

Fermented Dairy Products



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Abstract The microbiota of fermented dairy products contributes to the safety, flavor, and organoleptic qualities of the products. Moreover, metabolites obtained from the fermentation process enhance the milk nutritive value and digestibility, whereas dairy microorganisms could be the perfect carriers for reseeded the gut microbiota. The structural food matrix of fermented milk facilitates the delivery of viable microorganisms to the intestinal tract. Fermented dairy products may be beneficial to human health by improving lactose intolerance symptoms and for the production of bioactive compounds such as vitamins, gamma-amino butyric acid, exopolysaccharides, and bioactive peptides, among others. Also, fermented dairy products contribute to the modulation of the gut microbiota and the prevention of infections, inflammation, and cardiometabolic diseases. Furthermore, fermented dairy products constitute the hallmark of probiotics supply in the food market.

Keywords Fermented dairy · Yogurt · Kefir · Cheese · Probiotics · Bioactive compounds

Introduction

The fermented dairy products consumed today are generated through controlled microbial culturing and enzymatic conversions of major and minor milk components (see (Macori and Cotter 2018) for a recent review). Fermentation improves shelf life, increases microbiological safety, adds flavor, and enhances palatability and organoleptic qualities. The fermentation process involves a series of complex reactions carried out by microorganisms, which transform milk constituents rendering new molecules of

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enhanced nutritive value and digestibility. Moreover, fermentation generates metabolites that can be major contributors of a daily healthful diet (Marco et al. 2017).

The contributions of milk components and dairy products to human health have been comprehensively reviewed (Tunick and Van Hekken 2015). These can be summarized as enhancing muscle building, lowering blood pressure, reducing low density lipoprotein cholesterol, and preventing diabetes, obesity and cancer, among others. Additionally, due to the reduced consumption of dietary fiber in Western societies and the overall decrease of microbial diversity in processed foods, fermented dairy products could be the perfect carriers for reseeding the gut microbiota. The above listed health benefits and the fact that they are viewed as natural products have placed yogurt, kefir, and cheese in the forefront of consumers' preferences. In the present chapter we describe existing data regarding the microbiota found in fermented dairy products and the advances in knowledge of microbial properties that may benefit human health.

Fermented Dairy Products

Fermentation is one of the oldest forms of milk preservation and has been used by humans since ancient times (Markowiak and Slizewska 2017). The beneficial effects of fermented dairy products was empirically known by Romans, Greeks and Egyptians. They produced different types of sour milk from buffalo, cow, or goat's milk. However, it was not until the twentieth century that the beneficial properties of lactic acid bacteria (LAB) started to be scientifically substantiated by the immunologist Élie Metchnikoff, who was awarded the Nobel Prize in 1908. He concluded that the unusual high numbers of Balkan centenarians was due to the consumption of sour milk containing large numbers of the lactic acid-producing bacterium *Lactobacillus bulgaricus bacillus*, currently classified as *Lactobacillus delbrueckii* subsp. *bulgaricus*. In the book 'The Prolongation of Life' (Metchnikoff 1908), Metchnikoff recommended the daily consumption of milk fermented with pure cultures of this *Lactobacillus* to discourage microbial putrefactive growth in the colon, setting the stage for further studies on beneficial effects of LAB in fermented products.

Yogurt

Among fermented dairy products, yogurt is consumed the most in Western societies, being a common component in the daily diet of populations from the Netherlands and Scandinavian countries. Although yogurt has been manufactured commercially for over a century, its concept has changed over time into a very segmented market. Today fermented dairy products include a broad catalog of flavored, low-fat, drinkable, probiotic, and other products marketed as health-promoting. The European

Codex Alimentarius Commission explicitly defines yogurt as the product of milk fermentation by *Streptococcus thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*) (Codex-Alimentarius 2003). According to the Codex STAN 243-2003 these microorganisms must reach a minimum of 10^7 cfu/g, be viable, active, and abundant in the product until the set expiration date. However, differences in labeling laws allow, for example in the United Kingdom, to include any *Lactobacillus* species in fermented milks labeled as ‘yogurt’. In this case, the term ‘yogurt-like product’ is used and defined as an alternative dairy product in which *L. bulgaricus* can be substituted by other *Lactobacillus* species for the fermentation, or yogurt containing probiotic bacteria, when probiotic or alternative organisms are added to yogurt (Guarner et al. 2005).

Yogurt is an excellent source of macro- and micronutrients like high-quality, digestible proteins and carbohydrates, minerals, and vitamins. It contributes to the growth and fitness of muscle mass and helps maintaining bone health due to their calcium and phosphorus content. The nutritional value of yogurt has been recognized by the Canadian Food Guide (Health-Canada 2011), USA Department of Agriculture (USDA 2010), and the British Nutrition Foundation (BNF 2015). These international agencies recommend the inclusion of fermented dairy products in the daily diet. Furthermore, they emphasize that food guides must state whether dairy products are fermented or non-fermented since fermented dairy products have additional health claims compared to non-fermented products (Chilton et al. 2015). As an example, studies in the Netherlands and Sweden (Keszei et al. 2010; Sonestedt et al. 2011) showed that regular consumption of fermented dairy products, but not non-fermented dairy products, significantly decrease the risk of bladder cancer and cardiovascular disease. Likewise, a significant positive effect of calcium intake on teeth health was specifically associated with dairy fermented products (Adegboye et al. 2012). This may be explained by the breakdown of milk components during microbial fermentation into more bioavailable and new metabolites of potential health benefits.

Kefir

Kefir is a traditional fermented dairy beverage produced by a complex natural microbiota from kefir grains. These grains are traditionally obtained from periodic coagulation of cow’s milk with calf or sheep abomasum (forth stomach) in goatskin bags. Kefir originated in the Caucasus but gained popularity in Eastern and Central European countries starting in the second half of the nineteenth century. Kefir can also be made with milk from other sources including as goat, sheep and buffalo milk (Bourrie et al. 2016). Distinctive microorganisms in kefir are homofermentative lactobacilli (*Lactobacillus kefirifaciens*), which produces a kefiran complex that surrounds yeasts (*Saccharomyces cerevisiae*), and other bacteria (*Lactococcus*, *Leuconostoc*, thermophilic and mesophilic lactobacilli and acetic acid bacteria). Microbiological, technological, as well as nutritional, and health benefits of kefir have been recently summarized (Bourrie et al. 2016; Kesenkas et al. 2017).

Cheese

Animal skins and inflated internal organs, particularly the rumen, have provided storage vessels for a range of foodstuffs since ancient times (Marciniak 2011). Hence, we can presume that cheese making was discovered accidentally when storing milk in ruminant stomachs, which resulted in milk curdling by the residual gastric rennin. Most modern cheeses are manufactured from pasteurized milk coagulated in a vat by recombinant enzymes or proteases of vegetable origin with added *Lactococcus*, *Lactobacillus* and *Leuconostoc* as starters. However, traditional raw milk cheeses naturally fermented by its indigenous microbiota are still produced in some Mediterranean countries. For specific cheeses like blue or soft cheeses, bacteria of the genera *Brevibacterium* and *Propionibacterium* and molds of the genus *Penicillium* are added to develop their characteristic organoleptical properties. Raw milk can contain over 400 bacterial species. This microbial biodiversity decreases in the cheese core usually dominated by few species of LAB but persists on the cheese surface with high numbers of species of bacteria, yeasts and molds (Montel et al. 2014; Orla O'Sullivan 2017). It is commonly accepted that cheese flavor develops as a result of the overall microbial metabolism beginning during clotting, progressing further during cheese ripening (Weimer 2007).

Semi-hard cheeses typically contain non-starter lactobacilli (NSLAB), which can reach up to 10^7 – 10^8 cfu/g for long periods of time during production and storage (Pelaez and Requena 2005). NSLAB have been shown to generate bioactive peptides and gamma-aminobutyric acid (GABA) (Settanni and Moschetti 2010). *Propionibacterium freudenreichii*, a ripening culture in Swiss-type cheese, produces conjugated linoleic acid (CLA) and may have bifidogenic and immunomodulatory properties (Thierry et al. 2011).

The Microbes in Fermented Dairy Products

Microorganisms already present in the raw milk, from the environment or added from a previously fermented product were the main actors of the traditional fermentation process. Because humans have consumed fermented foods since ancient times, the human gastrointestinal tract (GIT) adapted to a constant supply of live bacteria on a nearly daily basis. In fact, many of the microbial species found in fermented foods are either identical to or share physiological traits with species known to promote GIT health (Marco et al. 2017). However, the industrialization of food production has reduced the variety of foods that humans consume and their associated microbiota. Hygienic industrial practices, including thermal treatment, have decreased the microbial diversity of fermented foods, modern dairy fermentations have to rely on standardized starter cultures and microorganisms as well as cultivation protocols that extend shelf life, improve food safety and enhance perceived health benefits (Hill et al. 2017). The consumption of these products limits the

traditional exposure of the human gut to the highly biodiverse traditional food ecosystem. In response, artisanal dairy fermentation and consumption of traditional fermented dairy products as part of Western diets have regained popularity (Prakash Tamang and Kailasapathy 2010).

In 1873, Lister (1873) first isolated *Bacterium lactis* [later renamed as *Streptococcus lactis* and more recently *Lactococcus lactis* subsp. *lactis* (Schleifer et al. 1985)]. This bacterium together with other species of the genera *Lactococcus*, *Lactobacillus* and *Leuconostoc* are usually included in well-defined starter cultures currently used in modern cheese fermentation. Likewise, most common starters for yogurt and fermented milks include species of *Lactobacillus*, *Streptococcus*, *Bifidobacterium* and some yeast used in the manufacture of kefir and koumiss (Carminati et al. 2016). Nevertheless, undefined starter communities composed of a complex undefined mixture of LAB strains are still in use in dairy fermentation due to advantages in reducing sensitivity to bacteriophage attack. State of the art advances in high-throughput DNA sequencing technology and the potential of predictive metabolic modelling of the multi-strain cultures, are emerging as powerful tools to investigate structure and function of these complex communities (Smid et al. 2014).

The structural food matrix of fermented milk facilitates the delivery of viable microorganisms to the intestinal tract by contributing to the microorganisms' survival during transit, enhancing their interaction with the gut microbiota, and participating in the reinforcement of the intestinal barrier. Similarly, the solid matrix of cheese and its buffering capacity may protect bacteria during the intestinal transit more efficiently than yogurt or fermented milks (Karimi et al. 2011). The fact that semi-hard ripened cheeses like Cheddar, Gouda or Manchego, maintain viable bacteria for up to 12 months, is a good reason to consider cheeses as excellent carriers of health promoting bacteria into the gastrointestinal tract (Ross et al. 2002).

Do Microbes in Fermented Dairy Products Survive Passage Through the Gastrointestinal Tract?

Upon entering the human gastrointestinal tract, fermentation-associated microorganisms must survive environmental challenges including acidity of the stomach and bile salts and enzymes in the small intestine to reach the colon. Hence, survival of fermentative bacteria within the gastrointestinal tract became an important research topic since the beginning of the last century. As early as 1920, Cheplin and Rettger (1920) were able to recover live *Lactobacillus acidophilus* but not *L. bulgaricus* from rat stools after daily consumption. Since then, there has been conflicting evidence concerning the viability of the yogurt cultures in the gastrointestinal tract. In a double-blind placebo prospective study including 114 healthy young volunteers that were usual yogurt consumers the authors did not detect *S. thermophilus* nor *L. bulgaricus* in feces by culturing or by specific PCR and DNA hybridization

of total fecal DNA (del Campo et al. 2005). Detection of LAB from yogurt in total fecal DNA was consistently negative, even after repeated yogurt consumption during 15 days. In a contrasting study, out of 39 samples recovered from 13 healthy subjects over a 12-day period of fresh yogurt intake, 32 and 37 samples contained viable *S. thermophilus* and *L. bulgaricus*, respectively (Mater et al. 2005). Furthermore, in a population of 51 healthy subjects living in the Paris area, the yogurt species *L. bulgaricus* was detected in 73% of fecal samples from consumers (200–400 g yogurt/day) vs. 28% from non-consumers (Alvaro et al. 2007). Accordingly, Elli et al. (2006) demonstrated that yogurt bacteria survived gastrointestinal transit being recovered in feces from 20 healthy volunteers fed commercial yogurt for 1 week.

Lactose Hydrolysis by Microbes in Fermented Dairy Foods

During milk fermentation, most lactose is converted into lactic acid by fermentative microorganisms. Although the final product still contains traces of the carbohydrate, lactose-intolerant populations are able to consume yogurt without experiencing adverse symptoms. The improvement in lactose absorption was also demonstrated when healthy subjects with lactose maldigestion consumed yogurt containing live bacterial cultures in comparison with heated yogurt (Rizkalla et al. 2000). This can be attributed to the intestinal release of β -galactosidase by yogurt cultures that must be viable when ingested (Guarner et al. 2005; Savaiano 2014). In the study carried out by Alvaro et al. (2007) with 51 healthy adult subjects of yogurt and non-yogurt consumers, they found that among the nine metabolic bacterial enzyme activities investigated, the only significant difference concerned β -galactosidases.

Lactose intolerance is caused by a decreased expression of the enzyme β -galactosidase or lactase normally secreted by the epithelial cells within the villi. This enzyme is required to digest the lactose from milk and dairy products. Undigested lactose consequently enters the colon where it is fermented by gas producing microbes, resulting in symptoms including abdominal pain, bloating, diarrhea, and flatulence (Misselwitz et al. 2013). Expression of lactase decreases after weaning in most individuals and as a result become relatively lactose intolerant. It is, therefore, not surprising that as adults, as much as 75% of the world's human population is intolerant to ingested dietary lactose (Silanikove et al. 2015). Over centuries of evolution, humans have adapted to lactose ingestion by several mechanisms including genetic mutations that allow lactose digestion in a classic example of evolutionary nutrigenetics. But also, colonic microbiome adaptation and the development of fermented dairy products have contributed to lactose tolerance in humans. As a result, the intolerance to lactose occurs in the Central European population at a 5% rate, while Asia and Latin America populations observe up to 90% intolerance rates. Furthermore, some authors have proposed to modulate the colonic microbiome of lactose-intolerant individuals, increasing the abundance of lactose metabolizing bacteria that are non-gas producers (i.e. *Bifidobacterium*), by administration of short-chain galactooligosaccharides (Azcarate-Peril et al. 2017).

The generation and release of β -galactosidases is a species-related trait in the yogurt-associated species (*L. bulgaricus* and *S. thermophilus*). The European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies in 2010 formally approved yogurt's beneficial effects on reverting lactose intolerance. They indicated that the dose of live microorganisms should be at least 10^8 cfu/g (EFSA 2010). This claim does not require survival and reproduction of the bacterial cells during intestinal transit.

Besides the improvement in lactose intolerance symptoms, the conversion of lactose into lactic acid reduces the intestinal pH and confers a protective effect against foodborne pathogens infection. The low pH also increases peristalsis, thereby indirectly removing pathogens by accelerating their transit through the intestine (Kailasapathy and Chin 2000). Moreover, LAB cultures enhance pathogens elimination by other mechanisms including competitive exclusion and production of antimicrobial metabolites like bacteriocins (Arqués et al. 2015). Alvaro et al. (2007) showed significantly lower numbers of Enterobacteriaceae in human feces of yogurt consumers versus non-consumers. Accordingly, Van der Meer and Bovee-Oudenhoven (1998) reported that lactic acid in yogurt and calcium in other dairy products inhibit the gastrointestinal survival and colonization of *Salmonella* Enteritidis.

Bioactive Compounds

One important mechanism by which the fermented dairy foods and its associated microbiota (LAB, propionibacteria, yeasts, and molds) may be beneficial to human health is through the production of bioactive compounds. Some of these bioactive compounds include vitamins, bioactive peptides, exopolysaccharides (EPS), GABA and CLA. See Fernández et al. (2015) for a recent overview of the impact of metabolites produced by microorganisms found in fermented dairy products.

Particular attention to bioactive components resulting from the fermentation of milk and their impact on health is rapidly been explored using high throughput, multi-omic approaches. Over the last decades these new technologies have allowed for more sophisticated metabolite analysis, which integrates fermented dairy products composition and functional assessments following ingestion (Zheng et al. 2015; Hagi et al. 2016). Still, we should consider that the acidic environment of the stomach and the subsequent stages of digestion can lead to early inactivation of certain bioactive compounds (Stanton et al. 2005).

Vitamins Vitamins are compounds essential for human individuals that are insufficiently or not synthesized at all by the human organism. Although they are present in foods, vitamin deficiencies still exist due to malnutrition or lack of a daily balanced diet (Arth et al. 2016). Furthermore, food processing and cooking may destroy or remove some vitamins such as vitamins of the B-group. This group of vitamins includes thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6), pantothenic

acid (B5), biotin (B7 or H), folate (B9, B11 or M) and cobalamin (B12). Although shown beneficial, programs with synthetic vitamin B fortification of foods to correct vitamin deficiencies has not been adopted in many countries due to the potential side effects from excessive intake in populations with normal vitamin B levels (Atta et al. 2016).

Folate vitamin deficiency is associated with megaloblastic anemia as well as congenital malformations, including spina bifida and anencephaly, although only a small fraction of these diseases is actively being prevented worldwide (Arth et al. 2016). Today, it is recognized that the yeast *S. cerevisiae*, used as starter in fermented milks like kefir or koumiss, is a folate producer (Moslehi-Jenabian et al. 2010). As for LAB, production of folate still remains controversial. It has been shown that *S. thermophilus* can produce folate whereas *L. bulgaricus* is a folate consumer. Still, the concentration of the vitamin in yogurt may reach values up to 200 µg/L (Wouters et al. 2002). In addition to *S. thermophilus*, other bacteria present in dairy fermentations such as *L. lactis*, *L. acidophilus*, *L. plantarum*, *L. fermentum*, *Leuconostoc lactis*, *Bifidobacterium longum*, and some strains of *Propionibacterium*, also have the ability to produce folate (Iyer and Tomar 2009; LeBlanc et al. 2017). Therefore, the consumption of fermented dairy foods could be an attractive approach for improving the world wide nutritional deficiencies of folate (Saubade et al. 2017).

Gamma-amino butyric acid Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the adult mammalian brain. GABA also has different functions in the central and peripheral nervous systems, and in some non-neuronal tissues (Watanabe et al. 2002). In general, anti-hypertensive and antidepressant activities are the major functions of GABA or GABA-rich foods. However, the mechanisms responsible for these activities are still unknown due to scarce studies carried out on the pathway for GABA absorption (Dhakai et al. 2012).

LAB can generate GABA as end product from the decarboxylation of glutamic acid. The enzyme that converts glutamic acid into GABA is the glutamic acid decarboxylase (GAD). Genes encoding GADs are broadly distributed in *Lactobacillus brevis*, *L. plantarum*, *L. fermentum*, *L. reuteri*, *S. thermophilus*, *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis* and some *Bifidobacterium* species, indicating that these bacteria may be able to synthesize GABA (Wu and Shah 2017). In fact, production of GABA has been demonstrated in *L. lactis*, *S. thermophilus*, and *L. bulgaricus* isolated from milk, a trait that could be used to produce GABA-rich fermented milk products. Other high GABA producers are *L. brevis* strains, which are not usually associated with the dairy environment. Hence, to enhance GABA concentrations in fermented dairy products, strategies have been proposed that include co-culturing *L. brevis* with conventional dairy starters in dairy fermentation (Wu and Shah 2017). Enhancement of GABA production in fermented products has shown hypotensive effects on rats (Quilez and Diana 2017). Additionally, with the aim of producing GABA enriched cheeses, GABA producing LAB have been isolated from several cheeses made from raw milk such as Spanish artisanal cheeses (Diana et al. 2014) and traditional alpine Italian cheeses (Franciosi et al. 2015).

Exopolysaccharides Exopolysaccharides (EPS) are high molecular weight carbohydrate polymers loosely connected to the cell surface of microorganisms. EPS protect against food processing promoting biofilm formation, and also mediate cell-to-cell interactions in the human gut, facilitating microbial adhesion to intestinal mucosa and preventing adhesion of pathogens. Multiple strains including the yogurt starter species *L. bulgaricus* and *S. thermophilus* have been reported generate EPS. Also many probiotic strains of *Lactobacillus* and *Bifidobacterium* are being investigated for their ability to produce EPS (Salazar et al. 2016). The use of EPS producers in dairy fermentation can be beneficial not only for intestinal health but also technologically to improve texture and flavor of the final products (Ryan et al. 2015; Nampoothiri et al. 2017).

Bioactive peptides Bioactive peptides are released from animal and plant proteins by endogenous proteolytic enzymes or enzymes of microbial origin. They usually contain between two and 20 amino acids, which can be naturally resistant to gastrointestinal digestion due to partial protection conferred by their high hydrophobicity and the usual presence of proline. The hydrolysis degree of bioactive peptides can vary depending on peptide chain length, nature of the peptide, presence of other peptides in the medium and the food texture (Fang et al. 2016).

Bioactive peptides have been the subject of intensive research due to their potential physiological effects on various human systems such as cardiovascular, digestive, endocrine, immune, and nervous systems [see recent review of (Martinez-Villaluenga et al. 2017)]. Furthermore, industrial-scale technologies suitable for the commercial production of bioactive milk peptides have been developed (Korhonen and Pihlanto 2006; Urista et al. 2011). By far, the most studied bioactive peptides are those derived from milk proteolysis (Nagpal et al. 2011; Beermann and Hartung 2013). Several peptides have been isolated from yogurt, kefir, other fermented milks and cheese and a number of them have shown to be released by the proteolytic system of the LAB from these fermented products (López-Expósito et al. 2017).

Microbial antihypertensive properties have been related to peptides with the capacity to inhibit the angiotensin converting enzyme (ACE) that suppresses angiotensin II-mediated vasoconstriction. The responsible inhibitor peptides of the antihypertensive effect derive mainly from β -casein and have been found in milk fermented by strains of *Enterococcus faecalis*, *L. lactis*, and *Bifidobacterium* (Martinez-Villaluenga et al. 2017). The most studied antihypertensive peptides are the tripeptides VPP and IPP, released from β -casein after fermentation of milk. VPP and IPP are now added to fermented sour-milk products that claim antihypertensive effects launched in Japan and Finland. The Japanese product called “Calpis” consists of milk fermented with *L. helveticus* CP790 and *S. cerevisiae* containing both peptides VPP and IPP. This fermented milk has demonstrated properties to prevent the development of hypertension (Sipola et al. 2002). The Finnish product called “Evolus” contains the tripeptide IPP and claims to have similar antihypertensive effects. It is produced by *L. helveticus* LBK-16H strain as starter in milk fermentation

(Seppo et al. 2003). A comprehensive meta-analysis of data from relevant human studies showed a modest reduction in blood pressure in individuals consuming VPP and IPP compared to antihypertensive drugs (Fekete et al. 2015) demonstrating a potential for use in cardiovascular therapy as a complement to traditional medications.

Other beneficial effects attributed to peptides derived from milk include immunomodulating, antioxidant, antimutagenic, mucin-stimulating, and opioid effects (Martinez-Villaluenga et al. 2017). Some peptides have multifunctional characteristics such as the peptide YQEPVLGPVVRGPFPIIV (fragment 193–209 of β -casein) obtained after fermentation of milk with the strain *L. casei* Shirota. This peptide showed an inhibition efficiency ratio for ACE (antihypertensive activity) of 0.14%/peptide concentration (mg/mL), and a thrombin inhibition efficiency ratio of 4.6%/peptide concentration (mg/mL) (Rojas-Ronquillo et al. 2012). Some strategies employed to enhance the release of bioactive peptides during fermentation by LAB include supplementing milk with milk peptide fractions. This strategy facilitates the proteolytic activity of *L. acidophilus*, *L. helveticus*, *L. bulgaricus*, and *S. thermophilus* and enhances the production of ACE-inhibitory peptides (Gandhi and Shah 2014).

Cheese can also contain peptides with antihypertensive, antioxidant, opioid, antimicrobial, antiproliferative, mineral absorption and modulatory effects (López-Expósito et al. 2017). As example, an intensive ACE-inhibitory activity (75.7%) was detected with peptides isolated from Gouda cheese aged for 8 months (Saito et al. 2000). Dimitrov et al. (2015) evaluated the ACE inhibitory activity of 180 LAB and selected as starters several *L. helveticus*, *L. bulgaricus* and *L. casei* strains for Bulgarian cheese production, which led to increased production of bioactive peptides. Finally, addition of *L. casei* 279 or *L. casei* LAFTI® L26 as adjuncts in Cheddar cheese production increased the ACE-inhibitory activity during ripening at 4 °C, possibly due to increased proteolysis (Ong et al. 2007).

Scientific Evidence for Health Promoting Effects by Fermented Dairy Products

Although the presence of beneficial microbiota and their biological active metabolites in fermented dairy products is well documented, many of their claimed physiological actions have only been assayed *in vitro* and in animal models. Thus, there is a significant challenge in trying to extrapolate animal studies to humans. Scientific evidence from human interventions and clinical trials that include the evaluation of the fermented products and their matrices are needed to validate their functional properties.

Modulation of the gut microbiota It has been postulated that the live microorganisms in yogurt and fermented milks benefit gastrointestinal health by modulating the resident gut microbiota. However, it is worth pointing out that this modulation is based on the notion that a “normal” healthy gut microbiota exists; however, a

normal healthy gut microbiota has not been defined, except perhaps as microbiota without a pathogenic bacteria overgrowth. The type and amount of microbes in the human intestine differ substantially between individuals, which means that the phylogenetic composition could be considered a subjective fingerprint (Schmidt et al. 2018). However, while our microbiota varies phylogenetically, metagenomic analyses have revealed that at the highest functional level, the functional potential of the microbiome of healthy individuals remains very similar (Heintz-Buschart and Wilmes 2018). This could be a good starting point for developing the concept of “normal” healthy gut microbiota.

To exert their benefits, transient dairy bacteria entering the human gastrointestinal tract not only must survive the hostile conditions of the stomach and the small intestine to reach the colon, they also need to survive and compete in a colon environment fully seeded with resident microorganisms (Hillman et al. 2017). Fermented food and beverages represent between 5 and 40% of the daily food intake in the world (Prakash Tamang and Kailasapathy 2010), which corresponds to 0.1–1.0% of the bacteria present in the gastrointestinal tract. The bacteria that survive the conditions of the gastrointestinal tract are an extra source of microbial metabolites being conceivable that they might alter the proportions of autochthonous bacteria, impacting diversity and functionality.

Unno et al. (2015) investigated changes in the human gut microbial community structure after consumption of fermented milk containing probiotics. The microbiota was stable at the phylum level, although the relative abundance of Bacteroidetes increased during the ingestion of the fermented milk and decreased during the non-ingestion period. This suggested that consumption of the fermented milk can temporarily alter the gut microbial community structure maintaining its stability. Nevertheless, interactions between resident and transient microorganisms are yet insufficiently clear and can be highly dependent on the colonization resistance of the autochthonous microbes. In another study, conventional and gnotobiotic rats fed fermented milk containing five strains of *Bifidobacterium animalis*, *L. lactis* subsp. *lactis*, *L. bulgaricus* and *S. thermophilus* over a 15-day period showed that the clearance kinetic of *L. lactis* was strongly dependent on the structure of the resident gut microbiota and its susceptibility to be modulated by the transient strain (Zhang et al. 2016). One group of rats promptly eliminated *L. lactis* after fermented milk ingestion, whereas another group shed the strain over an additional 24–48 h. Overall, the specific contribution of dairy fermentative bacteria to the human gut ecosystem composition and functionality remains unclear.

Prevention of infection by pathogens Cheese has been associated with the prevention of bacterial gastroenteritis caused by the foodborne pathogen *Campylobacter jejuni*. A case control study carried out in South Australia involving children aged 1–5 years diagnosed with *C. jejuni* infection, showed that frequent consumption of foods including Cheddar and soft processed cheese was associated with a lower risk of gastroenteritis symptoms (Cameron et al. 2004). This fact reinforces the body of evidence pointing to traditional fermented products such as cheese be considered and included regularly in the daily diet.

Inflammation Maturation, function and defense mechanisms of the immune system are built up over the first years of life and are greatly influenced by the established gut microbiota (Nash et al. 2017). Inflammation is part of the human host defense system for facing unwanted environmental challenges. The immune system reacts with the production of proinflammatory mediators that causes systemic inflammation.

Human cross-sectional studies have supported the premise that the consumption of yogurt can be associated with a lower inflammatory state. Meyer et al. (2007) studied the effect of daily intake of conventional yogurt containing *S. thermophilus* and *L. bulgaricus* on cytokine production in 33 healthy women aged 22–29 years. The subjects consumed 100 g of yogurt per day for 2 weeks, and then 200 g for the subsequent 2 weeks. Consumption of yogurt enhanced cellular immune function, stimulating significant production of TNF- α (63% compared with baseline). On the other hand, Olivares et al. (2006) studied the immunological effects of the dietary deprivation of fermented foods in 30 healthy adult human volunteers (15 females and 15 males) aged from 23 to 43 years. After deprivation for 2 weeks, a decrease in phagocytic activity in leukocytes was observed. The fall in immune response was counteracted after the ingestion of conventional yogurt. Nevertheless, methodological factors limit comparisons between these studies and do not allow differentiation between a beneficial or neutral impact of dairy products on inflammation. Hence, further studies specifically designed to assess inflammation-related outcomes are warranted.

Chronic intestinal inflammation has been associated with development of colorectal cancer. Several studies have shown that LAB present in fermented products may protect against cancer by binding mutagens, inhibiting bacterial enzymes that form carcinogens and reducing inflammation (Zhong et al. 2014). Accordingly, Perdigón et al. (2002) demonstrated in BALBc mice that yogurt may exert antitumor activity by decreasing the inflammatory immune response mediated by IgA(+), apoptosis induction and IL-10 release. Likewise, Pala et al. (2011) conducted a prospective study on 45,241 (14,178 men; 31,063 women) volunteers of the EPIC-Italy cohort and found that high yogurt intake can be significantly associated with decreased colorectal cancer risk in humans.

Cardiometabolic diseases Studies have concluded that yogurt may help to improve diet quality and maintain metabolic well-being as part of a healthy, energy-balanced dietary pattern. A cross-sectional study that examined whether yogurt consumption was associated with better diet quality and metabolic profile among American adults (n = 6526) participating in the Framingham Heart Study Offspring (1998–2001) and Third Generation (2002–2005) cohorts, concluded that yogurt consumption was associated with lower levels of circulating triglycerides, glucose, and lower systolic blood pressure and insulin resistance (Wang et al. 2013). Healthier insulin profile after frequent yogurt consumption was also observed with children in a cross-sectional study using data from the National Health and Nutrition Examination Survey (NHANES) in the USA (Zhu et al. 2015). Accordingly, the PREDIMED study following prospectively 3454 non-diabetic individuals

concluded that consumption of yogurt was inversely associated with type 2 diabetes risk in the elderly at high cardiovascular risk (Diaz-Lopez et al. 2016). Association of high yogurt intake with a reduced risk of diabetes type 2 was also observed in a meta-analysis of 14 prospective cohorts with 459,790 participants (Chen et al. 2014).

Unlike type 2 diabetes, an inverse correlation between yogurt consumption and obesity has not been clearly established. A prospective study in an elderly population at high cardiovascular risk (PREDIMED study), concluded that consumption of whole-fat yogurt was associated with positive changes in waist circumference and higher probability for reversion of abdominal obesity (Santiago et al. 2016). Furthermore, a comprehensive literature search on MEDLINE and ISI Web of Knowledge from 1966 through June 2016 (Sayon-Orea et al. 2017) indicated that an inverse association between yogurt consumption and the risk of becoming overweight or obese was not fully consistent in prospective cohort studies although the results showed a tendency to improvement of most parameters of weight gain, risk of overweight or obesity, and risk of metabolic syndrome associated to yogurt consumption.

Finally there is evidence suggesting a moderate cholesterol-reducing action by fermented dairy products (St-Onge et al. 2000). Nevertheless, there is still a need for more prospective studies and high-quality randomized clinical trials to confirm improvement of metabolic markers apparently associated to yogurt and fermented milk consumption.

Probiotics in Fermented Dairy Foods

The International Scientific Association for Probiotics and Prebiotics has agreed to define probiotics as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Hill et al. 2014). Traditionally, *Lactobacillus*, *Bifidobacterium* and the yeast *Saccharomyces boulardii* are added fermented milks as probiotics (Aryana and Olson 2017). However, new species recently identified as relevant to intestinal health like *Faecalibacterium prausnitzii*, *Akkermansia muciniphila* and *Roseburia* could be added to fermented products as probiotics (El Hage et al. 2017). These species contribute to intestinal homeostasis, mucus integrity, and generation of short chain fatty acids.

Viability and functionality of probiotics in both food matrix and the gastrointestinal tract are essential to exert their beneficial effects. Therefore fermented products containing probiotics must have a minimum 10^6 cfu/g live bacteria at the expiration date, since the minimum therapeutic dose per day is suggested to be 10^8 – 10^9 cfu. Nevertheless, the presence and activity of probiotics in fermented products is sometimes far from being optimal. Although specific strains are inherently resistant to the conditions of production and transit through the gastrointestinal tract, like *Lactobacillus salivarius* CECT5713 and PS2, both isolated from

human milk, which were shown to survive during storage for 28 days at 4 °C with only a significant reduction in their viable counts was observed after 21 days (Cárdenas et al. 2014), some strains of bifidobacteria added to commercial fermented milks do not survive gastric transit and their numbers decline during storage (Ladero and Sanchez 2017). Consequently, technological strategies for improving viability and functionality of probiotics have been developed (Tripathi and Giri 2014) and include control of the product's final pH, the addition of oxygen scavengers, the use of probiotics producing protective EPS, microencapsulation or addition of prebiotics to the fermented milk in order to improve survival (Fernández et al. 2015). The review by Castro et al. (2015) summarizes different aspects related to the technological stability barriers encountered in the development of cheeses containing live probiotics.

Bifidobacterium and *Lactobacillus* have been exploited as probiotic beneficial microorganisms for centuries; however, the molecular mechanisms by which these bacteria exert their beneficial effects are still under investigation. Genome sequencing has provided insights into the diversity and evolution of commensal and probiotic bacteria to reveal the molecular basis for their health-promoting properties (Ventura et al. 2009). Full genome sequences of lactobacilli and bifidobacteria are publicly available and have significantly expanded our understanding of the biology of these microorganisms and how they have adapted so well to the human gastrointestinal tract despite their distant original niches. Combined with advanced postgenomic mammalian host response analyses, the molecular interactions that underlie the host-health effects observed are being elucidated (Sommer and Backhed 2016). Following metagenomics, the current metaproteomic and metatranscriptomic analyses have revealed that although there are similarities at the highest functional level among individuals, the microbiota is affected by a variety of factors, including diet, host genetics, and health status. As example, a placebo-controlled intervention trial of 16 healthy subjects that consumed *Lactobacillus rhamnosus* GG for 3 weeks found a common core of shared microbial protein functions in all subjects but no significant changes in the metaproteome attributable to the probiotic intervention (Kolmeder et al. 2016).

A new era in the probiotic field to be considered is the link between bacteria and brain activity in humans, the so called psychobiotics (Bambury et al. 2018). The link between the gut microbiota and brain was first demonstrated in germ free mice showing impaired emotional behaviors and brain biochemistry (Diaz Heijtz et al. 2011). In this model, microbial colonization of the gut initiates signaling mechanisms that affect neuronal circuits involved in motor control and anxiety behavior. A recent randomized, double-blind placebo controlled clinical trial conducted among 60 patients with Alzheimer's disease showed that patients drinking 200 mL/day of a probiotic milk containing *L. acidophilus*, *B. bifidum* and *L. fermentum* during 12 weeks were positively affected in its cognitive function (Akbari et al. 2016). Although promising, this field is still in infancy and high-quality clinical trials are needed to provide enough evidence before probiotics could be therapeutically used in neurodegenerative and emotional disorders. Furthermore, human randomized

control trials and hypothesis-driven mechanistic-based experimental studies are needed to validate health claims before fermented dairy products including probiotics can be advocated for specific disease prevention.

Conclusions

Fermented dairy products are ideal carriers of live microorganisms and their metabolites allowing for specific delivery where they will exert their physiological functionality. Moreover, dairy products constitute the hallmark of probiotics supply in the food market. Although industrialized processes have reduced the microbial diversity in milk and dairy products contributing to the limited exposure to a traditionally rich and highly biodiverse microbiota in food products, recent efforts are addressing this issue by applying diversification methods to dairy starters and by enriching fermented dairy products with bioactive compounds. New omics technologies are providing a better understanding of the microbial metabolism, interaction between transient and resident microbiota, and contribution of the microbiota to the host homeostasis at a molecular level essential to increase our understanding of the beneficial effects of milk and their microbially generated products.

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References

- Adegboye, A. R., Christensen, L. B., Holm-Pedersen, P., Avlund, K., Boucher, B. J., & Heitmann, B. L. (2012). Intake of dairy products in relation to periodontitis in older Danish adults. *Nutrients*, *4*(9), 1219–1229.
- Akbari, E., Asemi, Z., Kakhaki, R. D., Bahmani, F., Kouchaki, E., Tamtaji, O. R., Hamidi, G. A., & Salami, M. (2016). Effect of probiotic supplementation on cognitive function and metabolic status in Alzheimer's disease: A randomized, double-blind and controlled trial. *Frontiers in Aging Neuroscience*, *8*, 256.
- Alvaro, E., Andrieux, C., Rochet, V., Rigottier-Gois, L., Lepercq, P., Sutren, M., Galan, P., Duval, Y., Juste, C., & Dore, J. (2007). Composition and metabolism of the intestinal microbiota in consumers and non-consumers of yogurt. *The British Journal of Nutrition*, *97*(1), 126–133.
- Arqués, J. L., Rodríguez, E., Langa, S., Landete, J. M., & Medina, M. (2015). Antimicrobial activity of lactic acid bacteria in dairy products and gut: Effect on pathogens. *BioMed Research International*, *2015*, 584183.
- Arth, A., Kancherla, V., Pachon, H., Zimmerman, S., Johnson, Q., & Oakley, G. P., Jr. (2016). A 2015 global update on folic acid-preventable spina bifida and anencephaly. *Birth Defects Research. Part A, Clinical and Molecular Teratology*, *106*(7), 520–529.
- Aryana, K. J., & Olson, D. W. (2017). A 100-year review: Yogurt and other cultured dairy products. *Journal of Dairy Science*, *100*(12), 9987–10013.

- Atta, C. A., Fiest, K. M., Frolkis, A. D., Jette, N., Pringsheim, T., St Germaine-Smith, C., Rajapakse, T., Kaplan, G. G., & Metcalfe, A. (2016). Global birth prevalence of spina bifida by folic acid fortification status: A systematic review and meta-analysis. *American Journal of Public Health, 106*(1), e24–e34.
- Azcarate-Peril, M. A., Ritter, A. J., Savaiano, D., Monteagudo-Mera, A., Anderson, C., Magness, S. T., & Klaenhammer, T. R. (2017). Impact of short-chain galactooligosaccharides on the gut microbiome of lactose-intolerant individuals. *Proceedings of the National Academy of Sciences of the United States of America, 114*(3), E367–E375.
- Bambury, A., Sandhu, K., Cryan, J. F., & Dinan, T. G. (2018). Finding the needle in the haystack: Systematic identification of psychobiotics. *British Journal of Pharmacology, 175*, 4430–4438.
- Beermann, C., & Hartung, J. (2013). Physiological properties of milk ingredients released by fermentation. *Food & Function, 4*(2), 185–199.
- BNF. (2015). *Healthy eating*. Retrieved March 20, 2015, from <http://www.nutrition.org.uk/healthy-living/healthy-eating.html>
- Bourrie, B. C., Willing, B. P., & Cotter, P. D. (2016). The microbiota and health promoting characteristics of the fermented beverage kefir. *Frontiers in Microbiology, 7*, 647.
- Cameron, S., Ried, K., Worsley, A., & Topping, D. (2004). Consumption of foods by young children with diagnosed campylobacter infection—A pilot case-control study. *Public Health Nutrition, 7*(1), 85–89.
- Cárdenas, N., Calzada, J., Peiroten, A., Jiménez, E., Escudero, R., Rodríguez, J. M., Medina, M., & Fernández, L. (2014). Development of a potential probiotic fresh cheese using two *Lactobacillus salivarius* strains isolated from human milk. *BioMed Research International, 2014*, 801918.
- Carminati, D., Giraffa, G., Zago, M., Marco, M. B., Guglielmotti, D., Binetti, A., & Reinheimer, J. (2016). Lactic acid bacteria for dairy fermentations: Specialized starter cultures to improve dairy products. In F. Mozzi, R. R. Raya, & G. M. Vignolo (Eds.), *Biotechnology of lactic acid bacteria: Novel applications* (2nd ed., pp. 191–208). Chichester, UK: Wiley.
- Castro, J. M., Tornadijo, M. E., Fresno, J. M., & Sandoval, H. (2015). Biocheese: A food probiotic carrier. *BioMed Research International, 2015*, 723056.
- Chen, M., Sun, Q., Giovannucci, E., Mozaffarian, D., Manson, J. E., Willett, W. C., & Hu, F. B. (2014). Dairy consumption and risk of type 2 diabetes: 3 cohorts of US adults and an updated meta-analysis. *BMC Medicine, 12*, 215.
- Cheplin, H. A., & Rettger, L. F. (1920). Studies on the transformation of the intestinal flora, with special reference to the implantation of *Bacillus acidophilus*, II. Feeding experiments on man. *Proceedings of the National Academy of Sciences of the United States of America, 6*, 704–705.
- Chilton, S. N., Burton, J. P., & Reid, G. (2015). Inclusion of fermented foods in food guides around the world. *Nutrients, 7*(1), 390–404.
- Codex-Alimentarius. (2003). *CODEX standard for fermented milks*. Codex Stan 243–2003. Retrieved from http://www.codexalimentarius.net/download/standards/400/CXS_243e.pdf, http://www.codexalimentarius.net/download/standards/400/CXS_243e.pdf
- del Campo, R., Bravo, D., Canton, R., Ruiz-Garbajosa, P., Garcia-Albiach, R., Montesi-Libois, A., Yuste, F. J., Abairra, V., & Baquero, F. (2005). Scarce evidence of yogurt lactic acid bacteria in human feces after daily yogurt consumption by healthy volunteers. *Applied and Environmental Microbiology, 71*(1), 547–549.
- Dhakar, R., Bajpai, V. K., & Baek, K. H. (2012). Production of GABA (gamma-aminobutyric acid) by microorganisms: A review. *Brazilian Journal of Microbiology, 43*(4), 1230–1241.
- Diana, M., Rafecas, M., Arco, C., & Quilez, J. (2014). Free amino acid profile of Spanish artisanal cheeses: Importance of gamma-aminobutyric acid (GABA) and ornithine content. *Journal of Food Composition and Analysis, 35*(2), 94–100.
- Diaz Heijtz, R., Wang, S., Anuar, F., Qian, Y., Bjorkholm, B., Samuelsson, A., Hibberd, M. L., Forssberg, H., & Pettersson, S. (2011). Normal gut microbiota modulates brain development and behavior. *Proceedings of the National Academy of Sciences of the United States of America, 108*(7), 3047–3052.

- Díaz-Lopez, A., Bullo, M., Martínez-González, M. A., Corella, D., Estruch, R., Fito, M., Gómez-Gracia, E., Fiol, M., García de la Corte, F. J., Ros, E., Babio, N., Serra-Majem, L., Pinto, X., Muñoz, M. A., Frances, F., Buil-Cosiales, P., & Salas-Salvado, J. (2016). Dairy product consumption and risk of type 2 diabetes in an elderly Spanish Mediterranean population at high cardiovascular risk. *European Journal of Nutrition*, 55(1), 349–360.
- Dimitrov, Z., Chorbadjiyska, E., Gotova, I., Pashova, K., & Ilieva, S. (2015). Selected adjunct cultures remarkably increase the content of bioactive peptides in Bulgarian white brined cheese. *Biotechnology & Biotechnological Equipment*, 29(1), 78–83.
- EFSA. (2010). Scientific opinion on the substantiation of health claims related to live yoghurt cultures and improved lactose digestion. *EFSA Journal*, 8(10), 1763.
- El Hage, R., Hernández-Sanabria, E., & Van de Wiele, T. (2017). Emerging trends in “Smart probiotics”: Functional consideration for the development of novel health and industrial applications. *Frontiers in Microbiology*, 8, 1889.
- Elli, M., Callegari, M. L., Ferrari, S., Bessi, E., Cattivelli, D., Soldi, S., Morelli, L., Goupil Feuillerat, N., & Antoine, J. M. (2006). Survival of yogurt bacteria in the human gut. *Applied and Environmental Microbiology*, 72(7), 5113–5117.
- Fang, X. X., Rioux, L. E., Labrie, S., & Turgeon, S. L. (2016). Commercial cheeses with different texture have different disintegration and protein/peptide release rates during simulated in vitro digestion. *International Dairy Journal*, 56, 169–178.
- Fekete, A. A., Givens, D. I., & Lovegrove, J. A. (2015). Casein-derived lactotriptides reduce systolic and diastolic blood pressure in a meta-analysis of randomised clinical trials. *Nutrients*, 7(1), 659–681.
- Fernández, M., Hudson, J. A., Korpela, R., & de los Reyes-Gavilán, C. G. (2015). Impact on human health of microorganisms present in fermented dairy products: An overview. *BioMed Research International*, 2015, 412714.
- Franciosi, E., Carafa, I., Nardin, T., Schiavon, S., Poznanski, E., Cavazza, A., Larcher, R., & Tuohy, K. M. (2015). Biodiversity and gamma-aminobutyric acid production by lactic acid bacteria isolated from traditional alpine raw cow’s milk cheeses. *BioMed Research International*, 2015, 625740.
- Gandhi, A., & Shah, N. P. (2014). Cell growth and proteolytic activity of *Lactobacillus acidophilus*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii* ssp *bulgaricus*, and *Streptococcus thermophilus* in milk as affected by supplementation with peptide fractions. *International Journal of Food Sciences and Nutrition*, 65(8), 937–941.
- Guarner, F., Perdigon, G., Corthier, G., Salminen, S., Koletzko, B., & Morelli, L. (2005). Should yoghurt cultures be considered probiotic? *The British Journal of Nutrition*, 93(6), 783–786.
- Hagi, T., Kobayashi, M., & Nomura, M. (2016). Metabolome analysis of milk fermented by gamma-aminobutyric acid-producing *Lactococcus lactis*. *Journal of Dairy Science*, 99(2), 994–1001.
- Health-Canada. (2011). *Eating well with Canada’s food guide*. Retrieved February 26, 2018, from <http://www.hc-sc.gc.ca/fn-an/food-guide-aliment/index-eng.php>
- Heintz-Buschart, A., & Wilmes, P. (2018). Human gut microbiome: Function matters. *Trends in Microbiology*, 26(7), 563–574.
- Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., & Sanders, M. E. (2014). Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews. Gastroenterology & Hepatology*, 11(8), 506–514.
- Hill, D., Sugrue, I., Arendt, E., Hill, C., Stanton, C., & Ross, R. P. (2017). Recent advances in microbial fermentation for dairy and health. *F1000Research*, 6, 751.
- Hillman, E. T., Lu, H., Yao, T., & Nakatsu, C. H. (2017). Microbial ecology along the gastrointestinal tract. *Microbes and Environments*, 32(4), 300–313.
- Iyer, R., & Tomar, S. K. (2009). Folate: A functional food constituent. *Journal of Food Science*, 74(9), R114–R122.

- Kailasapathy, K., & Chin, J. (2000). Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. *Immunology and Cell Biology*, 78(1), 80–88.
- Karimi, R., Mortazavian, A. M., & Da Cruz, A. G. (2011). Viability of probiotic microorganisms in cheese during production and storage: A review. *Dairy Science & Technology*, 91(3), 283–308.
- Kesenkas, H., Gursoy, O., & Ozbas, H. (2017). Kefir. In J. Frias, C. Martinez-Villaluenga, & E. Penas (Eds.), *Fermented foods in health and disease prevention* (pp. 339–361). London: Academic Press Ltd/Elsevier Science Ltd.
- Keszei, A. P., Schouten, L. J., Goldbohm, R. A., & van den Brandt, P. A. (2010). Dairy intake and the risk of bladder cancer in the Netherlands Cohort Study on Diet and Cancer. *American Journal of Epidemiology*, 171(4), 436–446.
- Kolmeder, C. A., Salojarvi, J., Ritari, J., de Been, M., Raes, J., Falony, G., Vieira-Silva, S., Kekkonen, R. A., Corthals, G. L., Palva, A., Salonen, A., & de Vos, W. M. (2016). Faecal metaproteomic analysis reveals a personalized and stable functional microbiome and limited effects of a probiotic intervention in adults. *PLoS One*, 11(4), e0153294.
- Korhonen, H., & Pihlanto, A. (2006). Bioactive peptides: Production and functionality. *International Dairy Journal*, 16(9), 945–960.
- Ladero, V., & Sanchez, B. (2017). Molecular and technological insights into the aerotolerance of anaerobic probiotics: Examples from bifidobacteria. *Current Opinion in Food Science*, 14, 110–115.
- LeBlanc, J. G., Chain, F., Martin, R., Bermudez-Humaran, L. G., Courau, S., & Langella, P. (2017). Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. *Microbial Cell Factories*, 16(1), 79.
- Lister, J. (1873). Further contribution to the natural history of bacteria and the germ theory of fermentative changes. *The Quarterly Journal of Microscopical Science*, 13, 380–408.
- López-Expósito, I., Miralles, B., Amigo, L., & Hernandez-Ledesma, B. (2017). Health effects of cheese components with a focus on bioactive peptides. In J. Frias, C. Martinez-Villaluenga, & E. Penas (Eds.), *Fermented foods in health and disease prevention* (pp. 239–273). London: Academic Press Ltd/Elsevier Science Ltd.
- Macori, G., & Cotter, P. D. (2018). Novel insights into the microbiology of fermented dairy foods. *Current Opinion in Biotechnology*, 49, 172–178.
- Marciniak, A. (2011). The secondary products revolution: Empirical evidence and its current zooarchaeological critique. *Journal of World Prehistory*, 24(2–3), 117–130.
- Marco, M. L., Heeney, D., Binda, S., Cifelli, C. J., Cotter, P. D., Foligne, B., Ganzle, M., Kort, R., Pasin, G., Pihlanto, A., Smid, E. J., & Hutkins, R. (2017). Health benefits of fermented foods: Microbiota and beyond. *Current Opinion in Biotechnology*, 44, 94–102.
- Markowiak, P., & Slizewska, K. (2017). Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients*, 9(9), 1021.
- Martinez-Villaluenga, C., Penas, E., & Frias, J. (2017). Bioactive peptides in fermented foods: Production and evidence for health effects. In J. Frias, C. Martinez-Villaluenga, & E. Penas (Eds.), *Fermented foods in health and disease prevention* (pp. 23–47). London: Academic Press Ltd/Elsevier Science Ltd.
- Mater, D. D., Bretigny, L., Firmesse, O., Flores, M. J., Mogenet, A., Bresson, J. L., & Corthier, G. (2005). *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* survive gastrointestinal transit of healthy volunteers consuming yogurt. *FEMS Microbiology Letters*, 250(2), 185–187.
- Metchnikoff, E. (1908). *The prolongation of life. Optimistic studies*. New York: G. P. Putnam's Sons/The Knickerbocker Press.
- Meyer, A. L., Elmadfa, I., Herbacek, I., & Micksche, M. (2007). Probiotic, as well as conventional yogurt, can enhance the stimulated production of proinflammatory cytokines. *Journal of Human Nutrition and Dietetics*, 20(6), 590–598.
- Misselwitz, B., Pohl, D., Fruhauf, H., Fried, M., Vavricka, S. R., & Fox, M. (2013). Lactose malabsorption and intolerance: Pathogenesis, diagnosis and treatment. *United European Gastroenterology Journal*, 1(3), 151–159.

- Montel, M. C., Buchin, S., Mallet, A., Delbes-Paus, C., Vuitton, D. A., Desmasures, N., & Berthier, F. (2014). Traditional cheeses: Rich and diverse microbiota with associated benefits. *International Journal of Food Microbiology*, *177*, 136–154.
- Moslehi-Jenabian, S., Pedersen, L. L., & Jespersen, L. (2010). Beneficial effects of probiotic and food borne yeasts on human health. *Nutrients*, *2*(4), 449–473.
- Nagpal, R., Behare, P., Rana, R., Kumar, A., Kumar, M., Arora, S., Morotta, F., Jain, S., & Yadav, H. (2011). Bioactive peptides derived from milk proteins and their health beneficial potentials: An update. *Food & Function*, *2*(1), 18–27.
- Nampoothiri, K. M., Beena, D. J., Vasanthakumari, D. S., & Ismail, B. (2017). Health benefits of exopolysaccharides in fermented foods. In J. Frias, C. Martinez-Villaluenga, & E. Penas (Eds.), *Fermented foods in health and disease prevention* (pp. 49–62). London: Academic Press Ltd/Elsevier Science Ltd.
- Nash, M. J., Frank, D. N., & Friedman, J. E. (2017). Early microbes modify immune system development and metabolic homeostasis—The “Restaurant” hypothesis revisited. *Frontiers in Endocrinology*, *8*, 349.
- Olivares, M., Diaz-Ropero, P., Gomez, N., Sierra, S., Lara-Villoslada, F., Martin, R., Rodriguez, J. M., & Xaus, J. (2006). Dietary deprivation of fermented foods causes a fall in innate immune response. Lactic acid bacteria can counteract the immunological effect of this deprivation. *Journal of Dairy Research*, *73*(4), 492–498.
- Ong, L., Henriksson, A., & Shah, N. P. (2007). Angiotensin converting enzyme-inhibitory activity in Cheddar cheeses made with the addition of probiotic *Lactobacillus casei* sp. *Le Lait*, *87*(2), 149–165.
- Orla O’Sullivan, P. D. C. (2017). Microbiota of raw milk and raw milk cheeses. In P. L. H. McSweeney, P. F. Fox, P. D. Cotter, & D. W. W. Everett (Eds.), *Cheese: Chemistry, physics and microbiology* (pp. 301–316). London: Elsevier Academic Press.
- Pala, V., Sieri, S., Berrino, F., Vineis, P., Sacerdote, C., Palli, D., Masala, G., Panico, S., Mattiello, A., Tumino, R., Giurdanella, M. C., Agnoli, C., Grioni, S., & Krogh, V. (2011). Yogurt consumption and risk of colorectal cancer in the Italian European prospective investigation into cancer and nutrition cohort. *International Journal of Cancer*, *129*(11), 2712–2719.
- Pelaez, C., & Requena, T. (2005). Exploiting the potential of bacteria in the cheese ecosystem. *International Dairy Journal*, *15*(6–9), 831–844.
- Perdigón, G., de Moreno de LeBlanc, A., Valdez, J., & Rachid, M. (2002). Role of yoghurt in the prevention of colon cancer. *European Journal of Clinical Nutrition*, *56*(Suppl 3), S65–S68.
- Prakash Tamang, J., & Kailasapathy, K. (2010). *Fermented foods and beverages of the world*. Boca Raton: CRC Press.
- Quilez, J., & Diana, M. (2017). Gamma-aminobutyric acid-enriched fermented foods. In J. Frias, C. Martinez-Villaluenga, & E. Penas (Eds.), *Fermented foods in health and disease prevention* (pp. 85–103). London: Academic Press Ltd/Elsevier Science Ltd.
- Rizkalla, S. W., Luo, J., Kabir, M., Chevalier, A., Pacher, N., & Slama, G. (2000). Chronic consumption of fresh but not heated yogurt improves breath-hydrogen status and short-chain fatty acid profiles: A controlled study in healthy men with or without lactose maldigestion. *American Journal of Clinical Nutrition*, *72*(6), 1474–1479.
- Rojas-Ronquillo, R., Cruz-Guerrero, A., Flores-Najera, A., Rodriguez-Serrano, G., Gomez-Ruiz, L., Reyes-Grajeda, J. P., Jimenez-Guzman, J., & Garcia-Garibay, M. (2012). Antithrombotic and angiotensin-converting enzyme inhibitory properties of peptides released from bovine casein by *Lactobacillus casei* Shirota. *International Dairy Journal*, *26*(2), 147–154.
- Ross, R. P., Fitzgerald, G., Collins, K., & Stanton, C. (2002). Cheese delivering biocultures—Probiotic cheese. *Australian Journal of Dairy Technology*, *57*(2), 71–78.
- Ryan, P. M., Ross, R. P., Fitzgerald, G. F., Caplice, N. M., & Stanton, C. (2015). Sugar-coated: Exopolysaccharide producing lactic acid bacteria for food and human health applications. *Food & Function*, *6*(3), 679–693.
- Saito, T., Nakamura, T., Kitazawa, H., Kawai, Y., & Itoh, T. (2000). Isolation and structural analysis of antihypertensive peptides that exist naturally in Gouda cheese. *Journal of Dairy Science*, *83*(7), 1434–1440.

- Salazar, N., Gueimonde, M., de los Reyes-Gavilan, C. G., & Ruas-Madiedo, P. (2016). Exopolysaccharides produced by lactic acid bacteria and bifidobacteria as fermentable substrates by the intestinal microbiota. *Critical Reviews in Food Science and Nutrition*, *56*(9), 1440–1453.
- Santiago, S., Sayon-Orea, C., Babio, N., Ruiz-Canela, M., Marti, A., Corella, D., Estruch, R., Fito, M., Aros, F., Ros, E., Gomez-Garcia, E., Fiol, M., Lapetra, J., Serra-Majem, L., Becerra-Tomas, N., Salas-Salvado, J., Pinto, X., Schroder, H., & Martinez, J. A. (2016). Yogurt consumption and abdominal obesity reversion in the PREDIMED study. *Nutrition, Metabolism, and Cardiovascular Diseases*, *26*(6), 468–475.
- Saubade, F., Hemery, Y. M., Guyot, J.-P., & Humblot, C. (2017). Lactic acid fermentation as a tool for increasing the folate content of foods. *Critical Reviews in Food Science and Nutrition*, *57*(18), 3894–3910.
- Savaiano, D. A. (2014). Lactose digestion from yogurt: Mechanism and relevance. *The American Journal of Clinical Nutrition*, *99*(5 Suppl), 1251S–1255S.
- Sayon-Orea, C., Martinez-Gonzalez, M. A., Ruiz-Canela, M., & Bes-Rastrollo, M. (2017). Associations between yogurt consumption and weight gain and risk of obesity and metabolic syndrome: A systematic review. *Advances in Nutrition*, *8*(1), 146S–154S.
- Schleifer, K. H., Kraus, J., Dvorak, C., Kilpperbalz, R., Collins, M. D., & Fischer, W. (1985). Transfer of *Streptococcus lactis* and related streptococci to the genus *Lactococcus* gen. nov. *Systematic and Applied Microbiology*, *6*(2), 183–195.
- Schmidt, T. S. B., Raes, J., & Bork, P. (2018). The human gut microbiome: From association to modulation. *Cell*, *172*(6), 1198–1215.
- Seppo, L., Jauhiainen, T., Poussa, T., & Korpela, R. (2003). A fermented milk high in bioactive peptides has a blood pressure-lowering effect in hypertensive subjects. *American Journal of Clinical Nutrition*, *77*(2), 326–330.
- Settanni, L., & Moschetti, G. (2010). Non-starter lactic acid bacteria used to improve cheese quality and provide health benefits. *Food Microbiology*, *27*(6), 691–697.
- Silanikove, N., Leitner, G., & Merin, U. (2015). The interrelationships between lactose intolerance and the modern dairy industry: Global perspectives in evolutionary and historical backgrounds. *Nutrients*, *7*(9), 7312–7331.
- Sipola, M., Finckenberg, P., Korpela, R., Vapaatalo, H., & Nurminen, M. L. (2002). Effect of long-term intake of milk products on blood pressure in hypertensive rats. *Journal of Dairy Research*, *69*(1), 103–111.
- Smid, E. J., Erkus, O., Spus, M., Wolkers-Rooijackers, J. C., Alexeeva, S., & Kleerebezem, M. (2014). Functional implications of the microbial community structure of undefined mesophilic starter cultures. *Microbial Cell Factories*, *13*(Suppl 1), S2.
- Sommer, F., & Backhed, F. (2016). Know your neighbor: Microbiota and host epithelial cells interact locally to control intestinal function and physiology. *BioEssays*, *38*(5), 455–464.
- Sonestedt, E., Wirfalt, E., Wallstrom, P., Gullberg, B., Orho-Melander, M., & Hedblad, B. (2011). Dairy products and its association with incidence of cardiovascular disease: The Malmo diet and cancer cohort. *European Journal of Epidemiology*, *26*(8), 609–618.
- Stanton, C., Ross, R. P., Fitzgerald, G. F., & Van Sinderen, D. (2005). Fermented functional foods based on probiotics and their biogenic metabolites. *Current Opinion in Biotechnology*, *16*(2), 198–203.
- St-Onge, M. P., Farnworth, E. R., & Jones, P. J. (2000). Consumption of fermented and nonfermented dairy products: Effects on cholesterol concentrations and metabolism. *The American Journal of Clinical Nutrition*, *71*(3), 674–681.
- Thierry, A., Deutsch, S. M., Falentin, H., Dalmasso, M., Cousin, F. J., & Jan, G. (2011). New insights into physiology and metabolism of *Propionibacterium freudenreichii*. *International Journal of Food Microbiology*, *149*(1), 19–27.
- Tripathi, M. K., & Giri, S. K. (2014). Probiotic functional foods: Survival of probiotics during processing and storage. *Journal of Functional Foods*, *9*, 225–241.
- Tunick, M. H., & Van Hekken, D. L. (2015). Dairy products and health: Recent insights. *Journal of Agricultural and Food Chemistry*, *63*(43), 9381–9388.

- Unno, T., Choi, J. H., Hur, H. G., Sadowsky, M. J., Ahn, Y. T., Huh, C. S., Kim, G. B., & Cha, C. J. (2015). Changes in human gut microbiota influenced by probiotic fermented milk ingestion. *Journal of Dairy Science*, 98(6), 3568–3576.
- Urista, C. M., Fernandez, R. A., Rodriguez, F. R., Cuenca, A. A., & Jurado, A. T. (2011). Review: Production and functionality of active peptides from milk. *Food Science and Technology International*, 17(4), 293–317.
- USDA. (2010). *Dietary guidelines for Americans*. Washington, DC: U.S. Government Printing Office.
- Ventura, M., O’Flaherty, S., Claesson, M. J., Turrone, F., Klaenhammer, T. R., van Sinderen, D., & O’Toole, P. W. (2009). Genome-scale analyses of health-promoting bacteria: Probiogenomics. *Nature Reviews. Microbiology*, 7(1), 61–71.
- Van der Meer, R., Bovee-Oudenhoven, I. M. J. (1998). Dietary modulation of intestinal bacterial infections. *International Dairy Journal*, 8(5-6), 481–486.
- Wang, H., Livingston, K. A., Fox, C. S., Meigs, J. B., & Jacques, P. F. (2013). Yogurt consumption is associated with better diet quality and metabolic profile in American men and women. *Nutrition Research*, 33(1), 18–26.
- Watanabe, M., Maemura, K., Kanbara, K., Tamayama, T., & Hayasaki, H. (2002). GABA and GABA receptors in the central nervous system and other organs. *International Review of Cytology*, 213, 1–47.
- Weimer, B. (2007). *Improving the flavour of cheese*. Boca Raton: Woodhead Publishing/CRC Press.
- Wouters, J. T. M., Ayad, E. H. E., Hugenholtz, J., & Smit, G. (2002). Microbes from raw milk for fermented dairy products. *International Dairy Journal*, 12(2–3), 91–109.
- Wu, Q. L., & Shah, N. P. (2017). High gamma-aminobutyric acid production from lactic acid bacteria: Emphasis on *Lactobacillus brevis* as a functional dairy starter. *Critical Reviews in Food Science and Nutrition*, 57(17), 3661–3672.
- Zhang, C., Derrien, M., Levenez, F., Brazeilles, R., Ballal, S. A., Kim, J., Degivry, M. C., Quere, G., Garault, P., van Hylckama Vlieg, J. E., Garrett, W. S., Dore, J., & Veiga, P. (2016). Ecological robustness of the gut microbiota in response to ingestion of transient food-borne microbes. *The ISME Journal*, 10(9), 2235–2245.
- Zheng, H., Yde, C. C., Clausen, M. R., Kristensen, M., Lorenzen, J., Astrup, A., & Bertram, H. C. (2015). Metabolomics investigation to shed light on cheese as a possible piece in the French paradox puzzle. *Journal of Agricultural and Food Chemistry*, 63(10), 2830–2839.
- Zhong, L., Zhang, X., & Covasa, M. (2014). Emerging roles of lactic acid bacteria in protection against colorectal cancer. *World Journal of Gastroenterology*, 20(24), 7878–7886.
- Zhu, Y., Wang, H., Hollis, J. H., & Jacques, P. F. (2015). The associations between yogurt consumption, diet quality, and metabolic profiles in children in the USA. *European Journal of Nutrition*, 54(4), 543–550.