
Development of Functional Dairy Foods

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Abstract

There has been a growing interest on functional foods, markedly recognized as being able to provide additional benefits on health promotion, wellbeing maintenance, and disease prevention. Based on this scenario, food industries have been increasingly focused in developing added-value foodstuffs, being dairy foods one of the most currently used food products for functional purposes. Different extraction and encapsulation technologies have been used to obtain target food bioactive ingredients and to ensure an effective functionalization of dairy products, respectively. Probiotics, prebiotics, mushrooms, and plant food bioactive extracts comprise the most commonly used food ingredients to produce functional dairy foods, mostly fermented milk, yogurt, and cheese. In fact, dynamic and promissory biological effects have been documented for these functional dairy foods, among them antioxidant, cardioprotective, antihypertensive, immunomodulatory, antimicrobial, antidiabetic, anti-inflammatory, neuromodulatory, and even bone protection. However, besides the impact of health benefits on consumers' acceptance and subsequent consumption of functional dairy foods, other factors, such as consumers' familiarity with new products and functional ingredients used on their formulation, consumers' knowledge and awareness

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about the credibility of shared health effects, and finally the organoleptic and sensory evaluation of the developed functional dairy foods, have also a determinant role. Anyway, consumers are considered self-contributors for this promising food innovation. Thus, the concept of functional dairy foods may represent an upcoming multiniche market and sustainable trend to be exploited.

Keywords

Functional foods • Dairy products functionalization • Food ingredients • Extraction/encapsulation technologies • Consumers' acceptance

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1 Introduction

Consumers' perception about the real importance of a balanced diet and healthy lifestyle on disease prevention, health maintenance, and longevity promotion has driven the development of multiple and increasingly deepen studies [1, 2]. Foods, food ingredients, and even bioactive molecules with health-promoting abilities present a large demand by worldwide consumers [3]. In fact, biotechnological and agro-food industries have been increasingly focused on the development of highly specific methodological procedures to incorporate certain bioactive molecules on daily products, making them more safe, effective, bioavailable, and even able to confer certain additional biological attributes [4, 5]. Despite the inexistence of a general consensus, these food products are commonly referred as functional foods, i.e., *"foods that when included in the normal diet display one or more target functions in the human body, being able to improve the health status and/or to reduce the likelihood of some disorders occurrence"* [6]. Thus, functional foods make part of daily diet, both as natural, whole, or unmodified foods; however, they may also be used and/or incorporated as food constituents, added and/or removed by technological or biotechnological procedures that modulate their bioavailability, focusing on the improvement of their biological effects [6, 7]. Several food groups, such as meat and fish products, ice cream, cheese, yogurt, and other dairy products present very interesting chemical characteristics and nutritional composition that triggers the focus of researchers to the incorporation and application of bioactive molecules in those food matrices [3].

Dairy products are amongst the most commonly tested and selected food groups toward the incorporation of food ingredients for functional purposes. Milk, cream, yogurt, kefir, powdered milk, condensed milk, ricotta, butter, casein, fermented dairy drinks, infant milk formula, colostrum, cheese, and ice creams are the most common traditional dairy products considered as functional foods [8, 9]. All these products have on their chemical composition recognized ingredients that provide, besides the nutritional benefits, a considerable improvement on human health and well-being [8]. Numerous nutrients are present on dairy food products, being vitamins, minerals, and proteins the most abundant ones. However, some preparations present active ingredients with low availability and/or nutritional deficits of these constituents, being milk fortified with calcium and vitamin-D (fortified product) one of the most representative examples of this situation [10, 11]. Moreover, the incorporation of milk supplements and milk-based food products, such as probiotic bacteria and prebiotic polysaccharides, omega-3 fatty acids, and other compounds, is becoming increasingly common (enriched product) [10, 11]. Moreover, there are some situations in which some deleterious components need to be removed, reduced, or replaced by another substance with beneficial effects, for example, fibers as fat releasers in ice cream products (altered products) [10, 11]. Curiously, functional dairy foods are virtually found in all these types of functional food categories, despite they are not homogeneously scattered over all segments of the growing market.

In this sense, the present chapter aims to provide an overview on functional dairy foods, emphasizing the role of biotechnological and agro-industrial prospects in their development, describing the most commonly used plant food bioactives on functional dairy foods formulation, and lastly their bioactive and health-promoting abilities.

2 Dairy Food Products: An Overview

Innovations in the agro-food sector are mostly focused on the improvement of the currently available food products, always taking into account some specific criteria, such as the quality, safety, and even distinct characteristics related with bioactive and health promoter effects conferred by these matrices [12–15]. Besides, the information about nutrients, ingredients, and food additives should also be prioritized [12, 14]. Specifically, the food industry has been the target of intensive and impressive innovative processes along with different parts of the food chain, being the most evident ones classified in five distinct sectors: (1) new food ingredients and materials; (2) innovation in fresh foods; (3) innovation in food quality; (4) new food process techniques; and (5) new packaging methods [12]. All these innovative aspects result from consumers' demand that trigger the search and development of highly effective products, techniques, and services by food science and technology researchers in articulation with the food industry (Fig. 1). Within the scope of food science and technology, the market of dairy products has gained a renowned niche of

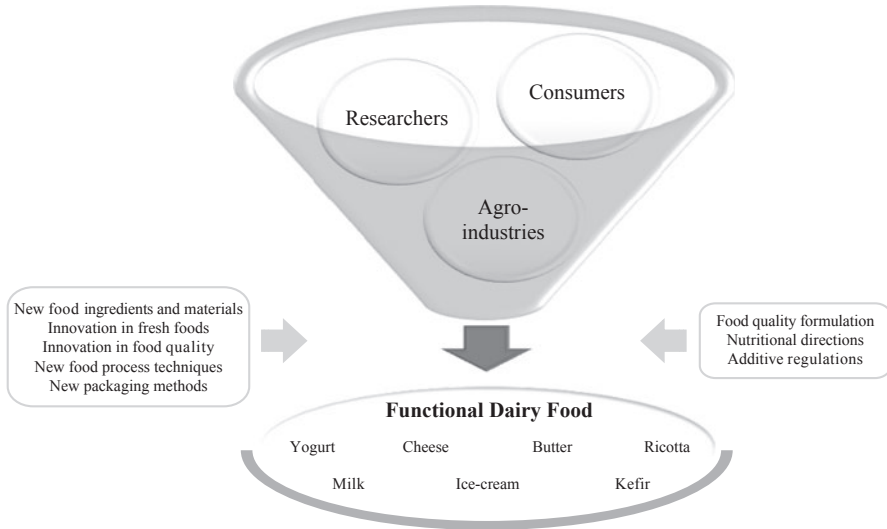


Fig. 1 Main actors and factors involved in the development of functional dairy foods

demand and recognition by consumers, besides of their increasing research interest and innovation procedures.

Milk is one of the most widely consumed dairy product, being used over centuries as part of daily diet [15, 16]. Despite a growing evidence showing that their unlimited ingestion is associated with several disorders, including some forms of cancer, asthma, diabetes, obesity, cardiovascular diseases, and even osteoporosis, more recent clinical data have demonstrated that calcium supplementation helps to promote bone health besides other benefits [9, 15, 17]. Nevertheless, there are some data that still remains inconclusive concerning to the linkage between high dietary calcium intake from milk and prevention of osteoporosis and bone loss [16]. On the other hand, and considering that many people are lactose-intolerant or even allergic to milk, the interest was progressively driven to milk fermented products, such as yogurt and cheese [16, 18, 19]. Fermented dairy products contain live beneficial microorganisms that can digest lactose and other nontolerable milk constituents, exert cholesterol-lowering effects, and also contribute to cancer risk reduction [15]. These promising modulatory effects are mainly reached through action of those microorganisms, namely probiotic bacteria, that significantly enhances the health-promoting abilities of dairy products, contributing for a longer and healthier lifetime [9, 16]. Not least important to highlight is that the benefits of probiotic bacteria may be achieved both directly and/or indirectly, being all these aspects carefully described in a specific section of this chapter.

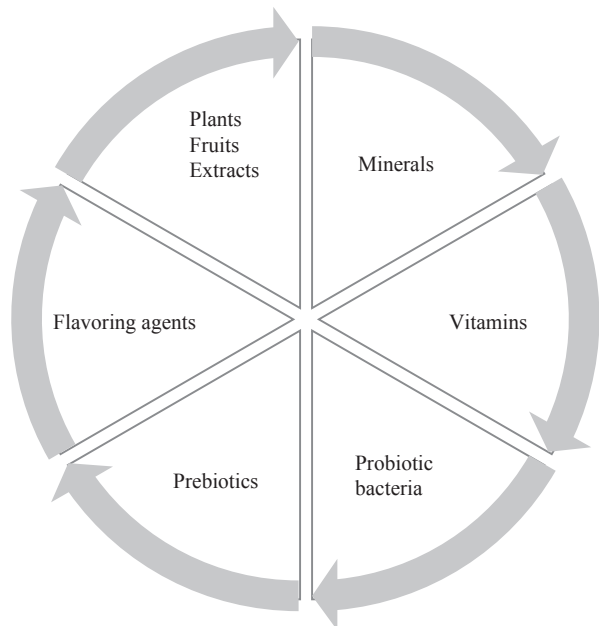
Considering the recent findings on this field, milk and other dairy products, and more specifically fermented dairy products, may have a crucial and determinant functional role in contributing to health and well-being of worldwide population. This aspect has driven an increasing interest of researches in developing functional

foods based on milk and other dairy products, which are known by humanity since medieval times due to their health benefits. More recently, the biologically active constituents have been increasingly studied and their benefits reported, remaining, however, the interest to deepen knowledge on this field, in order to promote their strengthening and valorization.

3 Functional Foods: Innovative Trends on Dairy Products

Functional foods have been the target of an intense investigation by researchers, highly demanded by consumers as also with increasingly strict regulatory processes applied to agro-industries. Despite dairy products present a broad history of use, in the last years a high demand by consumers for enhanced-dairy foods with additional health-promoting abilities has been observed [20, 21]. The large evidence that foods in general, and specifically dairy foods with balanced-enriched nutritional composition, may confer additional health benefits has stimulated the development of novel formulations of dairy products containing active ingredients [9, 18, 22]. Until now, prebiotics, probiotic bacteria, vitamins and minerals, flavoring agents, plants, fruits, and even its derived extracts are amongst the most frequently used food ingredients in the design of functional dairy food formulations (Fig. 2). In fact, the development and formulation of functional dairy foods are on the top of the current studies, being yogurt, followed by cheese, butter, milk through different preparations (powdered, processed, condensed, and even milk-based foods), ice cream, kefir, and ricotta the

Fig. 2 Most commonly used ingredients in the formulation of functional dairy foods



most widely investigated toward the incorporation of the previous mentioned ingredients for different functional purposes (Fig. 1). This information is briefly summarized in Table 1 and described in detail in the following paragraphs.

Studies with probiotics are on the top of dairy products' investigations, including the use of microorganisms, such as *Bifidobacterium*, *Lactobacillus*, *Streptococcus*, and *Saccharomyces*, individually or in cocultures [4, 14, 18]. Very interesting results have been reported related with food quality characteristics, effectiveness, and acceptance by consumers [4, 18]. There is an increasing evidence related with the effective biological potential and health benefits of probiotic bacteria, namely on the prevention of gastrointestinal disorders, microbiota modulation, selective inhibition of opportunistic microorganisms, on the metabolism of toxic substances, as anti-carcinogenic/antimutagenic agents, and even on the reduction of cholesterol levels and of lactose intolerance [3, 4, 7]. This last one has received a pivotal attention by food industries, due to the increasing rates of lactose intolerance, which directly interfere with food selection by worldwide consumers [4]. Different levels of lactose intolerance require specific dietary recommendations, including to avoid dairy products and derivatives [16]. Therefore, the incorporation of probiotic bacteria in various matrices of dairy products to reduce lactose intolerance is very important. Yogurts and cheeses are amongst the most investigated dairy products toward the incorporation of probiotic bacteria for functional purposes [4].

The incorporation of vitamins and minerals on dairy foods is not less common, both in bioavailable, stable, and soluble forms to enrich the nutritional value as also to extend the shelf life of dairy products [17]. Calcium, iron, and vitamins C, D, and B12 are among the most frequent ones [9]. Other B vitamins, such as folate and

Table 1 Types of food ingredients commonly incorporated in the formulation of functional dairy foods

Types of food ingredients	Detailed food ingredients	Dairy products incorporation
Prebiotics	Milk proteins, lactose, saccharose, lactulose, fructo-, glyco- and galactooligosaccharides, arabinose, galactose, inulin, raffinose, mannose, lactulose, staquiose, xylooligosaccharides, isomaltulose (palatinose), isomaltooligosaccharides, and soy oligosaccharides	Cheese, yogurt
Probiotics	Species: <i>Bifidobacterium</i> , <i>Lactobacillus</i> , <i>Streptococcus</i> and <i>Saccharomyces</i>	Cheese, yogurt
Vitamins and minerals	Calcium, iron, vitamins C, D, B2, B9, and B12	Milk, cheese, yogurt
Flavoring agents	Polyphenols, flavonoids, carotenoids	Milk, cheese, yogurt
Plants, fruits, and its derived extracts	Chamomile, fennel, honey, mushrooms, chestnut, lemon balm, rosemary, basil	Cheese, yogurt
Others	Omega-3 fatty acids are linoleic acid (LA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA); β -carotene	Milk, yogurt

riboflavin, have been also used; some of them are even synthesized from various nonvitamin precursors through action of certain bacteria in plant and dairy foods [14]. Although several years ago synthetic formulas were the most common, presently the incorporation of natural ingredients rich on these nutrients are on the top of agro-industrial selection and consumers' preference [3]. A similar scenario is observed for the addition of flavors and aromas, in which essences, plant fruits and/or plant fruit extracts, and even honey have been increasingly used, while artificial flavoring agents become secondary in the formulation of dairy products [2, 23, 24]. The addition of plants and fruits and/or its derived extracts seems to present modulatory effects on gut microbiota, i.e., favors the growth of beneficial bacteria and limits the growth of opportunistic microorganisms [14]. Still, these active ingredients are also able to contribute for the improvement of the dairy products shelf life at large extent, being therefore currently used for different purposes [25].

Plants and fruits are *per* definition, composed by a rich and complex pool of chemical molecules, most of them already characterized and properly identified as having important bioactive effects at different levels [26]. Within the different classes of bioactive molecules, phenolic compounds have received a special attention, due to their well-known antioxidant properties [26]. Nevertheless, most of these bioactive molecules present inactive forms, being transformed into active forms by metabolic processes; in this field lactic acid bacteria (*Lactobacillus* and *Streptococcus*) display a very important role, favoring the conversion of inactive phenolic compounds into their biologically active forms, via expression of glycosyl hydrolase, esterase, decarboxylase, and phenolic acid reductase enzymes [14]. These biochemical reactions lead to the formation of several biologically active molecules, namely pyranoanthocyanidins and 3-desoxypranoanthocyanidins that display determinant and regulator effects as health promoters and disease preventers [14]. Once again, probiotic bacteria seem to exert multiple contributing effects when combined with other food ingredients in dairy functional products' formulation. However, besides plant extracts, several other ingredients, such as milk proteins, inulin, oligosaccharides, and lactulose, have been incorporated for prebiotic purposes, i.e., to promote the growth of *Bifidobacterium* and other probiotic bacteria, to ensure gut health, to keep harmful bacteria under control, and to enhance immune system [9, 27]. Besides prebiotic effects, these active ingredients also show other important functions, such as improvement of minerals and vitamins absorption, decrease of triglycerides and cholesterol levels, and due to their high contents in dietary fiber, improvement of fecal transit time and stool weight, crucial to prevent constipation [9, 22]. Not least important to point out is that the above referenced health conditions are amongst the most common disorders observed in modern society.

The latest studies performed on this area have investigated different parameters related with the general quality and acceptability of the incorporation of honey to produce functional yogurts [2], mushrooms on functional cheese formulations [23], and even phenolic extracts derived from different plant parts on dairy functional yogurts and cheese formulation [25, 28–36]. Nevertheless, further studies are

necessary to evaluate the feasibility, efficacy, effectiveness, and bioavailability of bioactive ingredients used to functionalize dairy products, and not least important to evaluate the consumers' acceptance.

4 Functional Dairy Foods: Agro-Industrial and Biotechnological Prospects

A balanced and healthy diet is a key determining factor for overall quality of life, which has been increasingly evident for worldwide consumers [6]. In fact, the perception about the real importance of some specific foods and even food ingredients in the daily diet is increasingly common, up to a point that the consumers' demand of healthy and functional foods reached exponential levels [13, 14]. Being consumers directly involved on food selection, development, and evaluation of its overall quality and acceptability, the food industry is increasingly focused on the formulation of foods with abilities to improve the health and well-being of consumers, at the same time that favor digestive system (which is considered a key factor for overall quality of life), besides other physiological and metabolic effects [9, 11]. Therefore, the development of functional dairy foods provides a great opportunity to contribute to the improvement of food quality and consumers' health and well-being.

Among the wide variety of food ingredients, there are three main groups that have become increasingly common in functional foods formulation, such as: probiotics (live bacteria), prebiotics (compounds as fibers), and antioxidants [27, 37]. The latest group of antioxidants mainly includes vitamins, minerals, plants and fruits, and even its derivatives (Table 1). Most of the food ingredients currently incorporated in functional dairy foods formulation naturally occurs on these dairy matrices, such as bioactive peptides, probiotic bacteria, antioxidants, vitamins, specific proteins, oligosaccharides, organic acids, highly absorbable calcium, conjugated linoleic acid, and other biologically active components [9, 37]. Notwithstanding, and despite their natural occurrence, a limited content of bioactive compounds is present, which can compromise the effective value of these products [31]. Therefore, the incorporation of these active ingredients in the functionalization of milk and dairy products is of the utmost importance. Using these food ingredients, new functional dairy products may be formulated, through modification of the traditional formulas, adding, eliminating, or even substituting some constituents, and even though the addition of some wholesome compounds.

For example, Caleja et al. [31] observed that the incorporation of antioxidants from natural origin (from chamomile and fennel extracts) in yogurts not only improved their biological activity but also satisfied consumer demands, in comparison with the synthetic additive, potassium sorbate [31]. Also important to highlight is that the yogurt functionalization did not alter significantly its nutritional profile, external appearance, pH, and even fatty acids content, being therefore suitable for upcoming use both in food industry and in the dairy sector, where synthetic additives are commonly used [31]. On the other hand, Heleno et al. [36] performed an

experiment using *Agaricus bisporus* extracts obtained by ultrasound-assisted extraction, and ergosterol, to incorporate in dairy beverages at concentrations mimicking those that commercially occur in phytosterol-added yogurts. The authors observed that samples incorporated with the extract and with ergosterol showed a higher antioxidant capacity at same time that protected yogurt from oxidation, improving therefore their shelf life, without modifying the nutritional value in comparison with the original product [36]. Similar findings were reported by Carocho et al. [34] using chestnut flowers, lemon balm, and its respective aqueous extracts (decoction) to incorporate into “Serra da Estrela” cheese, aiming not only to assess their ability to maintain its nutritional value but mainly to promote additional characteristics toward new dairy foodstuffs formulation [34]. Caleja et al. [25] and Carocho et al. [34] incorporated, respectively, fennel in cottage cheese [38] and basil in “Serra da Estrela” cheese [35], aiming to assess their functional and preserving abilities. The authors found that both samples did not alter significantly the nutritional value of cheeses, at same time that improved their antioxidant properties, preserved the contents in unsaturated fatty acids and proteins, as also exerted a prominent functionalizing and preservative effect.

Concerning the incorporation of prebiotics and probiotics in functional dairy products formulation, multiple findings have reported that, by one hand the incorporation of prebiotics not only conferred protection but also improved the viability of probiotic bacteria, presupposing therefore their action as symbiotic ingredients [39]. On the other hand, it was also shown that the incorporation of stingless bee honey in goat yogurt containing *Lactobacillus acidophilus* positively affected several characteristics, among them the color, syneresis, viscosity, sensory acceptance, and even purchase intention [2]. But besides to these aspects, probiotic bacteria also exert other highly valuable biological effects, such as being responsible for reinforcing natural intestinal flora, decreasing serum triglycerides, cholesterol, transaminase, and total bilirubin levels, exerting immunomodulatory effects, protecting against gastrointestinal pathogens, contributing for lactose metabolism, infantile diarrhea, besides reducing the incidence of urogenital and respiratory diseases and preventing the occurrence of some cancers [3, 40].

It is clearly evident that these food ingredients may provide considerable nutritional and sensory attributes of quality and acceptability, despite having a great market potential to be exploited considering the functional properties. In this sense, and considering that the top research areas in the Food Science and Technology comprises the extraction and characterization of new natural ingredients with biological effects for further incorporation into functional foods formulation, it is very important to use proper extraction methodologies and even effective technologies in functional foods preparation. Therefore, there is not only interest in ensuring a proper extraction of the bioactive constituents, but also the use of effective encapsulation methodologies toward the preservation of all the characteristics presents in the developed functional food until the physiological site of action is reached.

Considering the existence of a wide variety of bioactive molecules with suitable characteristics for upcoming use as food ingredients on functional foods formulation, it is mandatory to select the most appropriate extraction methodology aiming to

achieve the highest extraction yield, without affecting both the chemical composition and even the final bioavailability and subsequent biological activity. The quality of the bioactive compounds is closely dependent on the suitability of the extraction process and the proper selection of separation steps, which will also affect the subsequent identification and characterization of those biomolecules [3, 4]. In this sense, the selected extraction technique will also need to convert bioactive compounds into more suitable forms both for detection and subsequent characterization, but also to provide a strong and reproducible method to be used in other molecules belonging to the same group [3, 4]. There are a wide variety of extraction techniques, but the most commonly used are briefly described in Table 2, namely Soxhlet extraction (SoE), Ultrasound-assisted extraction (UAE), Supercritical-fluid extraction (SFE), Accelerated solvent extraction (ASE), and finally Shake extraction (ShE).

Encapsulation technique is a complex process that includes the creation of a barrier, more or less complex, that acts covering bioactive components inhibiting the occurrence of chemical interactions, protecting against environmental factors (i.e., temperature, pH, enzymes, and oxygen), and even allowing the progressive release of the active components under certain conditions [3, 4].

There are three main groups of encapsulation techniques, i.e., microencapsulation, nanoencapsulation, and even emulsions, as briefly described in Table 3. The selection of a specific encapsulation procedure mainly depends on two distinct characteristics: the type of core material and characteristics of the final product where the technique will be applied. Moreover, the material of capsule wall, size, shape, and structure of capsule particles also determines the stability of the bioactive molecules during production, storage, and even releasing during consumption [3, 4]. However, the encapsulation technologies mainly aspire in ensuring a proper stabilization of the bioactive compounds, warranting that they reach consumers in appropriate levels, maintaining their bioavailability, avoiding the formation of harmful and unpleasant compounds and even to mask some undesirable sensorial attributes characteristic from some bioactive substances [3, 4].

5 Mushrooms and Plant-Food Bioactives: Key Factors on Functional Dairy Products Formulation

The world of plant-food bioactives has received a pivotal attention in the last years, being the assessment of their pharmacological effects and related biotechnological applications an exponential niche of market to be exploited. Regarding particularly their biotechnological applications, the sector of food industry and specifically the subsector of development of new food ingredients and materials are under dynamic innovation and overall exploitation. Inside this subsector, the area of functional foods' formulation is of largest demand not only by consumers but also of the utmost interest of researchers and industries (Fig. 1). Moreover, being the group of dairy products one of the most widely recognized food categories for functional purposes, considering the presence of promissory molecules with multiple benefits, a

Table 2 Most commonly used extraction technologies in functional foods formulation

Extraction techniques	
Soxhlet extraction (SoE)	Commonly used to extract lipophilic compounds (hexane or petroleum ether as solvents), but can be also used to extract polar compounds, such as some specific phenolic compounds that are effectively extracted using methanol. More recently has been also applied to extract volatile compounds. <i>Bioactive molecules</i> : carotenoids, phenolic compounds, sterols, and aromatic compounds.
Ultrasound-assisted extraction (UAE)	Based on ultrasonic vibrations, being a fast and simple method, allowing the extraction of multiple samples at same time. The solvent extraction used (mainly methanol, acetone, water, and ethyl acetate) displays a crucial role, but also sample size, pH, temperature, and pressure determines extraction quality and yield. <i>Bioactive molecules</i> : carotenoids, polysaccharides, proteins, phenolic compounds, aromatic compounds, and sterols.
Supercritical fluid extraction (SFE)	Currently considered a “green” methodology, this technique is used on the extraction of multiple nutraceutical ingredients, usually performed using carbon dioxide under high pressure. This technique allows the use of low temperatures, presents a high selectivity, needs low solvent volume, small samples, short extraction times, and great automation procedures. <i>Bioactive molecules</i> : carotenoids, sterols, tocopherols, and phenolic compounds.
Accelerated solvent extraction (ASE)	This technique is carried out under high pressure and temperatures, presenting, however, some advantages in relation to the previous ones, such as enable the extraction of fresh samples, short extraction times, higher automation procedures, and good extraction rates. <i>Bioactive molecules</i> : vitamins, phenolic compounds, and carotenoids.
Shake extraction (ShE)	This technique needs the use of a shaking device, allowing more effective extractions at short times. The main advantage is to increase the surface where solvent extraction interacts with the sample material. <i>Bioactive molecules</i> : phenolic compounds.

pivotal attention and intensive research work has been carried out on this field. On the other hand, and despite probiotics and prebiotics have been on the top of the latest studies on this area, a great attention has been given to plants, fruits, and its derived extracts on fortification of dairy food products and subsequent functionalization. As briefly described in Table 1, different plant, fruits, derived extracts, and even other food constituents have been used to functionalize dairy products. Yogurt and cheese are the most commonly selected to be investigated. Also interesting to point out is that these food ingredients are not only used to improve the nutritional value, health-promoting effects, and to confer additional benefits to dairy food products but also to improve their shelf life and even to remove some unpleasant and even nonbeneficial, nontolerated, and unaccepted characteristics by consumers.

Concerning the use of plant food bioactives in the development of functional dairy foods, chamomile and fennel have been incorporated both in yogurt [31] and cheese [25, 30, 38] samples for subsequent analytical and methodological studies.

Table 3 Most commonly used encapsulation technologies in functional foods formulation

Encapsulation technologies	
Microencapsulation	
Spray drying	This is the most commonly used preparation, destined to prepare dry and stable food additives and flavors. It is based on the injection of a liquid suspension of the bioactive compound at the top of a vessel, drying the droplet into a fine powder particle concomitantly with hot air. Then, liquid droplet solidified and entraps the bioactive molecule. The technique is economic, flexible, and suitable to be used in a continuous operation, besides to provide a high protection to short exposure periods to acids, humidity, and oxygen.
Freeze drying	Commonly known as lyophilization or cryodesiccation. This technique is great to be used in a wide variety of heat-sensitive ingredients. It consists in homogenize core materials from the initial solution and then originates colyophilizes, i.e., irregular particles. This technique is suitable to encapsulate water-soluble essences, natural aromas, and even drugs.
Coacervation	This technique is based on the phase separation of hydrocolloids present in an initial solution and further deposition of the newly formed coacervates around the suspended or emulsified bioactive molecule. It is considered an expensive method, being particularly suitable to be used in high-value and sensitive functional ingredients (i.e., polyphenols).
Liposome entrapment	This technique consists in the formation of a lipid membranous system that encapsulates a hydrophilic space, leading to the formation of a suitable place to encapsulate water-soluble, lipid-soluble, and amphiphilic functional ingredients. With this technique, it is possible to control the release rate and exact point of incorporated materials delivery. Encapsulated materials through this technique are protected from stomach digestion, so ensuring their subsequent bioavailability and bioactivity in the next section of the gastrointestinal tract.
Cocrystallization	This technique consists in the modification of the sucrose structure, leading to the formation of an irregular agglomerated crystal. Bioactive ingredient is therefore incorporated into the porous matrix of the newly formed microsized crystals. This technique improves the solubility, homogeneity, dispersibility, hydration, anticaking, stability, and flowability of the encapsulated functional ingredients. It also allows the conversion of liquid materials into powders, which facilitates their subsequent application in pharmaceutical industry.
Yeast-encapsulation	This technique is based on the use of yeast cells (<i>Saccharomyces cerevisiae</i>) as the encapsulating material, being also usually applied to essential oils, flavors, and polyphenols. The target ingredients can pass across cell wall and remains inside the cells, being also ensured the control of the diffusion of the incorporated functional ingredients. Finally, this technique is also embroiled with green chemistry principles, once no additives besides water, yeast, and target functional ingredients are used. It is also a low-cost technique that allows the processing of high volumes of bioactive ingredients.
Cold gelation	This is a recent method, consisting from an alternative way to develop protein microparticles in food industry. No organic solvents are necessary, being encapsulation achieved under mild conditions, minimizing the destruction of sensitive nutraceutical compounds. Moreover, it is also possible to denature, dissociate, and even aggregate under different conditions of pH, ionic strength, and temperature.

(continued)

Table 3 (continued)

Encapsulation technologies
Nanoencapsulation
This technique is more complex than microencapsulation, mainly because of the intricate morphology of the capsule and core material, and the demand in controlling the release rate of the nanoencapsulates. It involves the incorporation, absorption, or dispersion of the bioactive compounds and subsequent formation of the functional ingredients encapsulated in small vesicles (<100 nm). Nanoparticles ensure a greater surface area, increase the solubility and enhance the bioavailability of the target ingredients, due to their subcellular size and improved controlled release. They can also easily penetrate tissues through fine capillaries, crossing epithelial lining fenestration, being therefore efficiently taken up by cells and allowing an efficient delivery of the active compounds in the target sites of body.
Emulsions
In a broad sense, an emulsion is a mixture of at least two immiscible liquids, usually oil and water, where one of the liquids is dispersed as small spherical droplets in the other. Therefore, emulsions are turbid, with small droplets sizes ranging from 0.2 μ m to 10 μ m, may remain stable for a considerable time-period, despite being classified as thermodynamically unstable systems. Emulsions may be also classified into two distinct groups: microemulsions and nanoemulsions. Microemulsions are systems consisting from a mixture of water, hydrocarbons and amphiphilic compounds, forming kinetically and thermodynamically stable compounds, transparent, homogeneous, and isotropic solutions, ranging particle sizes from 5 nm to 100 nm. Nanoemulsions consist from fine dispersed emulsions and submicron emulsions, that in contrary of microemulsions, are not thermodynamically stable. They are produced by microfluidization or micelle formation techniques, exhibiting poor solubility and low bioavailability.

Lemon balm [33, 34], rosemary [32], chestnut [33, 34], and basil [35] were incorporated in cheese samples, while honey [2], wild blackberry [41], and seaweed extracts [42] were used to functionalize yogurt samples. More recently, dairy beverages were tentatively functionalized with pure ergosterol and mycosterols from *Agaricus bisporus* extracts [36], as an alternative to phytosterol-based beverages; and also incorporated antioxidant extracts from two mushrooms species, namely *Suillus luteus* (L.: Fries) and *Coprinopsis atramentaria* (Bull.), acting as food ingredients in the functionalization of cottage cheese [23]. Interestingly, in all the above described experiments, prominent results were achieved both in terms of protection against oxidation, improving consequently the shelf life of the functionalized dairy products, as also in terms of nutritional value, stronger antioxidant activity, moisture and sensory attributes, such as external color, syneresis, and viscosity [31, 32, 34]. Moreover, organoleptic characteristics also display a crucial role in overall sensory acceptance and purchase intention, which was achieved through the functionalization of these dairy products that provide beneficial characteristics both for consumers (contributing for healthier lifestyles) and producer industries (commercialization of added-value products) [2, 33]. From the point of view of consumers, two additional aspects should be also considered. The first one is related with the healthier substitution of artificial preservatives from natural ones, improving consequently the acceptability, quality, and security of the final product [11]. In fact, modern consumers are increasingly focused on the consumption of

naturally-derived and safer products and formulated with natural food ingredients. The second one is related with the health-promoting abilities attributed to the consumption of these functional dairy foods; it has been increasingly documented and widely recognized that daily diet exerts a determinant role in health maintenance and disease prevention [8, 22].

The last aspect that is not least important is related with the high interest by food industry in always searching for new products, being this goal markedly driven by the production of cheaper food products, but healthier for consumers also looking for longer storage periods [7, 14, 33]. Therefore, consumers' acceptance and subsequent selection of a specific functional dairy product is markedly determined by a host of factors. The first one is related with the primary health concerns, as already highlighted, the consumers' familiarity with the new functional dairy food and with the functional food ingredients used on their formulation (i.e., nature of the carrier product, delivery of health effects, etc.) [11, 43]. On the other hand, consumers are awareness about the health effects of multiple substances and food ingredients, being thus essential to provide specific and credible information about the latest documented studies on the area. Finally but also determinant is the overall organoleptic and sensory evaluation carried out by consumers about the new product. In fact, different surveys have been applied to consumers, being clearly evident the key conditions for acceptance, such as taste, product quality, price, convenience, and even the reliability of health claims [11, 43, 44]. Therefore, during the development of a functional dairy food, all these aspects should be taken into consideration, besides to ensure their added value in terms of biological activity.

6 Bioactive Effects of Functional Dairy Foods

There is a widespread knowledge about the real benefits of functional dairy foods at the level of health promotion, well-being maintenance, and longevity [27, 44]. By themselves dairy products can confer several biological effects due to the naturally occurrence of biologically active constituents on its chemical composition, such as bioactive proteins, lipids, oligosaccharides, immunoglobulins, enzymes, hormones, antimicrobial peptides, cytokines, growth factors, among others [37]. Nevertheless, these constituents exist in limiting quantities, being therefore extremely usefulness their incorporation in dairy products functionalization [31]. Furthermore, it has been increasingly clear that some fermented dairy foods promote human health not so much directly attributable to the starting materials, but instead from the outcomes of fermentation, thus conferring additional properties beyond basic nutrition [14].

In fact, the formulation of dairy food products able to promote health and well-being is one of the main key research priorities by food industries, that has mostly favored the selection and subsequent consumption of foods enriched with physiologically active components, such as probiotics, prebiotics, and more recently plant food bioactives [3, 14, 37]. One of the main and most widely recognized biological effects of functional dairy products are their recognized modulatory activities, through the action of probiotic bacteria, also existent in fermented milk products

[14, 37]. Besides other aspects, they exert an important role in gut fermentation metabolites, and therefore contributes to local and systemic beneficial effects in humans, not only at a level of digestion, absorption, and metabolism but also elimination, once favor a healthy bowel movement [14, 15]. More interestingly is that the functional effect of dairy products may be achieved directly through the action of probiotic bacteria or even indirectly because of their metabolic activity, i.e., microbial metabolites, such as vitamins, proteins, peptides, oligosaccharides, organic acids, and bacteriocins [14, 37]. In fact, the modulatory effects conferred by probiotic bacteria are both related with the stimulation of the natural immunity as also modulation of the production of cytokines, antimicrobial peptides, and inhibition of the carcinogenicity of multiple substances [45]. Another pronounced bioactive effect of functional dairy products is their ability to minimize the incidence of hypertension [14, 46]. In fact, dairy products, such as milk and cheese, contain significant amounts of antihypertensive peptides, including lactopeptides isoleucine-proline-proline and valine-proline-proline, that mainly acts as antihypertensive angiotensin-converting-enzyme (ACE) inhibitors [14, 46, 47]. On the other hand, whey and casein fractions of yoghurt have also shown strong antihypertensive effects being documented by several studies their role in curtail several syndromes among which in controlling blood pressure [46]. Other studies have even revealed that other peptides from dairy fermented products also exert antithrombotic, satiety, opioid, immunomodulatory, antimutagenic, osteogenic, and antioxidative effects [14, 48]. Additionally, interesting reports have revealed a positive association between fermented dairy foods' consumption and weight maintenance and body fat reduction, besides their ability to reduce the risk of type 2 diabetes and other metabolic disorders, and overall morbidity and mortality, mainly from yogurt consumption. The effects on glucose metabolism seems to be mainly related with the improvement of glucose metabolism and insulin sensitivity, and reduction of muscle soreness [14, 46]. The positive effects on inflammatory bowel diseases and other immune-related pathologies, including arthritis, sclerosis, fibromyalgia, migraine, and depression have been also increasingly evident, as also their modulatory effects at a level of brain and mood [14, 49], still, however, being essential to deepen knowledge on this field. All the above described biological effects are briefly summarized in the Table 4.

7 Concluding Remarks

The world of functional foods development has led to one of the most promising and dynamic development on a specific segment of food industry. The increasing consumer awareness and demands for healthy products, in association with the latest advances on the area of food science and technology are the most important factors supporting the new revolution on food science and innovation. Nevertheless, consumers' acceptance and preferences are not homogenous, being observed a large distribution and differences on functional foods acceptance. Specifically, the development and commercialization of functional dairy products is not easy, it involves

Table 4 Biological activity of functional dairy foods

Bioactive effect	Detailed activity	Functional dairy food
Cardiovascular disease	Improvement of total cholesterol, non-HDL-C, and LDL concentrations	Fermented milk
Hypertension	Antihypertensive peptides acting as angiotensin-converting-enzyme (ACE) inhibitors	Fermented milk, Cheese, Yogurt
Immunomodulatory	Stimulation of the natural immunity, modulation of the production of cytokines, inhibition of carcinogenic effects, gut fermentation, metabolites	Yogurt, Cheese, Kefir
Infection control	Peptides exerting antimicrobial effects, reduction of the incidence of fever and constipation	Fermented milk
Inflammatory bowel syndrome	Reduction of symptoms of flatulence, abdominal pain, cramps, and stomach rumbling	Fermented milk, yogurt
Mood and brain activity	Modulation of the activity of brain regions responsible for controlling central processing of emotion and sensation	Fermented milk
Muscle soreness	Suppression of muscle soreness	Fermented milk
Obesity	Improvement of body fat percentage, waist circumference and waist-to-hip ratio, lean body mass	Yogurt, Fermented milk
Osteoporosis	Increase of bone mineral density and short-term changes in turnover	Kefir
Type-2 diabetes	Decrease of insulin resistance and increase of insulin sensitivity, improving glucose tolerance, and metabolism	Yogurt, Fermented milk

complex, expensive, and specific requirements. Besides the consumers' demand, during the development of a dairy functional product, the technical conditions as also legislation conditions should also be considered. Special requirements established to apply during the development and marketing of functional dairy foods should be met, as also their economic potential; health impact, acceptance, and overall interest should not be forgotten. Particularly, consumers' acceptance has been considered a key success factor on market orientation, brand new product introduction, and consumer-led product development, besides to be successful in negotiating market opportunities. Therefore, by purchasing functional dairy foods, consumers may have the overall health benefits of functional foods, besides to achieve a positive and valuable impression of themselves. In fact, they are self-contributors for this achievement, once they interfere both on selection, formulation, evaluation, and overall qualification of these products. Besides to the highlighted aspects, consumers may follow a balanced diet and acquire a healthy lifestyle, which differ from the conventionally established healthy diet. Finally and not least important is that the concept of functional dairy foods may represent a sustainable trend in a multiniche market to be exploited.

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