations showed that soymilk fermentation with LAB in combination with probiotic yeast *Saccharomyces boulardii* could increase the bioactive aglycones form of soy isoflavone (Rekha and Vijayalakshmi 2010). Investigations

showed that glucoside conjugates of isoflavones exist principally in soya foods which is poorly absorbed in the body and their biological effect are mainly attributed to their glycosides form (Rekha and Vijayalakshmi 2010; Zubik and Meydani 2003).

Various studies revealed an increase in total phenols after fermentation of different foods, and observed that the increase in antioxidative activity may be due to the increase in the total phenolic compounds (Călinoiu et al. 2019; Hur et al. 2014; Zou et al. 2017). Probiotic LAB are naturally present in legume grains; they have also been traditionally used for legume fermentation. Evidences showed that fermentation of legumes with *Lactobacillus* genera can encourage the production of bioactive compounds, improving health benefits beyond basic nutrition. Fermentation of cowpeas with *Lactobacillus plantarum* resulted in the modification of phenolic compounds and improvement of antioxidant activity (Dueñas et al. 2005; Limon et al. 2015).

A research showed that complex polyphenols were hydrolyzed to simpler and more biologically active compounds during fermentation of cowpea flour, and the concentration of phenolic compounds in fermented has significantly increased (Dueñas et al. 2005). In humans, isoflavones bioavailability depends on the relative ability of gut microflora to degrade these compounds. Variation in the intestinal bacterial community as a result of illnesses, diet, or age could significantly influence isoflavones bioavailability (Rekha and Vijayalakshmi 2010; van der Velpen et al. 2014). A research carried out by Dudonné et al. (2015) consumption of showed thatcranberry extract co-supplemented with probiotic *Bacillus subtilis CU1* resulted in the significant change in the composition of gut microbial communities of high-fat fed diet mice through the inhibition of pathogenic bacteria and stimulation of beneficial bacteria (de Souza et al. 2019). According to a study performed by Parkar et al. (2014), anthocyanin-rich blackcurrant juice stimulated the in vitro growth and adhesion properties of *Salmonella Typhimurium 450*.

It has been reported that fermentation can significantly improve total phenolic content and antioxidant activity of cereals and pseudocereals, which is highly dependent on the species of microorganism, on the grains types,, fermentation conditions, particularly time, temperature, and pH values (Hur et al. 2014; Rollán et al. 2019). The enzymes involved in the phenolic metabolism by LAB are mainly decarboxylases (PAD), reductases (PAR), esterases, and/or glycosidases (Rollán et al. 2019). Fermentation of cowpeas with *Lactobacillus plantarum* resulted in the modification of phenolic compounds and improvement of antioxidant activity (Dueñas et al. 2005; Limon et al. 2015) (Fig. 10.1).

Catabolic products of orange juice flavanones identified by HPLC–HR–MS showed that probiotication of orange juice by *Bifidobacterium longum R0175* could significantly enhance the aglycone form of flavonones in orange juice which could eventually augment the bioavailability of orange juice flavanones, and, therefore, their potential beneficial effects on health. A study on the effect of probiotic fermentation of pomegranate juice revealed that fermentation of the juice using *L. plantarum* and *L. acidophilus* as probiotic starter organisms increased the antioxidant activity significantly (Mousavi et al. 2013). In a similar study, investigations

showed that fermentation of liquorice root extract could effectively improve the antioxidant activity of the extract from 53% to a maximum level of 73% (Mousavi and Mousavi 2019).

## 10.10.3 Allergens

Hydrolysis of proteins into smaller peptide fragments during lactic acid fermentation by probiotics could also suppress the potential allergenicity of parent proteins in different foods (Verhoeckx et al. 2015; Xiang et al. 2019). For instance, despite the high protein content, balanced amino acid composition, and high level of lysine in comparison with other vegetable protein sources, soybean meals contain antinutritional factors (ANFs) and allergens, which cause decrease in protein digestibility and absorption in animals (Gu et al. 2010). The soybean is one of the "Big 8" food allergens. The allergen proteins account for 65–80% of total protein content in the soybean and approximately 30% in soybean. The major allergen proteins are beta conglycinin, the 30-kDa allergen (GlymBd 30), and glycinin. In human subjects, these allergens can induce symptoms ranging from skin, gastrointestinal, or respiratory reactions to anaphylaxis. They also cause hypersensitivity in weaned piglets, with the primary adverse effect being diarrhea (Adachi et al. 2009).

*Lactobacillus kefiranofaciens* M1 isolated from Kefir grains has an anti-allergic effect. Digestion of caseins during maturation of fermented milk products has shown to facilitate loss of allergenic reactivity (Chen et al. 2012).

Fermentation of soybean meal enhanced the bioavailability of nutritious components and decreased the incidence of diarrhea in weaned pigs due to the degradation of allergens into peptides (Chi and Cho 2016). The absorption of peptides was significantly improved by the animal. In addition, soybean protein hydrolysate also exhibited antioxidative, metal-chelating activity and lipid peroxidation inhibitory activity attributed mainly to the low-molecular-weight (3 kDa) peptide (Chi and Cho 2016).

The probiotic *B. coagulans GBI-30, 6086* has the capacity to produce enzymes degrading proteins and a wide of carbohydrates. These enzymes can increase the amount of digested milk protein available for absorption. *B. coagulans GBI-30,* 6086 could be exploited to improve protein quality in plant protein sources with lower essential amino acid such as Leucine (Jager et al. 2018).

## 10.10.4 Cyanogenic Glucosides

Galactosidase is recognized to metabolize cyanogenic glucosides present in some vegetal matrixes such as cassava roots, bitter almonds, or whole sorghum. Cyanogenic glycoside linamarin and lotaustralin in cassava tubers can be detoxified by species of *Leuconostoc*, *Lactobacillus*, and *Streptococcus* during traditional method to Gari and Fufu productions to yield hydrocyanic acid (HCN). This compound is

volatile and can escape from the dewatered pulp during toasting rendering the product safe for human consumption (Tamang et al. 2016).

## 10.10.5 Tannins

Tannins are polyphenols widely available in cereals and legumes. They can easily bind to proteins making indigestible complexes with reduced bioaccessibility of nutrients. Various probiotic *Lactobacillus* species such as *L. plantarum*, *L. paraplantarum*, and *L. pentosus* have been confirmed to have tannase activity (Osawa et al. 2000). Therefore, the exploitation of these bacteria in the fermentation of plant-based foods rich in tannins can cleave the protein-tannin complexes rendering protein more available to the cells (Nkhata et al. 2018).

## 10.11 Conclusion

During food fermentation with probiotic bacteria, a number of chemical changes occur in the structure of components of the raw matrix, which thus results in the improvement of the functional properties of foods. This improvement is resulted from several mechanisms such as the elimination of anti-nutritional factors, production of metabolites with a positive effect (bioactive peptides, exopolysaccharides), improvement of the bioavailability through biopolymers hydrolysis (esters of phenolic compounds), and increased vitamin, mineral, and phenolic compounds, leading to an increase in the antioxidant capacity of the final product.

A higher bioactive molecule content and an improved antioxidant activity were found in fermented milks, cereals, fruit and vegetables, meat and fish. Antihypertensive peptides were detected in fermented milk and cereals. Changes in vitamin content were mainly observed in fermented milk and fruits. The imparted health benefits of probiotic fermentation to consumers make this category of foods worthy of recommending for regular dietary guidelines. However, it seems that molecular mechanisms behind the bioavailability and the potential health effects of the newly formed compounds by probiotic fermentation are not deeply investigated yet. Therefore, development of molecular tool analysis such as metabolomics, proteomics, and transcriptomics would considerably help in that respect. Analysis of food composition and enzyme activity evaluation in the gastrointestinal tract would be helpful to evaluate the extent of molecular changes at each stage. Eventually, clinical trials would be useful to measure the health effect of probiotic-fermented foods on different groups of the population.

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