Chapter 3

Potential of High Pressure Homogenization and Functional Strains for the Development of Novel Functional Dairy Foods

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Abstract

Functional foods are one of the fastest increasing fields in the global food industry since they are positively perceived by the consumers as dietary strategies to reduce the incidence of illness in the humankind. Actually, the use of biotechnological strategies, based on the use of functional and specific strains and sustainable technologies, such as high-pressure homogenization, can be a great chance to create innovation in the dairy field. Critical discussion on the actual scenario is the main topic of this chapter.

Keywords: high pressure homogenization, functional strains, dairy applications, novel product application, innovation

1. Introduction

Functional foods are one of the fastest increasing fields in the global food industry since they are positively perceived by the consumers as dietary strategies to reduce the incidence of illness in the humankind as established by the European Commission's Concerted Action on Functional Food Science in Europe (FuFoSE) (**Figure 1**) [1, 2].

Currently, the most important and interesting applications have been studied and applied for the dairy ingredients and products due to their great potential as functional and nutraceuticals. Although the health-promoting dairy products can be represented by several food types such as products with intrinsic functionality and products fortified with natural ingredients

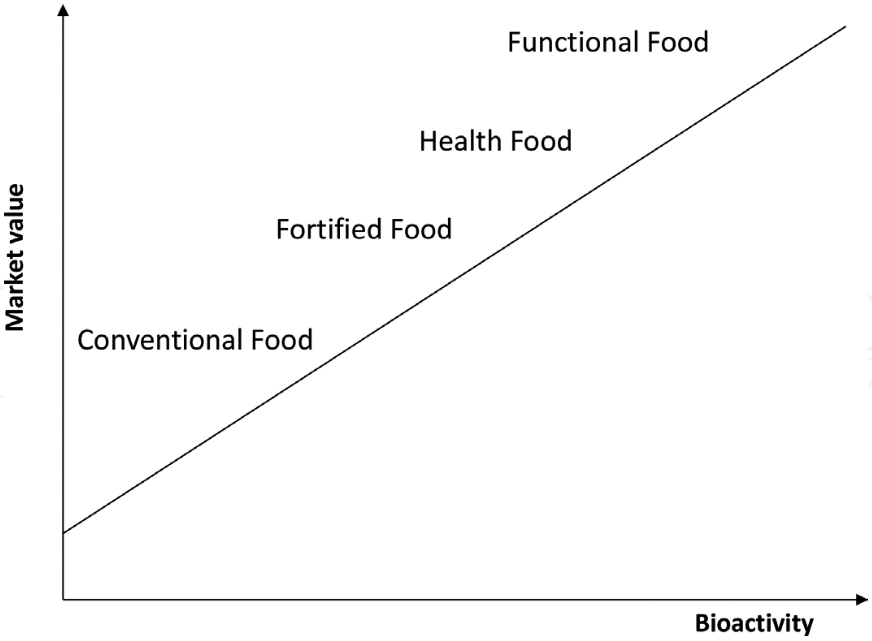

Figure 1. Functional food market. Source: Adapted from koronen, 2002.

to obtain a desired functionality, the probiotic products have received the highest attention due to their importance as a suitable vehicle for probiotic microorganisms, defined as "*live microorganisms which when consumed in adequate numbers confer a health benefit on the host*" [3]. In fact, it is scientifically proved that the intake of probiotics, in relation to the species and strain, can positively affect the host at gastrointestinal level and in the respiratory and urogenital tracts. Also lactose intolerance, diarrhea, gastroenteritis, irritable bowel syndrome, inflammatory bowel disease, depressed immune function, cancer and genitourinary tract infections can take benefit with probiotic-based diet. Thus, the development of dairy products containing probiotic bacteria has become an important challenge in the food industry and several attempts have been made to identify the most suitable carriers, strains and process conditions for probiotic products [3]. The increasing demand for high-value health-promoting dairy foods has encouraged the industries and consequently, the research field, to investigate new emerging food processes and biotechnological strategies to obtain functional but innovative products without detrimental effect on safety, quality and nutritional value. In particular, the literature has pointed out the potential of non-thermal technologies, such as high-pressure processing treatments, in the development of new functional dairy foods. Also the use of selected microbial strains used as fermentation starters, co-starters, adjuncts or bio-control agents, imparting specific functionalities but maintaining high product quality and safe standards, can contribute to designing new bio-functional dairy foods. Moreover, the literature has underlined the potential of tailor-made lactic acid bacteria to create gender foods with defined functionalities in relation to the strains used [4]. Thus, the use of an integrated biotechnological approach, based on new technologies and functional microbial cultures, could be the new challenge and an alternative to the engineered strains that are always lacking robustness and characterized by high production costs. Among the non-thermal technologies, the high-pressure homogenization (HPH) represents a potential technology to develop new functional applications, also easily implementable at industrial level. Several literature reports have highlighted its use in dairy field to increase product functionality by both treating the milk designated to cheese making or fermented product preparation and treating the bacteria cells to increase their probiotic features [5–8]. Moreover, high pressure homogenization was used also to develop suitable microcapsules to carry the probiotic cultures in order to increase their viability during dairy product storage under refrigeration conditions [9, 10].

The innovation of dairy products can be also achieved using specific microorganisms able to generate specific functionalities. In fact, functional foods could differently affect human health in relation to the gender, and this research aspect is particularly interesting for women since, in recent decades, research on the female gender has been neglected, and the results obtained in men have been directly translated to women both in medicine and in nutrition fields. In this sense, a recent contribution of Siroli et al. [4] has highlighted the challenge to study the technological properties of some *Lactobacillus* strains isolated from the vagina of healthy women and endowed with anti-Candida and anti-Chlamydia activities for potential use in dairy food to contribute to women's well-being by a dietary strategy. The exploitation of a *Lactobacillus salivarius* strain, with a strong anti-*Helicobacter pylori* activity, and a nisin producer *Lactococcus lactis* strain were used as co-starters in cheese making in order to contribute to product innovation and to increase the overall welfare [11].

Thus, this chapter will outline the most important findings on the attempts made in dairy product innovation, exploiting both an emerging technology such as high-pressure homogenization and the use of functional strains endowed with specific functionalities.

2. Biotechnological strategies for dairy innovation

2.1. Innovation in dairy field throughout technological approach

Innovation is the major driving force of the economic growth worldwide and it is directly connected with the needs of the consumers or final users. In 2008, Grunert et al. [12] created the term "user-oriented innovation" defining it "*as a process towards the development of a new product or service in which an integrated analysis and understanding of the users' wants, needs and preference formation play a key role*". In this last decade, the consumer interests and needs have been directed towards food able to confer specific functionalities to human well-being. For this reason, the scenario has notably changed in many food sectors, pointing out the fast development of new products being able to satisfy the consumer needs. In particular, the dual request for guaranteeing the safety and meeting the consumer demands for more fresh and high-quality functional foods necessitated the development of new strategies such as non-thermal technologies able to inactivate microorganisms at room or near-room temperatures but preserving flavor, color and nutritional value. Much attention has been focused on new food processing trends to offer interesting solutions to these challenges. Currently, the most important and interesting applications have been studied and applied for the dairy ingredients or products due to their innate great potential as functional and nutraceuticals. The first product innovation in this sector was represented by the use of different types of milk, to cow milk, recognized for their intrinsic functionalities, to produce new functional dairy products. For example, sheep dairy products have gained market size due to the product's quality, high yield and nutritional value due to the high concentrations of proteins, fats, vitamins and minerals [13]. However, these types of productions have grown over the years reaching a plateau in the market. For this reason, new dairy products have been developed and, among these, probiotic ones have gained major attention due to their driving force on the market [1, 2]. The literature data show that many attempts have been made to increase both functionality and product differentiation throughout biotechnological approaches. Among technologies, pulsed electric field (PEF), high hydrostatic pressure (HHP), high-pressure homogenization (HPH), ultrasounds, oscillating magnetic fields and high-intensity light pulses have been exploited in dairy product application with the purpose not to decontaminate milk but to increase the final product functionalities [14]. Among these technologies, most of them investigated and tested at least at laboratory level, the sector of high-pressure processing (HHP and HPH) [15], together with the PEF field [16], is probably one the most scientifically developed and with already-established applications at industrial level for the production of probiotic/prebiotic ingredients and foods.

2.2. Principles of high-pressure homogenization and its application

Although HHP and HPH share some action mechanisms, the latter induces major changes to macromolecules of the system with respect to HHP, throughout cavitation, turbulence and viscous shears, which seem the most probable mechanisms of action. Moreover, HHP can be applied both on liquid and on solid matrix while HPH can be used only for liquid foods. However, both offer interesting possibilities to restructure food proteins, affecting protein conformation, leading to protein denaturation, gelation or aggregation and, consequently, creating new products with new/improved texture [17, 18]. They are also used on dairy proteins for providing low-temperature enzyme activity modification and stabilization of fermented dairy products and also to improve coagulation of milk and to prepare dairy gels and emulsions characterized by novel textures. Moreover, according to the literature, they are involved in functional dairy formulation, and for HHP the most evidences are on bioactive milk proteins [19]. Although HHP is more consolidated at the industrial level in many fields, the potentialities of HPH in the dairy sector are multiple. In addition, this process can be applied, contrary to HHP, in a continuous manner, offering a great advantage from an industrial point of view and increasing the competition among the enterprises in process innovation and products. The word "homogenization" is referred to the ability to produce a homogeneous size distribution of particles suspended in a liquid, by forcing the liquid under the effect of pressure through a specifically designed homogenization valve (**Figure 2**) [17, 18].

Nowadays, homogenizers able to treat fluid matrices for pressure ranging between 10 and 40 MPa are well implemented in different sectors, that is, dairy, beverage, pharmaceutical and cosmetic industries, with the principal aim to reduce particle size and increase stability. However, the first application of HPH dealt with the cell disruption and recovery of intracellular bioproducts [17] reaching pressures of 100 MPa. The successful results obtained on cell rupture of microbial cultures motivated researches on the application of HPH for food safety and shelf life extension. In the food industry, the interest in mild non-thermal processes, able

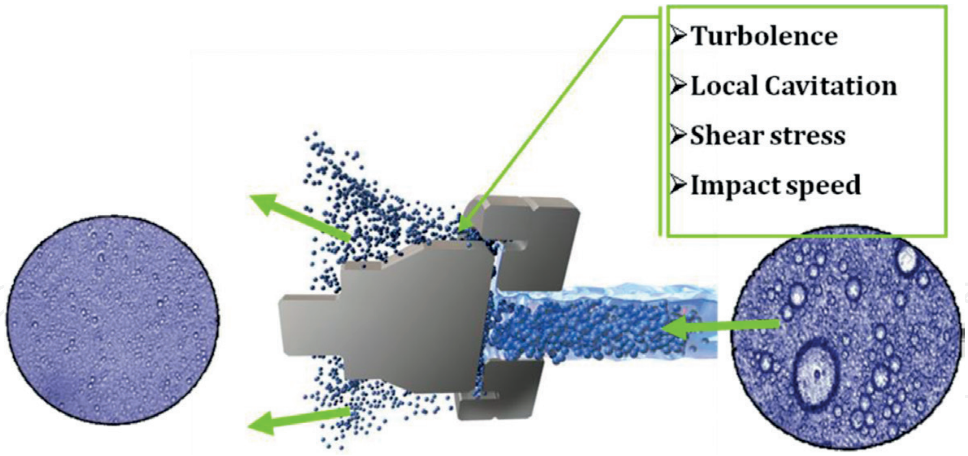

Figure 2. Principal action mechanisms of high-pressure homogenization.

to achieve efficient microbial reduction with a maximal retention of physicochemical product properties, as well as nutritional and sensory feature retention, is very high. Among the non-thermal treatments, HPH is regarded as one of the most encouraging alternatives to traditional heat treatments for food preservation and product diversification for dairy and beverages. Its efficacy against spoilage microorganisms in model and real systems has been well proven since 1994 [17].

A homogenizer is composed above all of a pump and a homogenizing valve. The pump is used to force the fluid into the valve where the homogenization takes place. In the homogenizing valve, the fluid is forced under pressure through a small orifice between the valve and the valve seat. The operating pressure is controlled by adjusting the distance between the valve and seat. Pressure, temperature and flow rate are the main parameters influencing the success of homogenization for microbial inactivation. As in HHP, the level of microbial inactivation by the application of HPH increases with the pressure level. Temperature effects have to be necessarily taken into account in HPH, since during homogenization, there is an increase of temperature (about 2.5°C per 10 MPa) related to the fluid food treated. This increase is due to the viscous stress caused by the high speed of the fluid flow and due to the loss of a significant fraction of the mechanical energy which is lost as heat in the fluid [17]. According to the literature, the HPH has been used instead of the conventional homogenization for the modification of the microstructure and rheology of food emulsions [20, 21], the improvement of the body and texture of yogurts and cheeses [22, 23], the increase of cheese yield [5] and the reduction of cheese ripening time due to the enhanced susceptibility of proteins and triglycerides to proteolysis and lipolysis, respectively [24]. Some papers report also the exploitation of HPH for the activation or inactivation of enzymes [25, 26] and to reduce the biogenic amine content of ripened cheeses.

2.3. Potential of high-pressure homogenization in dairy sector for the development of functional dairy products

HPH has demonstrated great potential in the dairy sector also for the development of new products, differentiated from traditional ones by sensory and structural characteristics or functional properties [27]. Moreover, Iordache and Jelen [28] showed HPH as a suitable approach for producing soluble whey protein concentrates/isolates for the production of several dairy products, as well as meat or egg substitutes. Bury et al. [29], comparing sonification, bead milling and HPH, showed that HPH was suitable for the large-scale disruption of *Lactobacillus delbrueckii* ssp. *bulgaricus* 11842 for the recovery of β-galactosidase for the production of lactose-hydrolyzed dairy products. In addition to the production of yeast autolisate for food aroma improvement, HPH has been used for the recovery of (1–6)-β-ᴅ-glucan from Saccharomyces cerevisiae. This molecule is a well-known immune modulator with a positive influence on the human and animal immune system. Because HPH has been extensively used to emulsify, mix [30] and reduce the mean droplet size, simultaneously narrowing the width of the size distribution by reducing the number of microparticles and the polydispersity index, one of the most recent applications regards the use of HPH processes to produce nanodispersions and nanoparticles containing bioactive compounds, including functional lipids, with substantial health benefits [31].

In the functional dairy sector, HPH has been proposed to produce probiotic fermented milk, bio-yogurt and probiotic cheeses with improved sensorial or functional properties [3, 5, 6, 22]. In general, fermented dairy products are considered by consumers as healthy foods since they are good sources of vitamins and minerals and have low lipid content. The use of probiotic (health-promoting) microorganisms in different fermented milk or yogurt-like products can also amplify their acclaimed healthful properties. In fact, *Streptococcus thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus*, the traditional starter cultures of yogurt, improve themselves the nutritional content and digestibility of yogurt, although they are not usually part of the native microbiota of the mammalian intestine and have limited survival after oral intake. In order to beneficially affect the host, improve the balance of intestinal bacterial ecosystem and influence positively human immune responses, the probiotic microorganisms need to be viable, active and sufficiently abundant (i.e. in concentrations of at least 10^7 cfu/g in the product) throughout the specified shelf life [24]. Also other properties of fermented milk, such as acidity, lipid content, the presence of diacetyl, acetaldehyde and acetoin and the nutritional value are important and affect acceptability by consumers. Thus, the ability to impart good sensory properties to the final product is included in the criteria of selection for probiotic strains [24], because fermented milk obtained from the direct and sole use of probiotic strains are often characterized by the lack of desirable sensory features [7].

Several options have been proposed in order to increase the textural properties of fermented milk. Among these, the exploitation of exopolysaccharide-producing strains has been suggested as an option to additives such as xanthan gum, gelatin, pectin, and carrageenan [24], which can adversely influence the product flavor, aroma and mouth feel [6]. Also, co-inocula of probiotic strains with *Lb. delbrueckii* ssp. *bulgaricus* and *Strep. thermophilus* has been reported to improve the sensorial properties of fermented milk [6]. Moreover, *Strep. thermophilus* is applied to sustain the mild acid fermentation of yogurt containing probiotic lactobacilli. Also the modulation of some physicochemical and technological variables permitted to increase the technological strain features and sensory properties of fermented milk. Mainly, some papers indicate response surface methodology as a good approach to evaluate the simultaneous effects of some important technological, compositional and microbiological variables on the acidification rate of starter bacteria (including probiotic strains), on their viability losses during product storage and on product sensory traits [6].

Among the technological variables that are potentially useful, the HPH of milk has been regarded: (1) to increase or modulate the sensorial features of probiotic fermented milk and cheese without detrimental effects on shelf life and safety [5, 6]; (2) to improve the technological performances of probiotic strains used alone or in combination with yogurt starter cultures [24]; and (3) to modify the functional features of lactic acid bacteria used as starters [32] and of probiotic bacteria [7, 8]. Regarding the use of homogenizing pressure to milk, the use of HPH tested between 20 and 100 MPa showed ability to improve the sensorial features of fermented milk using a probiotic strain, *Lactobacillus paracasei* BFE 5264, as starter culture in combination with compositional variables such as milk fat content and non-fat milk solids content as reported by Patrignani et al. [33]. These authors highlighted that the rheological parameters, such as firmness, viscosity index and consistency of probiotic fermented milk, increased with the increase in pressure level for added non-fat milk solid concentrations lower than 3%. When non-fat milk solids were higher than 3%, different rheological behaviors were observed. The improvement of textural properties of fermented milk can be explained with the progressive increase in the extent and strength of protein hydrophobic associations which the pressure can promote. Also, the content of characteristic molecules such as diacetyl and acetaldehyde, able to affect the flavor and taste of fermented milk, increased with the use of homogenizing pressure. Moreover, the HPH treatment affected significantly also some technological performances of the employed probiotic strain such as fermentation rate and its viability loss during fermented milk storage at refrigeration temperature. In fact, Patrignani et al. [33] found that *Lb. paracasei* BFE 5264 coagulation times were significantly affected, in addition to the added milk fat, by the increase of pressure. However, the technological performances of the probiotic strain were affected by the addition phase of milk fat (before or after the pressure treatment). When the addition of milk fat was performed before HPH treatment, the strain fermentation rate decreased and its viability during the refrigerated storage was reduced. The addition of UHT cream before high pressure homogenization treatment decreased the nutrient diffusion, the microbial growth and the acidification rates since it generated compartmentalization of the aqueous phase in the lipid-protein gel matrix. HPH showed good potentialities also when used to produce fermented milk containing the traditional yogurt starters (i.e. *Strep. thermophilus* and *Lb.s delbrueckii* subsp. *bulgaricus*) and probiotic strains of *Lactobacillus acidophilus* 08 and *Lb. paracasei* A13. In particular, Patrignani et al. [6] studied four types of fermented milk, obtained from HPH-treated and heat-treated (HT) milk with and without added probiotics. The results showed that HPH treatment favored the viability of starter cultures, particularly *Strep. thermophilus*, even at the end of the storage period without detrimental effect on the viability of probiotic bacteria. Higher levels of viable lactic acid bacteria LAB at the end of the shelf life is, in any case, an interesting feature for this type of product due to the now recognized probiotic characteristics of yogurt cultures. In addition, the probiotic strains of *Lb. acidophilus* and *Lb. paracasei*, employed by Patrignani et al. [6], were found at levels of 5 and 7 log orders, respectively, at the end of the shelf life of the fermented milk from HPH-treated milk. Moreover, the fermented milk obtained from HPH milk was characterized by significant higher values of firmness ($p < 0.05$) with respect to those from HT milk. Similar results were observed for consistency, cohesiveness and viscosity indexes. All the samples obtained from HPH milk received high sensory analysis scores for each descriptor considered. Also, significant higher amounts of acetaldehyde and 2-propanone were detected in fermented milk obtained from HPH milk than those from HT milk.

According to the literature available, HPH technology has shown good potential for the manufacturing of probiotic cheeses. In fact, soft cheeses have a number of advantages over yogurt and fermented milk as a delivery system for viable probiotic microorganisms because they generally have higher pH and buffering capacity, more solid consistency and relatively higher fat content [27]. On the other hand, in order to give protection to probiotic bacteria during storage and passage through the gastrointestinal tract, some cheese varieties such as Gouda [34], Argentinean Fresco cheese [35], white cheese [36], Arzua-Ulloa [37], Minas fresh cheese [38], Cheddar [39] and cottage cheese [40] have also been studied as vehicles of probiotic microorganisms. In addition, Burns et al. [5] studied the potential of HPH treatment of milk for the production of Crescenza cheese carrying probiotic bacteria. More specifically, these authors studied the viability of commercial probiotic cultures of *Lb. acidophilus* and *Lb. paracasei*, added as adjunct cultures during production, and the implications of their addition in the physicochemical and sensory characteristics of the product obtained. In fact, a previous work performed by Gobbetti et al. [41] showed that Crescenza cheese is suitable to serve as a carrier for probiotic bacteria because no prolonged periods of ripening are necessary, and storage occurs at refrigeration temperatures. However, the inclusion of probiotic bacteria affected negatively the organoleptic properties of this traditional Italian cheese. To evaluate the potential of milk treated by HPH for the production of probiotic Crescenza, Burns et al. [5] compared four types of cheeses made from HPH and pasteurized milk with and without probiotics, respectively. A strain of *Strep. thermophilus* was used as starter culture for all the cheese types. The results of compositional analyses carried out during the refrigerated storage (4°C) showed no significant differences for gross composition (protein, fat, moisture) and pH. Differently, the HPH treatment to milk induced an increase in cheese yield of about 1%. Also the viability of the used probiotic strain maintained cell loads of 8 log cfu/g after 12 days of refrigerated storage. *Lb. acidophilus* 05 showed, in probiotic Crescenza cheese from pasteurized milk, a cell load decrease of about 1 log cfu/g with respect to the cell loads reached in Crescenza-obtained HPH milk. Moreover, the hyperbaric treatment had a significant positive effect on release of free fatty acids, cheese proteolysis and organoleptic properties of cheeses tested by a sensorial analysis.

The modification of the rheological and sensorial properties of fermented milk and cheeses induced by HPH can be explained mainly with the modification induced by HPH treatment on casein-casein or casein-fat interactions. The ability of HPH treatment to increase the exposure of the hydrophobic regions of proteins and extent and strength of hydrophobic associations between proteins is well documented. Moreover, HPH of milk is reported to improve the coagulation characteristics of milk due to the modification of the balance between insoluble and soluble forms of calcium, phosphorus and nitrogen [27]. Also, the modification of sensorial profile in terms of volatile molecules and the different retention of flavor compounds can be dependent on the different gel networks of proteins. The release of flavor compounds and their perception during consumption, which are key quality parameters for foodstuff, are undoubtedly affected also by the food matrix and microstructure [27]. Moreover the different volatile profiles of fermented milk and cheeses obtained from HPH-treated milk could be due to the combination of events associated with homogenization. In fact, HPH is reported to increase the nitrogen fraction soluble at pH 4.6, the susceptibility to proteolysis of whey proteins and caseins, and, consequently, the availability of free amino acids regarded as several aroma precursors including acetaldehyde. The increase of viability for yogurt starters and probiotic cultures observed by Patrignani et al. [6] and Burns et al. [5], respectively, can be attributed to the increased precocious availability in the products obtained from HPH-treated milk of low molecular weight peptides and/or free fatty acids such as oleic acid, essential for the growth of many LAB. Moreover, Patrignani et al. [33] reported levels ranging between 60 and 80 MPa as optimal both for the viability of probiotic and for the sensorial features of fermented milk obtained with the sole use of probiotic strains. Moreover, because some literature papers proposed HPH to control and enhance the proteolytic and fermentative activities of some *Lactobacillus* species [32], Tabanelli et al. [8] have adopted this technology to modify/enhance some functional properties of already-known probiotic strains, considering that probiotic properties are related to the cell wall, also the principal target of HPH. Thus, viability and cell-surface hydrophobicity of *Lb. paracasei*. A13, *Lb. acidophilus* DRU, *Lb. delbrueccki* subsp. *lactis* 200 and their bile-resistant derivative DRU+ and 200+, inoculated in phosphate buffer solution and buttermilk, were evaluated. Moreover, at the same conditions, bacterial aggregation characteristics, response to simulated stomach duodenum passage and resistance to simulated gastric acid conditions were evaluated. The strains were treated by HPH at 50 MPa and the results were compared with control data obtained under the same conditions but without the pressure application. Among the tested strains, only *Lb. paracasei* A13 increased its hydrophobicity when subjected to HPH, while bacterial aggregation characteristics, viability and resistance were strain dependent and affected by the media employed. In particular, among the treated strains, the more resistant ones to gastric acid conditions were those inoculated in phosphate buffer solution. This study documented that HPH treatment could increase some important probiotic characteristics such as hydrophobicity and resistance to simulated gastric juice but the response varied according to the species and the characteristics of the individual strains as well as the time of refrigerated storage and the media containing probiotic strains.

2.4. Use of high-pressure homogenization to develop new carriers for probiotic strains

From the technological point of view, probiotic strains have to maintain not only a good viability but also a good functionality during manufacture, storage and even during consumption. Since probiotic cultures run across acidic conditions already in food products and during gastric transit, their tolerance to low pH is a critical factor that has an influence on probiotic functionality. The use of an appropriate technology for the preservation of probiotic viability is a key step for the industrial production of functional foods, since probiotic microorganisms are subjected to lose their viability during the fermentation process or during the product storage. In general, this is affected by several factors such as from 'strain sensitivity to process factors (low pH, oxygen and fermentation temperature), food matrix composition (water activity, pH, presence of natural antimicrobials and nutrient availability) and packaging and storage conditions (i.e. refrigeration temperature). Also, the gastrointestinal tract conditions can influence the viability of the probiotic bacteria during the passage. Many attempts have been performed by many researchers to maintain high viability of probiotic strains in food products. Recently, the literature data pointed out the use of polymers such as pectin, alginate, carrageenan, chitosan, whey, gelatin and lipids for microencapsulation of bacteria with positive effects in protection of probiotic cells during storage condition and gastric intestinal environment. Patrignani et al. [10] investigated the microencapsulation of two probiotic bacteria, *Lb. paracasei* A13 and *Lb. salivarius* CET 4063, performed by HPH at lab scale for the manufacture of functional fermented milk endowed with high functional strain viability. Specifically, the two probiotic bacteria were encapsulated by HPH at 50 MPa using 5 cycles, using sodium alginate vegetable oil emulsion. The microencapsulated bacteria were used as adjuncts for the production of functional fermented milks. The viability of the strains in the product was monitored over 2 months of refrigerated storage. The survival of lactic acid bacteria following the simulation of the gastric duodenal passage were evaluated. Textural parameters of fermented milk and the presence of exo-polysaccharides were also determined over storage. In addition, the profiles of volatile compounds of the products were evaluated by the GC/MS/SPMR (solid phase micro extraction) technique. The obtained microcapsules resulted in homogeneous and having a size <100 μM without detrimental effect on the sensory properties of the fermented milk (**Figure 3**).

The main effect of the encapsulation resulted in the decrease of the hyperacidity phenomena generally connected to the addition of probiotic bacteria in fermented milk. This result was fundamental for the improvement of the viability of the starter culture and the sensorial features of the products. Moreover, the microencapsulation conditions preserved the viability of the two used probiotic bacteria, even if the strain *Lb. paracasei* A13 showed a higher resistance

Figure 3. Micro-beads containing *Lb. salivarius* CET 4063 obtained by high-pressure homogenization.

to the gastric barrier with respect to *Lb. salivarius* CECT 4063. In contrast, the data obtained showed a reduction of exo-polysaccharides production in presence of microencapsulated bacteria. The results of this study underlined the applicative potential of HPH for microencapsulation of probiotic microorganisms to produce fermented milk with improved functionality and with enhanced sensory properties.

2.5. Future trends: gender foods based on biotechnological approach

Another great challenge to create new functional foods and develop product innovation is the exploitation of microbial strains, with great technological potential, able to provide specific functionalities in relation to the gender. In fact, today, foods are not intended to only satisfy hunger and to provide necessary nutrients for humans but also to prevent nutrition-related diseases and improve physical and mental well-being of the consumers [2]. The literature has suggested that functional foods could differently influence the male and female health. Until the last decade, research on women has been neglected and the results obtained in men were directly translated to women in medicine and nutrition fields [42]. Reproductive-aged women are often subjected to gynecological disturbances due to abnormalities in vaginal or gut microbiota and the occurrence of vaginal infections, including vulvovaginal candidiasis, bacterial vaginosis and aerobic vaginitis. Vulvovaginal candidiasis (30–35% due to Candida albicans) is a yeast infection compromising the life quality of many women. Bacterial vaginosis is an imbalance in the ecology of the normal vaginal microbiota, characterized by a decrease in *Lactobacillus* species, which predominate in the healthy vagina, and an increase of several pathogenic bacteria, mainly anaerobes. Aerobic vaginitis is another major abnormality of the vaginal microbiota where lactobacilli are replaced with aerobic organisms, that is, streptococci, enterococci, Escherichia coli and Staphylococcus aureus. Bacterial vaginosis and aerobic vaginitis interfere with female reproductive health, abortion, preterm delivery, premature rupture of membranes and chorioamnionitis. Conventional therapies failed in the treatments of these disorders. The administration of probiotics has been shown to be effective in restoring a normal vaginal microbiota. Recent studies highlighted the anti-Candida and anti-Chlamydia activities of strains, belonging to *Lactobacillus crispatus*, *Lactobacillus gasseri* and *Lactobacillus vaginalis*, isolated from the vagina of healthy women. Those strains were also characterized for some functional and technological properties by Siroli et al. [4] in order to evaluate their potential inclusion in a dairy products. Several strains of *Lb. crispatus* showed a significant antagonistic activity against spoilage and pathogenic microorganisms of food interest, as well as against the principal urogenital pathogens. Moreover, their fermentation kinetics in milk and their ability to survive at 4°C indicated the potential application of the selected strains as adjunct cultures for the production of female gender foods. In this view, their use for the preparation of a functional gender food could be a great scientific challenge. In particular, it is known that soft cheese could be an optimal carrier and dietary strategy to transfer of probiotic strains able to control the healthy status of the human vaginal microbiota to protect the woman from vaginal dysbiosis and infections. In fact, cheese is characterized by a protective protein and fat matrix, neutral or sub-alkaline pH and higher buffering capacity [27]. These properties allow for a great protection towards probiotic bacteria during their gastrointestinal transit. Indeed, cheeses represent a good source of calcium and vitamins. In this regard, dairy products produced with functional lactic acid bacteria strains used as co-starters have been recognized as functional foods able to provide potential benefits in preventing some diseases. Thus, in this scenario the formulation of a soft cheese containing *Lb. crispatus* BC4, isolated from the vagina of healthy women and selected for its antimicrobial activity and its ability to survive during the refrigerated storage, has been assessed in order to develop a new functional soft cheese able to promote the woman's well-being. The results obtained showed that the strain could survive very well in cheese during its refrigerated storage and also survive the simulated stomach duodenum passage maintaining cell loads higher than 6 log cfu/g over the storage. The ability of this strain to interact with the gut microbial population was also studied by using a dynamic model (called SHIME) of the gastrointestinal tract to study physicochemical, enzymatic and microbial parameters, in a controlled in vitro setting, able to affect the viability of *Lb. crispatus* BC4.

2.6. Conclusions

The results presented in this chapter have highlighted that the innovation in dairy field is achievable using different strategies. The field of high-pressure homogenization certainly represents one of the most important technological tools to reach this aim due to the tangible effects of this non-thermal approach. Also the use of safe and well-characterized probiotic/health-promoting strains can contribute to the development of products able to increase the general human well-being. Also the interaction between these two strategies could represent a challenge in the future for the sector's innovation.

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