Journal of Functional Foods 72 (2020) 104059

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

# New perspectives in fermented dairy products and their health relevance

María García-Burgos, Jorge Moreno-Fernández, María J.M. Alférez, Javier Díaz-Castro*, Inmaculada López-Aliaga

*Department of Physiology (Faculty of Pharmacy, Campus Universitario de Cartuja) and Institute of Nutrition and Food Technology "José Mataix Verdú", University of Granada, E-18071 Granada, Spain*

ARTICLE INFO

*Keywords:*
Fermented dairy product
Bioactive components
Lactic acid bacteria
Therapeutic effects

ABSTRACT

Fermented dairy products are obtained from fermentation of milk, through the action of suitable and harmless microorganisms. In addition to lactic acid bacteria, fermented dairy products have bioactive compounds as well as bacteria derived metabolites produced during fermentation. Fermented dairy products, due to their special characteristics, are an excellent matrix for the incorporation of ingredients and/or nutrients that give the final product properties beyond purely nutritional, making them true functional foods, interesting in a healthy diet. This review is focused on existing scientific evidence regarding the consumption of fermented dairy products and their health benefits, principally hypocholesterolemic, antioxidant, bone, hypotensive effects and immunological benefits, such as effects on the hosts intestine and microbiome, anti-carcinogenic, immunomodulation and anti-allergenic effects. This review has extensively assessed the fermented dairy products which could be used as possible nutraceutical agents in food and health industries.

## 1. Introduction

Fermented dairy products are foods widely consumed worldwide and they have shown a substantial consumption increase in recent years and market trends suggesting that this will even increase. There is a growing consumer interest in fermented dairy products due to the nutritional and health benefits offered by these products because their effect on the bacterial microbiota of the intestine contributes to a healthy life and to increase life expectancy (Bourrie, Willing, & Cotter, 2016; Chen, Ye, Shen, & Ma, 2019).

Fermentation processes generally enhance the nutritional interest of many foods and increase the bioavailability of nutrients. The fermentation action of specific lactic acid bacteria (LAB) strains may lead to removal of toxic or antinutritional factors, such as lactose and galactose, from fermented milks to prevent lactose intolerance and accumulation of galactose (Shiby & Mishra, 2013).

The transformation of lactose into lactic acid is the most important fact, in addition to other bioactive components. Triglyceride lipolysis is not a significant activity due to LAB has not lipase, but it has a casein proteolytic activity and produces release of amino acids and peptides. Furthermore, bacterial enzymes transform the milk carbohydrates into oligosaccharides, some of which have prebiotic properties (Granier, Goulet, & Hoarau, 2013).

In addition to the production of lactic acid, the production of other compounds produced by LAB depends on the bacterial strains, the conditions of the fermentation process and the fermentation medium. The most common strains of LAB used for fermentation of milk are *Streptococcus thermophilus*, usually in association with Bifidobacteria, such as *Bifidobacterium breve* C50*, Bifidobacterium lactis, Bifidobacterium longum* and *Bifidobacterium animalis*, or with Lactobacilli such as *Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus johnsonii* and *Lactobacillus casei* (Granier et al., 2013).

Different LAB produce different fermentation products, although they have in common that they are alive in the product and can interact with microbiota during intestinal transit and the cells of the intestinal wall (Granier et al., 2013). In this sense, fermented dairy products are an excellent matrix for developing a large variety of innovative health-promoting products and functional foods.

The actual trends in the food industry and the increasingly demand for healthy foods have led to the development of products providing functional components, such as prebiotic substances or probiotic bacteria. Functional foods containing prebiotics and probiotics have sparked the interest of the dairy industry due to scientific evidence related to their positive health benefits. In fact, many of the foods containing probiotics and prebiotics are fermented dairy products, such as yogurt, which is the most studied fermented dairy product, kumys, skyr, yakult, and kefir (Bourrie et al., 2016; Chen et al., 2019).

Taking into account that use of functional food is an emerging area

* Corresponding author.
*E-mail address:* javierdc@ugr.es (J. Díaz-Castro).

https://doi.org/10.1016/j.jff.2020.104059
Received 6 April 2020; Received in revised form 27 May 2020; Accepted 8 June 2020
Available online 16 June 2020

**Table 1**
Types of fermented milks. .
Adapted from Codex Alimentarius (2011)

| | |
|---|---|
| Yogurt | Symbiotic cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* |
| Yogurt based on alternative cultures | Cultures of *Streptococcus thermophilus* and all species of *Lactobacillus* |
| Acidophilus milk | *Lactobacillus acidophilus* |
| Kefir | Culture prepared from kefir granules, *Lactobacillus kefiri*, species of the genus *Leuconostoc*, *Lactococcus* and *Acetobacter* that grow in a close specific relationship. The kefir granules constitute lactose fermenting yeast (*Kluyveromyces marxianus*) and lactose-free fermenting yeasts (*Saccharomyces unisporus*, *Saccharomyces cerevisiae* y *Saccharomyces exiguus*) |
| Kumys | *Lactobacillus delbrueckii* subsp. *Bulgaricus* y *Kluyveromyces marxianus* |

to the development of fermented dairy products, this review aims to show a complete picture of current knowledge about the characteristics and advantages of fermented dairy products as a potential functional product and their benefits for human health. It also provides necessary background and some details on the composition of these products. Thus, fermented dairy products could be used on account of their nutraceutical effect and because it contributes to health benefits widely evaluated in this review.

## 2. Types of fermented dairy products

### 2.1. Fermented milk

Fermented milk is a dairy product produced by fermentation of milk, which has been made thanks to suitable and harmless microorganisms. The cultures of microorganisms will be viable, active and abundant in the fermented milk. When the fermented milk is heat treated the microorganisms will not be viable. Each fermented milk has a specific culture (or specific cultures) used for fermentation (Codex Alimentarius, 2011) (Table 1).

In addition to the traditional yogurt starters, many other microorganisms can be added to the specific culture (Codex Alimentarius, 2011). Kefir requires a special mention because it is thought to confer health benefits for hundreds of years. Kefir differs from other fermented dairy products because it has a kefir grain in fermentation and a large yeast population. They contain fermentation products and metabolites such as kefiran and exopolysaccharides which induce additional benefits associated with their consumption (Bourrie et al., 2016).

Kefir provides numerous positive effects including immunomodulation, anti-allergenic, cholesterol metabolism/angiotensin converting enzyme (ACE) inhibition, wound healing, anti-carcinogenic, antimicrobial and gastrointestinal health (Bourrie et al., 2016).

### 2.2. Cheese

Cheese is defined as the product in which the ratio between whey proteins and casein is not higher than milk. It is composed of cultures of ferments of harmless bacteria producing lactic acid and/or modifiers of taste and aroma, cultures of other harmless microorganisms; and also innocuous and suitable enzymes (Codex Alimentarius, 2011). The consumption of cheese has been proven beneficial in the building of muscle, reduction on blood pressure and low-density lipoprotein cholesterol, and prevention of tooth decay, diabetes, cancer, and obesity (Tunick & Van Hekken, 2015).

## 3. Components of fermented dairy products

The most important biogenic metabolites include proteins, peptides, oligosaccharides, vitamins and organic acids, including fatty acids (Ebringer, Ferencik, & Krajcovic, 2008) (Fig. 1).

### 3.1. Proteins

Cultured dairy products are composed of high-quality proteins, such as casein (α-s1, α-s2, β-casein, κ-casein) and whey proteins (β-lactoglobulin, α-lactoalbumin, lactoferrin, immunoglobulins, glucomacropeptide, enzymes and growth factors). Specific peptides are released during proteolysis of LAB. These peptides are bioactive and have immunomodulatory, antifungal, antimicrobial, antioxidant and anti-carcinogenic activities (Fernandez, Picard-Deland, Le Barz, Daniel, & Marette, 2016). In addition, due to this proteolytic effect of some LAB, the digestive process increases the digestibility and biological value of the protein (Tojo Sierra, Leis Trabazo, & Barros Velázquez, 2006). The milk LAB proteinases, such as those of *Lactococcus lactis* emit biologically active oligopeptides from α- and β-caseins, which have amino acid sequences that are present in casomorphines, lactorphines, casokinines and immunopeptides that are peptides with some biological activities, some similar to morphine. The oligopeptides have similar characteristics to analgesics, stimulate the excretion of insulin and somatostatin, prolong the gastrointestinal reabsorption of nutrients, modulate the transport of amino acids in the intestine and also act as antidiarrheal agents. These atypical opioid peptides differ from endogenous opioids, such as enkephalins and endorphins, only in their N-terminal sequences (Ebringer et al., 2008; Verruck, Dantasb, & Schwinden-Prudencioa, 2019)

In addition, Bacteriocins act against other bacteria with bactericidal or bacteriostatic activity. This ability can be very useful and represent an opportunity to search for new bacteriocins in complex microbiota, such as those of a traditional fermented product (Hill et al., 2017).

In addition, bioactive peptides are encrypted in larger proteins and, when released after proteolysis, have been associated with health promotion through a number of mechanisms such as ACE inhibitor, antithrombotic, antihypertensive, antioxidant, immunomodulation, modulation of apoptosis and opioid and anti-opioid activities. LAB has proteases and peptidases that can release encrypted peptides during milk fermentation or after ingestion of cultured products containing LAB in the intestinal lumen (Hill et al., 2017).

### 3.2. Lipids

Yogurt has a lower fat content than milk, due to the dairy ingredients used in its preparation. Fatty derivatives are also found by the bacteria, which contribute to the aroma (Tojo Sierra et al., 2006). Depending on the origin of the milk and the manufacturing process, the lipid content in the yogurt can vary in quantity, but the quality does not change significantly compared to the original milk.

Triglycerides are more than 95% of lipids of yogurt. In spite of the content of saturated fats (72%), the health benefits appear to be attributed to yogurt lipids, which also contain 25% monounsaturated and 3% polyunsaturated fatty acids and are vectors of fat-soluble vitamins A, D, E and K. In addition, dairy products contain high levels of conjugated linoleic acid (Fernandez et al., 2016).

### 3.3. Carbohydrates

Lactose is the main carbohydrate available in dairy products which gives rise to lactic acid after fermentation. Depending on the type of product and industrial additives, this disaccharide can reach up to 98% of the total carbohydrates in natural yogurt. Its hydrolysis in glucose and galactose occurs mainly in the digestive tract by the β-galactosidase

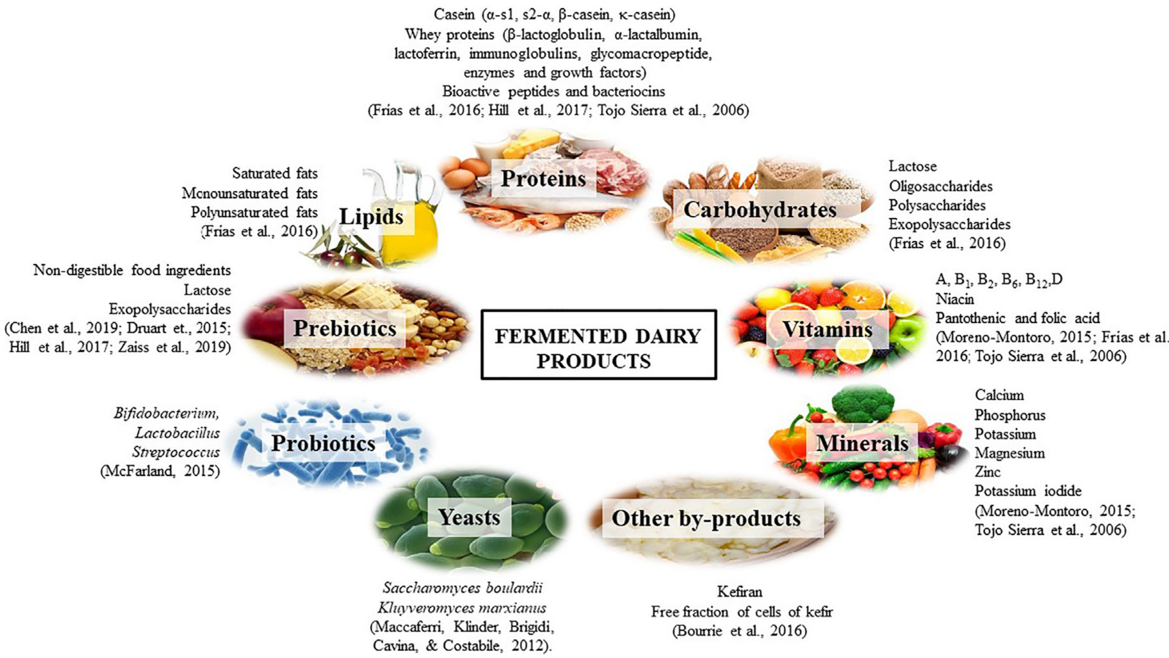


**Fig. 1.** Main components of fermented milks.

of the intestinal brush border. In addition, it contains oligosaccharides, polysaccharides and depending on the strains, some types of exopolysaccharides (homo and heteropolysaccharides) produced by LAB (Fernandez et al., 2016).

### 3.4. Vitamins and minerals

Fermented dairy products are rich in many vitamins and minerals highly bioavailable (Fernandez et al., 2016). They represent an important contribution of vitamins A, $B_1$, $B_2$, $B_6$, $B_{12}$, niacin, pantothenic acid and folic acid, as well as vitamin D, calcium, phosphorus, potassium, magnesium, zinc and potassium iodide (Moreno-Montoro, 2015; Tojo Sierra et al., 2006). Many of these micronutrients have a higher bioavailability in the fermented milk products than in raw milk due to the process of acidity and fermentation, which mainly affects the content of vitamins (Fernandez et al., 2016).

In addition, the contribution of lactic acid seems to play an important role in the absorption of calcium, inhibition of the microbiota pathogenic and in the stimulation of intestinal secretion (Tojo Sierra et al., 2006).

### 3.5. Prebiotics

A prebiotic is a component with selective fermentation that produces specific changes in the composition of the gastrointestinal microbiota, as well as in its activity with beneficial effects on the health of the host (Hill et al., 2017). The gastrointestinal microbiota produces a selective fermentation of prebiotics which modulates intestinal health through the production of bioactive metabolites, (Druart et al., 2015) especially short-chain fatty acids (acetate, propionate, butyrate, lactate) (Chen et al., 2019) which are generated by fermentation of complex carbohydrates, (Zaiss, Jones, Schett, & Pacifici, 2019); and poly-unsaturated fatty acids (Druart et al., 2015) showing an efflux from the gut into the systemic circulation. Short-chain fatty acids have a profound impact on intestinal cells and participate in the control of various processes such as mucosal proliferation, inflammation, colorectal carcinogenesis, mineral absorption and elimination of nitrogenous compounds (Zaiss et al., 2019). Different pathways are proposed to increase the amount of short fatty acids including, through probiotics, prebiotics and symbiotics. (Chen et al., 2019) Metabolites of bacterial origin account for about 10% of circulating metabolites and they have emerged as key regulatory metabolites (Zaiss et al., 2019). These components are non digestible and selectively stimulate the growth of specific microorganisms (Hernández & Guzmán, 2003).

The symbiotic is a combination of probiotics and prebiotics in the same dairy product. The function of the prebiotic is to improve the survival, growth and performance of probiotics or other beneficial bacteria in the colon inducing health benefits (Hill et al., 2017). Both lactose and exopolysaccharides are a known source of prebiotics (Aryana & Olson, 2017; Hernández & Guzmán, 2003). Another source of prebiotics are galactooligosaccharides, polydextrose, sialyllactose and sialyllactose that could significantly improve the absorption and synthesis of B vitamins (Allen et al., 2019), of particular interest are micronutrients, such as B vitamins, precursors of indispensable metabolic cofactors, that are produced *de novo* by some gut bacteria but must be provided exogenously in the diet for many other bacterial strains (Rodionov et al., 2019).

The use of probiotics, prebiotics and symbiotics in the treatment or prevention of cancer is being largely investigated. There is evidence that these act as anti-carcinogenic agents or antimutagenic agents through the diet (Hill et al., 2017).

### 3.6. Probiotics

Probiotics are live microorganisms that confer a benefit to the health of the host when administered in adequate amounts. In the global guidelines on probiotics and prebiotics published in 2013 by the World Gastroenterology Organization it confirmed that the effectiveness of probiotics is specific to the strain and the specific dose, dispelling the myth that any yogurt can be considered a probiotic. Three broad categories of probiotics were defined in 2014 (McFarland, 2015).

Those who not have health claims (generally they are considered safe, does not require proof of effectiveness).

As a dietary supplement with a specific health claim (define the strain used, evidence-based effectiveness of clinical trials or meta-analysis and use to strengthen the immune system).

As a probiotic drug (clinical trial for specific indication or disease,

**Table 2**
Different types of probiotic products. .
Adapted from McFarland, 2015

| Probiotic strain (single-strain probiotics) | Formulation | Evidence-based efficacy | Reference |
|---|---|---|---|
| *Bifidobacterium animalis* subsp *lactis* DN-173010 | **Yogurt** | Constipation | (Yang et al., 2008) |
| *Bifidobacterium animalis* subsp *lactis* Bb-12 | Capsules, powder in sticks, **fermented milk** | Eczema | (Varga, Süle, & Nagy, 2014) |
| *Lactobacillus casei* subsp *Shirota* | **Fermented milk** | Constipation, *H. pylori* infection | (Maragkoudakis, Chingwaru, Gradisnik, Tsakalidou, & Cencic, 2010) |
| *Lactobacillus casei* DN-114001 | **Fermented drink, yogurt** | Antibiotic-associated diarrhea, prevention of pediatric diarrhea, respiratory infections | (Dietrich, Kottmann, & Alavi, 2014) |
| *Lactobacillus johnsonii* La1 | **Milk** | *H. pylori* infection | (Sgouras et al., 2005) |
| *Lactobacillus plantarum* 299v (DSM9843) | **Fermented oat gruel in fruit drink,** capsules | Irritable bowel syndrome, *Clostridium difficile* infection | (Klarin, Johansson, Molin, Larsson, & Jeppsson, 2005) |
| *Lactobacillus reuteri* DSM 17,938 | Capsules, **yogurt** | Acute pediatric diarrhea, cholesterol | (Spinler, Taweechotipatr, Rognerud, Ou, Tumwasorn & Versalovic, 2008) |
| *Lactobacillus rhamnosus* GG (ATCC 53013) | **Yogurt**, capsules | Acute pediatric diarrhea, antibiotic-associated diarrhea | (Jia, Chen, Chen, & Ding, 2016) |
| Probiotic strain (mixtures of probiotic strains) | Formulation | Evidence-based efficacy | Reference |
| *Lactobacillus acidophilus* CL1285 + *Lactobacillus casei* Lbc80r + *Lactobacillus rhamnosus*CLR2 | **Fermented drink**, capsules | Antibiotic-associated diarrhea, *Clostridium difficile* infection | (Auclair, Frappier, & Millette, 2015) |
| *Lactobacillus acidophilus* + *Bifidobacterium animalis* subsp *lactis* | **Yogurt** | Improves microbiota | (Simpson et al., 2018) |

define the strain used, risk–benefit and compliance with the regulations that legislate the drugs).

It was found that probiotics are especially indicated in the prevention of diarrhea associated with antibiotics, treatment of *Helicobacter pylori* infection, treatment of pediatric acute diarrhea, prevention of allergies, treatment of chronic disease irritable bowel syndrome, treatment of inflammatory bowel disease, treatment of vaginitis and bacterial vaginosis, prevention of necrotizing enterocolitis in newborns, prevention of traveler's diarrhea, treatment of acute diarrhea in adults, treatment of constipation, treatment of *Clostridium difficile* infection, sepsis, dental infections, obesity. The findings of clinical effectiveness vary according to the probiotic strain and the type of indication (McFarland, 2015) (Table 2).

### *3.7. Yeasts*

A unique feature of the traditional production of fermented dairy products is a large amount of yeast in the grain and the fermented milk. Although most of the microorganisms probiotics marketed are bacteria such as lactobacilli and bifidobacteria, there are some other like *Saccharomyces boulardii* (Bourrie et al., 2016).

It has been shown that *S. boulardii* improves symptoms of diarrhea associated with *Clostridium difficile*, as well as that it reduces inflammation and it alters the immune status and reactions in the intestine, for this reason is diarrhea treatment's caused by *C. difficile*. Some yeasts of the kefir have immunomodulatory characteristics, *Kluyveromyces marxianus* B0399 has the ability to adhere to Caco-2 cells. When the yeast is co-incubated with Caco-2 cells stimulated with lipopolysaccharide, it was observed a reduction in the secretion of IL-10, IL-12, IL-8 and IFN-γ. In addition, *K. marxianus* B0399 caused a reduction in the secretion of proinflammatory cytokines TNF-α, IL-6 and MIP1α in peripheral blood mononuclear cell which had been stimulated with lipopolysaccharide (Maccaferri, Klinder, Brigidi, Cavina, & Costabile, 2012).

### *3.8. Other byproducts (Kefiran and free fraction of cells of kefir)*

Besides to microbial populations that there are in the probiotic kefir and other fermented foods, there are also products of fermentation and other byproducts of the metabolism of these microorganisms that possess bioactivity. Some of these byproducts can have benefits in the health of the consumer without the presence of the microbial population. These byproduct are free fraction of cells of kefir and kefiran, an exopolysaccharide produced by *Lactobacillus kefiranofaciens* during fermentation (Bourrie et al., 2016).

## 4. Immunological effects

The health promoting mechanisms are based on the beneficial effect on the modulation of immune function, generally due to stimulation of innate immune responses. In this case, they mainly modulate the production of cytokines and antimicrobial peptides (Ebringer et al., 2008).

### *4.1. Effects on the guest's intestine and microbiome*

#### *4.1.1. Modulation of the intestinal microbiota*

The effects of the products from the fermentation of LAB on the composition of the intestinal microbiota showed that bifidobacteria were greater in the feces of the group who received a cell-free concentrated whey from *Bifidobacterium breve* C50 fermented milk compared with the control group consuming diluted milk. Components produced during fermentation have benefits in the intestinal microbiota (Granier et al., 2013).

Several studies have shown that the feces of children fed with formula based on fermented milks versus who receive a standard formula not fermented had a higher average proportion of bifidobacteria with less adult species (Mullié et al., 2004). Children were colonized more frequently by *Bifidobacterium longum infantis* and *B. breve*, species that are mainly during breastfeeding and associated with an improved barrier function and immune priming. In fact, it is known that the proper maturation of the immune system is connected to the colonization of the intestinal microbiota and especially to some specific species of *Bifidobacterium* (Granier et al., 2013).

A randomised controlled trial showed that the episodes of acute diarrhea in healthy infants were less severe in the group intaking the formula based on fermented milk, reducing dehydration, medical visits and oral rehydration needs (Thibault, Aubert-Jacquin, & Goulet, 2004).

Infant formula supplementation with *Bifidobacterium lactis* and *Streptococcus thermophilus* have shown to be effective against nosocomical diarrhea in infants. Pediatric beverage contains *Bifidobacterium animalis, Lactobacillus acidophilus* and *Lactobacillus reuteri* have shown to be effective in prevention of rotavirus diarrhea.

The use of *Lactobacillus rhamnosus* GG as a treatment improves the local systemic immune response against rotavirus, which could be useful against infections. *Bifidobacterium longum, Bifidobacterium lactis, Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus* La5 and *Streptococcus faecum* and *Saccharomyces boulardii* yeast are probiotics that can prevent antibiotic-induced diarrhea (Shiby & Mishra, 2013).

#### *4.1.2. Exclusion of pathogens*

Probiotics can change the intestinal microbiota when installed in the intestinal transit, or by promoting the growth of beneficial microorganisms present. The consumption of kefir or kefiran in an animal model increased count of beneficial bacteria, mostly of the *Lactobacillus* and *Bifidobacterium*, in addition to reducing harmful microbial species such as *Clostridium perfringens*. The consumption of kefir makes less severe the infection by *Giardia intestinalis* in C57BL/6 mice, due to the modulation of the immune system. The *Lactobacillus* specific strains that have been isolated from kefir can adhere to Caco-2 cells preventing them from adhering to *Salmonella typhimurium* and *Escherichia coli* O157: H7 (Bourrie et al., 2016) In addition, *Lactobacillus* have the ability to protect vero cells from shiga toxin type II produced by *E. coli* O157: H7 and inhibit the ability of *Bacillus cereus* extracellular factors to cause damage to Caco-2 cells. The consumption of LAB can decrease the effect of *Escherichia coli* thanks to the anti-E metabolites that avoid colonization by pathogenic bacteria (Shiby & Mishra, 2013).

#### *4.1.3. Antibacterial and antifungal properties*

The bioactive peptides produced during fermentation of milk by *Lactobacillus helveticus* have been proposed as an alternative for the control of the bacterial infection due to its antimicrobial and immunostimulating properties (Matar, Valdez, Medina, Rachid, & Perdigon, 2001).

The potential stimulatory effect of cell-free supernatant of milk fermented by *Lactobacillus helveticus* LH-2 and its fraction peptide F5 was investigated in macrophages. The free fraction of cells from the fermented milk increased the production of IL-6, TNF-α, IL-1β through stimulation of macrophages, accompanied by a greater production of nitric oxide and phagocytic activity. The nitric oxide, whose synthesis is induced by TNF-α, is one of the cytotoxic agents by which macrophages can kill bacteria and other pathogens, as well as tumor cells. In addition, the TNF-α is a main component of host defense against trauma and infection and is of great importance because it induces the gene expression of various cytokines and nitric oxide synthesis. These effects suggest that the F5 peptide fraction could exert a modulation of macrophage functions (Tellez, Corredig, Brovko, & Griffiths, 2010).

Supplementation of a replacement diet with goat's milk fermented with *Lactobacillus rhamnosus* CRL1505 increases resistance to infections by *Salmonella typhimurium* and *Streptococcus pneumoniae* in immunocompromised hosts. In addition, it accelerated the recovery of the clinical nutritional parameters such as body weight and thymus. The recovery of the serum protein profile studied could be the result of a higher bioavailability of peptides and amino acids, which would facilitate its absorption at the intestinal level. In addition, the inclusion of the fermented goat milk in repletion diet improved the hematological parameters. The early normalization of leukocytes, neutrophils and lymphocytes in micés blood treated with fermented goat milk it would be important for the recovery of the immunity against infections. It is likely that the stimulation of immunity by the fermented goat milk - depends of *L. rhamnosus* and also of byproducts such as bioactive peptides (Salva et al., 2011).

On the other hand, the kefir has been shown to have a multitude of antibacterial and antifungal activities. It has been found that the kefir fermented milk has a similar function to ampicillin, amoxicillin, azithromycin, ceftriaxone and cetoconazol. Besides the antimicrobial effects of kefir fermented milk, there are microorganisms that have themselves antimicrobial properties. *Lactobacillus plantarum* ST8KF produces the bacteriocin ST8KF that exhibits antimicrobial action against *Enterococcus mundtii* and *Listeria innocua*. Other *Lactobacillus* species derived from kefir grains, such as *Lactobacillus acidophilus* and *Lactobacillus kefiranofaciens*, as well as some strains of *Streptococcus thermophilus*, have demonstrated antimicrobial activity against a range of pathogens including *Echerichia coli, Listeria monocytogenes, Staphylococcus aureus, Salmonella typhimurium, Salmonella enteritidis, Shigella flexneri, Pseudomonas aeruginosa* and *Yersinia enterocolitica* (Bourrie et al., 2016).

Other lactobacilli of the kefir also have antimicrobial activity against *Salmonella typhimurium* and *E. coli*. Lacticin 3147 has antimicrobial activity, affecting *Bacillus subtilis, Bacillus cereus, Clostridium sporogenes, Clostridium tyrobutyricum, Enterococcus faecium, Enterococcus faecalis, Listeria innocua, Listeria monocytogenes, Staphylococcus aureus* and *Clostridium difficile* (Bourrie et al., 2016).

## 5. Anti-carcinogenic *effects*

The effects of yogurt and LAB on cancer and intestinal inflammation have been extensively studied. The preventive effect of probiotics on the carcinogenesis may be associated with the modulation of the immune response and changes in the intestinal microbiota, preventing the increase of bacteria that become procarcinogens in carcinogens (De Moreno De Leblanc & Perdigón, 2010).

### *5.1. Colon cancer*

In a murine colon cancer model, the consumption of yogurt inhibits tumor growth through the reduction of the inflammatory response through the increase of IL-10-secreting cells, apoptosis and the decline of the procarcinogenicas enzymes (De Moreno De Leblanc & Perdigón, 2010).

The intestinal enzyme activities of β-glucuronidase and nitrorreductasa in a model of colon cancer were increased after dimethylhydrazine (DMH) injection, contributing to its power mutagenic. The injection of DMH produced lower levels of enzymatic activity in mice fed with yogurt than in the control group with tumor. However, this effect was not observed when mice were given the bacteria derived metabolites of yogurt. These results show that the bacteria in yogurt can be involved in the decline of the procarcinogens enzymatic activities and in the intestinal microbial changes (De Moreno De LeBlanc & Perdigón, 2005).

The administration of yogurt to mice injected with DMH increases the number of cells that encode IgA and CD4 T lymphocytes in the lamina propria of large intestine with a decrease in the IgG and CD8 T-cells. The increase in the number of cells that secrete IgA and no increase in IgG cells in the intestine of mice fed with yogurt could limit the inflammatory response, because the IgA is considered an important barrier in colonic neoplasia (Moreno, de LeBlanc, Matar, & Perdigón, 2007).

In addition to yogurt, kefir produces high level of TNF-α and IFN-γ that will lead to high level of IgA secretion (Sharifi et al., 2017).

On the other hand, bioactive peptides in kefir induce activation of macrophages and phagocytosis and nitric oxide (NO) production (Sharifi et al., 2017). The mice carriers of tumors have high amounts of inducible nitric oxide synthase (iNOS) (+) cells, which suggests an increase in the production of nitric oxide (NO) by these cells. The synthesis of the enzyme iNOS could be induced by IFN-γ that increase in the intestinal tissue in the DMH group. In the mice with tumor fed with yogurt the cells iNOS (+) lowered when the inflammation decreased (Moreno et al., 2007).

The lack of enzymatic induction of iNOS in DMH-yogurt group and control group with yogurt alone can show the way through which the yogurt can regulate the immune system modulating the inflammatory response. Despite the increase in the number of secretory cells of IFN-γ (+), the production of NO does not increased and, as a result, it was not observed tumor growth, only cellular infiltration. Therefore, it is suggested that the large number of IFN-γ (+) cells in mice fed with yogurt could be related to the increased number of immune cells in the intestine and it could be regulated by other cytokines such as IL-10. Feeding with yogurt produced by itself the greatest number of cells IL-10 (+), which shows that the administration of this fermented milk contributed to maintaining a regulated immune response in the intestine of mice fed with yogurt (Perdigón, De Moreno de LeBlanc, Valdez, & Rachid, 2002).

The ability of kefir supernatant to exert protective effects in DNA damage induced by carcinogen agents was shown at all the concentrations used in human colon adenocarcinoma cells (Rafie, Hamedani, Ghiasv, & Miraghajani, 2015; Sharifi et al., 2017).

Fermented milks may modulate the immune system of the mucosa. The administration of fermented products may have an impact on the intestinal microbiota, stimulate immune cells associated to the intestine and it is useful against the intestinal inflammation and colon cancer (De Moreno De Leblanc & Perdigón, 2010).

### 5.2. Breast cancer

In recent years considerable progress has been made in the understanding of the molecular factors involved in the development of breast cancer. There are genetic and environmental factors that increase the chances of breast cancer and the types of breast cancer most common are dependent on estrogen. Some factors, such as diets rich in - fermented dairy products, can inhibit the growth of many types of cancer, including breast tumors (De Moreno et al., 2007).

Fermented milks have fractions of peptides produced during fermentation that can stimulate the immune system and inhibit the growth of tumors (Matar et al., 2001). Milk fermented with *Lactobacillus helveticus* R389 slows the growth of breast tumor. This fermented milk decreases IL-6 and increases IL-10 and IL-4 in serum, mammary glands and immune cells (De Moreno De Leblanc & Perdigón, 2010). The decrease in IL-6 also occurred in mice fed with kefir or KF (the free fraction of cells of the kefir) (De Moreno, de LeBlanc, Matar, Farnworth, & Perdigon, 2006; Rafie et al., 2015). Microbial proteolysis could result in bioactive peptides since the peptides are different after LAB fermentation. This fact can be demonstrated through the mutagenic effect exerted by fermented milk by *Lactobacillus helveticus* R389, a bacterium with high protease and peptidase activity, while a mutant strain (L89), which is deficient in proteolytic activity, did not exert this effect. Similarly, the number of IgA secretory cells in the small intestine increased (Moreno et al., 2007) thanks to the fact that fermented dairy products induce the secretion of TNF-α that leads to a high level of IgA secretion (Sharifi et al., 2017).

The number of CD4 cells increased, while the number of CD8 cells remained unchanged in the group fed with fermented milk by *Lactobacillus helveticus* R389 and injected with tumor cells. This result was different in the control group with tumor, which had more CD8 cells than CD4 cells (Moreno et al., 2007).

Seven days of cyclic administration of fermented milk with *Lactobacillus helveticus* R389 delayed or stopped the development of breast cancer. Tumor growth decreased in mice after 2 days of feeding cyclical with kefir, and the same cyclic feeding with KF showed the most significant delay of tumor growth. The pattern of cytokines was similar for all three products in connection with the delay in the development of the tumor (De Moreno De Leblanc & Perdigón, 2010).

KF had a significant impact on the size of the tumor, the apoptosis and the immune recruitment in a model of murine breast cancer, resulting in an increase in apoptosis of the tumor cells and an increase of the population CD 4 of T-cells (Bourrie et al., 2016). Kefir show significant dose-dependent suppressive effects on breast cancer cells proliferation with no inhibitory effects on normal cells (Rafie et al., 2015).

In addition, *Lactobacillus acidophilus* isolated from yogurt reduced tumor growth rate and increased lymphocyte proliferation in a mouse model of breast cancer (Pei, Martin, DiMarco, & Bolling, 2017). Sharifi et al. (2017) reported that fermented dairy products induce apoptosis, cell cycle arrest and reduce tumor growth in breast cancer cells; therefore, it may be suitable in the prevention or treatment of breast cancer

### 5.3. Other cancers

Fermented dairy products have a significant anti-carcinogenic activity against multiple types of cancer cells. *Lactobacillus kefiri* increased apoptosis of human myeloid leukemia cells resistant to multiple drugs *in vitro* through the activation of caspase 3 with a dose-dependent way (Sharifi et al., 2017). Kefir inhibits cell proliferation in patients with leukemia in a dose and time-dependent manner with induction of apoptosis in cancerous cells without affecting healthy cells (Rafie et al., 2015).

In addition, KF have demonstrated anti-carcinogenic activity *in vitro* when it was observed that it had an antiproliferative effect dose-dependent in the gastric cancer cell line SGC7901. A study showed that KF was capable of inducing apoptosis in cells SGC7901 through the regulation of gene BAX and the promoter of apoptosis and antioncogene, and to induce the regulation of the bcl-2 gene, which is an inhibitor of apoptosis and oncogene known. In addition to the promotion of cell death in cancer cells, antimutagenic effects have been shown in studies with carcinogens (Gao et al., 2013; Sharifi et al., 2017).

The size of the tumor was reduced in the murine models of spindle cell sarcomas in mice that received intraperitoneal kefir with some tumors disappearing completely during a treatment period of 20 days. These discoveries has not yet been determined if can be repeated in oral consumption (Bourrie et al., 2016). On the contrary, soy tumor kefir inhibits tumor growth in female mice bearing sarcoma tumor cells (Sharifi et al., 2017). In another study, the beneficial therapeutic effects of kefir decreases tumor size in sarcoma cells in mice (Rafie et al., 2015).

As far as skin cancer is concerned, kefir has the ability to protect human melanoma cells from ultraviolet damage. These effects are related to active substances of kefir other than antioxidant agents (Rafie et al., 2015).

In addition, Pei et al. (2017) found that the risk for bladder cancer was lowest in individuals consuming the highest levels of yogurt It is important to highlight that the consumption of fermented milk products can stimulate the immune system and can keep you in a state of vigilance, which could be helpful in coping with different types cancers (De Moreno De Leblanc & Perdigón, 2010).

## 6. Immunomodulatory effects

The interactions between the bacteria, immune system and specific metabolites that occur during fermentation are not fully understood. Dendritic cells are known to play an important role in this interaction as antigen presenting cells. Due to these properties and its ability to elicit different responses to different stimulus, so dendritic cells can perform immunomodulatory functions (Granier et al., 2013).

LAB may interact with dendritic cells derived from monocytes to modulate its properties. This fact is related to the characteristics of bacterial infections, as well as components of the cell wall, cytoplasmic compounds and DNA fragments, while the natural fermentation process that occurs in the intestine could also produce active components. The actives bacterial components of *Bifidobacterium breve* C50 can pass through intestinal epithelial cells and regulate the production of cytokines. Unlike other strains, the supernatant of *Bifidobacterium breve* C50 induced maturation and activation of human dendritic cells, but only after the fermentation of a means on the basis of serum of milk (Granier et al., 2013).

The role of probiotic in the modulation of the intestinal immune system is yet poorly understood. Oral administration of probiotic *Lactobacillus casei* DN114001 in BALB/c mice showed that all the bacteria or their fragments come in contact with intestinal immune cells. Probiotic fermented milk stimulus the intestinal cells with release of IL-6, as well as cells related to the non-specific barrier and with intestinal immune cells, increasing T lymphocytes and B lymphocytes IgA (+), and the expression of cell markers related to the innate and macrophage-mediated adaptive responses (De Moreno De Leblanc & Perdigón, 2010; Galdeano, De Moreno de Leblanc, Carmuega, Weill, & Perdigón, 2009).

Macrophages have a central role as mediators of innate immune responses against microbial pathogens, including phagocytosis, secretion of cytokines and production of nitric acid.

When an infection occurs, IL-6, IL-1β and TNF-α are released, and the immune system is activated. However, an uncontrolled overproduction of these proinflammatory mediators can also lead to vasodilation, increased vascular permeability, hypotension, multiple organ failure and death.

Due to the bioactive peptides of fermented milk, the functioning of the innate immune system is improved since macrophage activation (Tellez et al., 2010).

Cultured milks that are considered beneficial for health should have a minimum number of viable microorganisms at the time of consumption (more than 107 CFU/g). LAB and *Propionibacterium freudenreichii* have probiotic properties and an interesting potential immunostimulator. Most of the studies on the potential immunostimulant bacteria were initiated after its growth in a laboratory environment, and often studied as a single culture. The real situation is different since the ecosystem has several bacterial species that have different immunological properties. In the ecosystems of dairy products synergistic effects occur which results in a modification of the proteome and therefore of the immunomodulatory properties. Different strains of LAB in combination with *P. freudenreichii* presents immunostimulatory properties and it has been demonstrated that this last species presents some interesting anti-inflammatory properties and is adapted to the stress digestive tract, including acids and bile salts (Foligné et al., 2016).

In addition, it is difficult to achieve the growth of LAB and *P. freudenreichii* in milk. The fermented milk that produced in this study allowed only limited growth of *P. freudenreichii*, whose population remained stable during 14 days of storage at 4 °C, this means that a fermented milk is a good vector for the supply of *P. freudenreichii.* However, there is a need for more technological improvements in the processes if it is used *P. freudenreichii* in fermented milks to increase the final count (Foligné et al., 2016).

One of the main ways in which the probiotic products, like kefir, confer health benefits, is because they modulate the immune system of the gastrointestinal tract. When the rats inoculated intra-duodenal with cholera toxin (CT) were fed with kefir, serum levels of IgA anti-CT enhanced as well as the secretion of IgA anti-CT in the Peyer's patches, mesenteric lymph nodes, the spleen and in the intestinal lamina propria, in comparison with mice fed with kefir (Bourrie et al., 2016).

Other studies show rises in IgG and IgA cells in the small intestine of rats that were fed with both usual as with pasteurized kefir, besides rises in the levels of IL-4, IL-10, IL-6 and IL-2 in cells in the lamina propria, which promote a Th2 response. These effects occur in both solid parts of kefir that contained live bacteria as well as in KF and in kefiran. Interestingly, IFN-γ, TNF-α and IL-12 increased only in rats fed with pasteurized kefir. If the pasteurized kefir has an effect, the mechanisms that produce immune modulation are not completely related to living cells and may be due to the products that are present in the kefir (Bourrie et al., 2016).

On the other hand, the bacteria derived metabolites of the milk fermented by *Lactobacillus helveticus*, which contain specific peptides and oligosaccharides, modulate mucosal immunity. The IgA production enhanced in the intestine of mice fed with free of bacteria fermented milk. The production of cytokines, specifically IL-10, was affected in the cells of the mucous membranes. The exopolysaccharide produced by *Lactobacillus kefiranofaciens* could modulate the immune responses in a mouse model. In addition, mice infected with *Salmonella enteridis*, improved survival in mice fed a bacteria derived metabolites of cultured milk in comparison with the control group (Granier et al., 2013).

It should also be added that the probiotic cultured milk and the supernatant of probiotic cultured milk enhanced bifidobacteria in the intestinal microbiota, the number of macrophages, IgA cells and, especially with the supernatant, the number of dendritic cells in a model of mice. Specific products of fermentation can modulate the mucosal immune response. These products could promote T-helper cell type 1 or regulatory T cells induction and, therefore, they could participate actively in the maintenance of homeostasis of the immune system (Granier et al., 2013).

Other compounds are those contained in small amounts in fermented infant formulas which feature properties and effects on immune response stimulation. These formulas contain different growth factors and hormones and cell wall components of LAB (peptidoglycan, lipoteicoic acid) or cytoplasmic content (bacterial DNA). Some fermentation products are metabolized by the microorganisms that produced them, while others accumulate in formulas for infants based on fermented milk and they may exhibit functional properties. The infant formula that contains products of fermentation may modify systemic immune responses particularly Th1 and IL-10, were increased in mice by feeding infant formula based on fermented milk after immunization with ovoalobumin in comparison with the control mice. In addition significantly higher levels of IFN-γ, IL-12p40 and IL-10 were found in mice fed fermented infant milk formula (Granier et al., 2013).

The anti-poliovirus response may be improved by the formulas for infants based on fermented milk, which are able to promote intestinal bifidobacteria. IgA poliovirus levels increased after vaccination with Pentacoq, but in the group fed with formulas for infants based on fermented milk the increase was significantly higher. Antibodies levels were correlated with bifidobacteria, especially with *Bifidobacterium longum infantis* and *Bifidobacterium breve* (Mullié et al., 2004).

A previous study in subjects with HIV suggested that the probiotic *Lactobacillus rhamnosus* GR-1 could improve immunity and relieve diarrhea when it added to the yogurt. Most participants had significant improvements in body weight, and eight showed an improvement in body weight class, changing from severe to moderate low weight or moderate to slight low weight, when they started with the consumption of yogurt. The probiotic yogurt consumers had significantly higher levels of thiamine, riboflavin, biotin, pantothenic acid, vitamin C, calcium, copper, phosphorus and potassium than controls at the end of the study. The probiotic yogurt consumers had significantly fewer infections fungal infections, fewer episodes of diarrhea and degree of fatigue substantially lower. In addition, the 84% of consumers of probiotic yogurt did not show symptoms of diarrhea during the study period, in comparison with 69% of non-consumers of yogurt. Finally, the 52% of consumers of probiotic yogurt did not report gastrointestinal symptoms in comparison with the 39% of non-consumers. The further increase in CD4 T lymphocytes was 0.73 CD4 cells/μl/day during the first 70 days and it continued to increase to 0.2 CD4 cells/μl/day. The results are not sufficient to state that CD4 T lymphocytes count can be enhanced and it is necessary more studies to explore this phenomenon (Reid, 2010).

## 7. Anti-allergens effects

Allergic diseases have increased in the developed world over the past decades, which have resulted in an increased incidence of asthma and food allergy. Some studies have reported that the complexity and heterogeneity of human microbiome is an important factor to develop an allergic disease (West, 2014). The high levels of *Bifidobacterium* and group 1 lactobacilli in the childreńs intestine show lower incidence of allergic disease later, and it has been observed that both the kefir as kefiran have these effects on the intestinal microbiota in animal trial (Hamet et al., 2013). Supplementation with *Bifidobacterium* influences the intestinal microbiota of infants because it reduces levels of *Bacteroides* and it has been associated with a lower incidence of food allergy (Bourrie et al., 2016).

One of the main mechanisms of food allergy is Th1/Th2 cells imbalance, which leads to a higher production of IgE antibodies. The total Ig E and specific Ig E to ovalbumin were significantly lower in mice that had been sensitized with ovalbumin and then they fed with a mixture of lactobacilli, than in control mice that had also been sensitized with ovalbumin but they did not receive the mixture. This study show that treatment can help alleviate all of the symptoms of allergy (Bourrie et al., 2016).

In a mouse model of asthma sensitized with ovalbumin, the mice that received intragastric kefir showed lower levels of airway hyperresponsiveness (AHR) than control mice and they had lower levels of AHR than the control group that received a drug antiasthmatic. The mice that received kefir had significantly lower levels of infiltration of eosinophils in the lung tissue as well as in the bronchoalveolar lavage fluid together with low levels of Ig E, IL-4 and IL-13 which are associated with the Th2 response that is responsible for the allergic reaction (Lee et al., 2007). Oral feeding with kefir in ovalbumin sensitized mice significantly reduced anti-ovalbumin Ig E and Ig G1 antibodies levels of serum than those found in mice that received non-fermented milk or water (Bourrie et al., 2016).

## 8. Hypocholesterolemic effects

Clinical studies have shown that regular administration of certain probiotics may have hypocholesterolemic effects. It is assumed that probiotic bacteria can metabolize cholesterol and thus reduces its resorption in gastrointestinal tract. *In vitro* and *in vivo* studies indicates that lactobacilli, bifidobacteria and other milk bacteria assimilate cholesterol, by incorporation into the cellular membranes, deconjugation and precipitation of cholesterol with bile acids. Deconjugated bile acids are less soluble and therefore are less absorbed from the intestinal lumen than their conjugated forms (Ebringer et al., 2008). Al-Sheraji et al. (2012) indicate that yogurt containing *Bifidobacterium pseudocatenulatum* G4 and *Bifidobacterium longum* BB536 has shown beneficial effects reducing principal cardiovascular risk factors. Moreover, *Bifidobacterium pseudocatenulatum* G4 and *Bifidobacterium longum* BB536 decreased plasma levels of cholesterol by increasing bile acids excretion (Al-Sheraji et al., 2012). The probiotic yogurt consumption containing *Lactobacillus acidophilus* and *Bifidobacterium lactis* caused a significant reduction in cholesterol serum levels compared with ordinary yogurt (Ataie-Jafari, Larijani, Alavi Majd, & Tahbaz, 2009).

However, no all bacterial strains and species in fermented dairy product present a hypocholesterolemic effect (Ebringer et al., 2008). *Lactobacillus fermentum* SM-7 isolated from koumiss shows cholesterol-reducing efficacy in *in vitro* and *in* vivo experiments (Pan, Zeng, & Yan, 2011). In a clinical study, the administration of Danish yogurt Gaio fermented with *Streptococcus thermophilus* and *Enterococcus faecium* showed a significant reduction in cholesterol serum levels (8.4%). Yogurt produced with *Streptococcus thermophiles* and *Lactobacillus acidophilus* or *Lactobacillus rhamnosus* did not exhibit the hypocholesterolemic effect. A long-term daily consumption of fermented milk products increases HDL cholesterol serum levels, and improves the LDL/HDL ratio. This fact has also been observed in the traditional Slovak cheese Liptauer (bryndza). The Enterococcus and Lactobacillus families metabolize cholesterol by 12 to 56%. The results show a statistically significant decrease in the level of total cholesterol and LDL cholesterol that decreases more significantly in people with higher initial cholesterol levels. In addition, there was a decrease in the level of blood glucose and serum creatinine, C-reactive protein and blood pressure. Some products derived from β-lactoglobulin, such as the Ile-Ile-Ala-Glu-Lys pentapeptide, also have hypocholesterolemic effects (Ebringer et al., 2008).

## 9. Antioxidant effects

Fermented goat milk increased serum melatonin levels and total antioxidant capacity and reduced glutathione peroxidase 1 expression in the duodenal mucosa, limiting biomolecular oxidative damage (Moreno-Fernandez et al., 2016, Moreno-Fernandez et al., 2017).

Probiotic bacteria are particularly effective trapping reactive oxygen species. The intracellular extracts from *Lactobacillus* sp. have been demonstrated to be effective in curing vitamin E deficiency in rats. *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* inhibit lipid peroxidation through scavenging reactive oxygen species and free radicals. Different strains and species of LAB present in fermented milk with antioxidant activity can significantly affect human health. This efficacy has also been supported by clinical studies with fermented goat milk with *Lactobacillus fermentum* ME-3. In addition, sour milk has shown significant improvement of the total antioxidant activity as compared to unfermented milk, including antioxidant status, prolonged oxidative resistance of lipoprotein fraction, reduced lipoprotein peroxides and oxidized low-density lipoprotein, reduced glutathione redox ratio, and increased total antioxidant activity. Several factors can be involved in the anti-atherogenic activity of fermented milk, such as antioxidant factors produces by some lactobacillus in the human gastrointestinal tract and also various peptides released from α-lactalbumin and β-lactoglobulin and α-casein (Ebringer et al., 2008).

Exopolysaccharide isolated from kefir were shown to possess antioxidant activities. Studies suggest a possible mechanism of microbial exopolysaccharide improving oxidative status of the host by regulating microbiota to selectively inhibit or eliminate microorganisms related to oxidative stress. *Lactobacillus plantarum* YW11 isolated from Tibet kefir produced an exopolysaccharide that showed a great anti-oxidant activity both *in vitro* and in *vivo* with increased serum levels of glutathione peroxidase, superoxide dismutase, catalase and total antioxidant capacity but decreased malondialdehyde level (Zhang et al., 2017). Kefir consumption has shown a protective effect on oxidative stress, by increasing the level of glutathione peroxidase and decreasing the level of malondialdehyde, and it was also effective in reducing lipid peroxidation. Moreover, kefir has also anti-cancer properties by acting as an antioxidant in protecting cellular DNA from oxidative damage (Sharifi et al., 2017). The majority of milk bacteria show antioxidant behavior producing superoxide dismutase, or glutathione (Ebringer et al., 2008).

## 10. Bone effects

Intervention trials in children and adolescents have shown that fermented dairy product has a positive effect on bone health, particularly on bone mineral content and bone mass density. Rizzoli and Biver (2018) have shown that cheese supplements may be more beneficial for cortical bone accumulation than supplying calcium in the form of tablets

Consumption of vitamin D with or without calcium-fortified yogurt or soft cheese reduced serum bone resorption markers (TRAP 5b and CTX) and PTH compared to usual diet or non-fortified fermented dairy products (Bonjour, Benoit, Payen, & Kraenzlin, 2013). In conclusion cultured dairy products fortified or not, enhance IGF-I and are effective in promoting the accumulation of bone minerals in children and adolescents. In adults, it affects the balance of calcium and resorption (Rizzoli & Biver, 2018). A systematic review and meta-analysis support a significant positive association of yogurt intake whit hip fracture risk, improving bone formation, increasing bone mass density and preventing bone loss (Bian et al., 2018). Kefir-fermented milk consumption was associated with short-term changes in bone turnover biomarkers and enhanced bone mineral density in osteoporotic patients (Tu et al., 2015).

The action of prebiotics involves the fermentation of the fibers in the large intestine, which leads to the production of short chain fatty acids such as acetate, propionate, valerate, isovalerate or butyrate and isobutyrate. Bioavailability of calcium is enhance through a reduction in the pH of the intestinal content, an increase in the weight of the cecum and the surface of the bristles, the acetylation of histones with epigenetic modulation, as well as modifications of the microbiota, with an increase in the species of bifidobacteria capable of metabolizing phytoestrogens phyto-estrogens. Cultured products also improve intestinal barrier function by stimulating the assembly of tight junctions.

Fermentation of the fibers in the large intestine produces short chain fatty acids such as acetate, propionate, valerate, isovalerate or butyrate and isobutyrate. In this way the bioavailability of calcium is improved since the pH of the intestinal content is reduced, the weight of the cecum and the surface of the sows increases, in addition to modifications of the microbiota, with an increase in the species of bifidobacteria capable of metabolizing phytoestrogens. Cultured products stimulate assembly of tight junctions improving intestinal barrier function. Daily consumption of prebiotics (inulin or oligofructose-enriched inulin-type fructans) enhances calcium absorption and influences bone turnover in postmenopausal women and bone mineralization during pubertal growth. Rizzoli and Biver (2018) showed that consumption of milk products fortified with prebiotics (FOS-inulin) was more effective than regular milk to decrease bone resorption.

The products of the metabolism of short-chain fatty acids can directly modify the local intestinal metabolism, support the function of the intestinal barrier and modify the pH of the intestine, which influences the availability of calcium and increases its absorption (Rizzoli & Biver, 2018).

When calcium absorption increases, there is a reduction in PTH production and therefore bone resorption decreases. The gut endocrine system could be affected too, as germ-free animals have a lower serotonin secretion that has been shown to lower bone formation (Rizzoli & Biver, 2018).

## 11. Hypotensive effects

Probiotic bacteria hydrolyzes milk proteins to oligopeptides which present diverse biological functions, including small peptide with hypotensive effect based on inhibition of ACE activity, such as Val-Pro-Pro and Ile-Pro-Pro. The proteolytic activity of milk bacteria during milk fermentation generates hypotensive peptides with a larger number of amino acid units - casokinins and lactokinis (Ebringer et al., 2008).

Small and non-basic bioactive peptides in fermented goat milk would be considered responsible for the ACE inhibitory, antioxidant and antibacterial activities. These findings reinforce the potential benefits of the consumption of fermented goat milk in the prevention of cardiovascular diseases associated with oxidative stress and hypertension (Moreno-Montoro et al., 2017, 2018). Fermented goat milk has nutraceutical properties with potential positive health effects on cardiovascular system, to reduce response related with the inflammatory signaling, macrophages activation and their role in the atherosclerosis (López-Aliaga et al., 2018).

It has been shown that some fermented milks *in vitro and* in vivo assays and in clinical studies are antihypertensive agents, and their effects in most cases have been attributed to milk peptides. These fermented milks are as effective as synthetic ACE inhibitors. Therefore, they can be considered hypotensive agents because they can be part of the daily diet. Although a large amount of research related to antihypertensive peptides has been carried out, it is necessary to find and that LAB has the capacity to generate this bioactivity because commercial fermented milks with antihypertensive effects are scarce and most are based on *Lactocacillus helveticus*, as well as good technological properties for the production of fermented milk products (Beltrán-Barrientos, Hernández-Mendoza, Torres-Llanez, González-Córdova, & Vallejo-Córdoba, 2016).

## 12. Conclusions and future perspectives

In recent decades, the possible therapeutic effects of cultured dairy products in the prevention and treatment of diseases have been extensively studied due to the wide range of products available in the food industry. Numerous studies and clinical trials with fermented dairy products suggest that they feature functions such as immunomodulatory agents, anti-carcinogenic agents, hypocholesterolemic agents, antioxidants and hypotensive agents. According to scientific evidence, fermented dairy products are one of the best natural ingredients to be used as an effective treatment that in turn has fewer adverse side effects.

Products containing prebiotics can act by interacting with the mucosa, whose action will depend on the nature, number and physiological state of the LAB strain that reaches the mucosa. These modulatory effects would be reduced if fermented products are supplied directly, because their activity does not depend on other metabolic pathways within the intestine. To ensure reproducibility, fermentation products should have precise control of the technological process. However, research should focus on the molecular characterization of fermentation products and bacterial strains.

Due to the variety of strains and complexity of the fermentation substrate, the exact composition and the molecular structure of the fermentation products are not fully known. These approaches are limited by the fact that not all microorganisms are easily cultured in the laboratory and there is no culture medium that allows the simultaneous growth of all strains present. The culture-independent techniques allow access to the genetic information contained in the species of the fermented products. The molecular identification of the compounds could be an objective of the research and that is necessary to understand the effects. This identification could be carried out by nanotechnology. Thus, future products will improve and adjust their organoleptic characteristics or nutrient content according to the taste or health needs of each consumer. Furthermore, smart packaging could provide a dose of additional nutrients to those it identifies as having special dietary needs and detect when a consumer is allergic to avoid them. Metatranscriptomics can also be used to study the gene expression of microbial populations in fermented dairy products. The information obtained through this science can be combined with proteomics and metabolomics, to provide a detailed insight into the microbiology of food, the core metabolic pathways, microbiome specific functionality in fermented dairy products and their relationship with flavour, aroma and other characteristics.

There is a need for randomised controlled trials of high quality to distinguish the immunological effects of fermentation products of those produced by the bacterial cell walls and to confirm specific immunomodulatory properties.

A suggestion for future studies is to provide detailed descriptions of the probiotic tested, including genus, species and strain daily dose and duration used, so that the appropriate data can be grouped and analyzed. Metabolomics can be easily applied to identify the detailed composition of fermented milk and record the biochemical changes due to bacterial activity during the fermentation and storage process.

The beneficial effects of prebiotics, probiotics and fermented have different mechanisms of action. Its action on the intestine and mucosa should be elucidated for a better understanding of the immunological maturation of the mucosa and the relation to the use of cultured dairy products aimed at immunomodulation, modulation of the intestinal microbiota and the promotion of the development of bifidobacteria.

Functional foods are products that can provide a health benefit beyond the traditional nutrients it contains. Dairy products are functional foods and this is a key opportunity for the dairy sector. The use of milk to produce functional foods is related to the composition of milk, which is a good matrix for probiotic bacteria due to lactic acid bacteria and bifidobacteria. Related to the food matrix and probiotic bacteria are probioactives that are bioactive compounds. An example of a probioactive is bioactive peptides released specifically by the hydrolysis of casein in milk appearing in fermented dairy products. Further research is required to obtain a better knowledge of how fermented dairy products could be used as a nutraceutical agent and an alternative potent therapeutic in a large number of pathologies. More attention should be paid to the mechanisms by which cultured dairy products may exert eventually beneficial health effects and also to know which organisms or fractions of the product are responsible for each benefit. Furthermore, most of the health effects discussed in this review are based on biochemical or laboratory tests and should be corroborated in further studies to be more conclusive.

## 13. Ethics statement

The papers cited involving the use of human subjects, have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The papares cited involving animal research have been carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, . EU Directive 2010/63/EU for animal experiments , or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

## Declaration of Competing Interest

The author declare that there is no conflict of interest.

## Acknowledgments

Maria Garcia-Burgos and Jorge Moreno-Fernandez are supported by a fellowship from the Ministry of Education, Culture and Sport (Spain), and are grateful to the Excellence Ph.D. Program “Nutrición y Ciencias de los Alimentos” from the University of Granada. The authors also thank Susan Stevenson for her efficient support in the revision with the English language.

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