The role of milk proteins in the structure formation of dairy products

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Abstract

Introduction. The structure of dairy products is a complex of proteins, fat, minerals and water that determines the texture and sensory properties of the product.

Material and methods. The fermented milks (using the example of yogurt), cheese, ice cream, aerated milk and frozen fruit desserts have been researched. Scientific articles, published during 2000 and 2014 years, as well as theses and monographs of dairy science have been analysed too. Methodology of the investigation is based upon the use of the methods of analysis, comparison and synthesis.

Results and discussion. The scientific understanding of the milk proteins' role in the structure formation of dairy product has been summarized. Negligible changes of structure as a result of compositional or technological changes can lead to shifts in the stability, texture and rheology of products, which are closely related to each other. The allowance of these properties has significant influence on the manufacturing.

Acid coagulation is a major functional property of milk proteins, which used in the structure formation of cheese and fermented dairy products. However, the form and properties of milk curd depend on the heat treatment of milk before fermentation. Milk proteins exhibit other functional properties (emulsification and partial coalescence of fat globules, aeration and foam stability during a churning, viscosity increasing of external phase) in the development of structure in the ice cream, aerated milk and frozen fruit desserts.

Conclusions. It is expedient to use results into a further study of the structure formation mechanism of dairy products and the development of recommendations in order to an efficient production.

Introduction

For all dairy products including curd, cheese, yoghurt, ice-cream, whipped cream and desserts structure governs the quality, in particular the texture, solubility, flow, viscoelasticity and fracture characteristics. The structure of dairy products is the spatial arrangement and interaction of constituents and structural elements (such as proteins, carbohydrates and lipids) of at the nano-, meso- and microscale. Different methods can be employed to modify structure and texture, which may then influence the creation (generation) and the release of flavour volatiles [1-5]. This in turn influences the sensory perception of texture and flavour by consumers. In recent years, there have been significant advances in our understanding of milk systems and milk components' interaction. Improvements in structure formation have been accompanied by massive changes in the scale of many milk/dairy processing operations, and the manufacture of a wide range of new dairy products.

Milk proteins play a crucial role in many food products, especially in dairy product [6-13]. Milk proteins are the best characterized and most widely used food proteins due to their abundance, ease of isolation and crucial role in human nutrition [14, 15]. Milk proteins distinguish themselves from most other food proteins in that they naturally exist in an aqueous environment and are thus readily soluble. This solubility is combined with a high nutritional value, bland flavour profile and wide array of desirable functional properties, such as emulsification, foaming, gelation and heat stability [14, 16-18].

Material and methods

The fermented milk (the case of yogurt), cheese, ice cream, whipped milk and frozen fruit desserts have been studied. Scientific articles, published during 2000 and 2014 years, as well as theses and monographs of dairy science have been analysed. Methodology of the investigation is based upon the use of the methods of analysis, comparison and synthesis.

Results and discussion

Milk is an emulsion of fat globules in an aqueous phase. The aqueous phase consists of three major groups of components which participate in the formation of dairy products: proteins (casein and whey proteins), milkfat (milkfat globules, lipoproteins), and lactose (milk sugar) [19]. Two classes of milk proteins exist. These are the caseins and the whey proteins, which represent ~75 % and 25 % of protein in bovine milk, respectively. The most abundant casein family consists of several fractions (mainly α_{s1}-, α_{s2}-, β-, κ-casein) and most of them exist in a colloidal particle known as the casein micelle [20]. These casein micelles are highly hydrated, containing ~75 % of water and consist of ~10000 casein molecules, as well as small quantities of calcium phosphate (fig. 1).The second protein group in milk is the whey proteins which include heat-sensitive, globular, water soluble proteins, of which α-lactalbumin, β-lactoglobulin, serum albumin and the immunoglobulins are the most abundant [21].

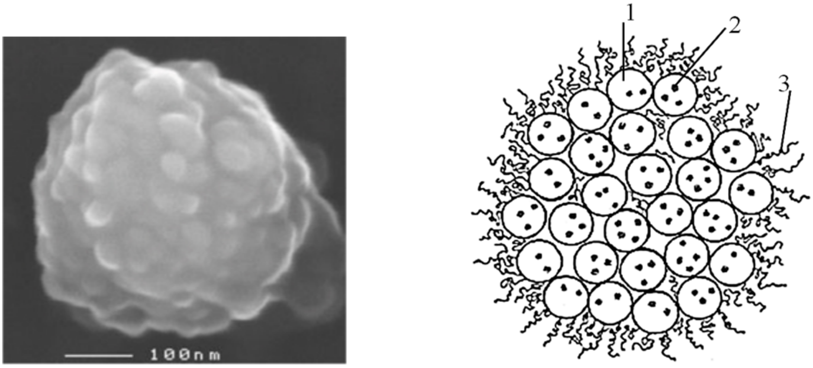

Fig. 1. Scanning electron micrograph (left) [22] and schematic outline (right) of a casein micelle: 1– submicelle, 2 – protruding peptide chain, 3 – calcium phosphate [23].

Functional properties of milk proteins. The functional properties of proteins may generally be classified into two main groups, hydrodynamic or hydration related, which includes water absorption, solubility, viscosity, and gelation. Functional properties such as emulsification, foaming, and film formation are related to the surface-active properties of proteins [16]. Proteins usually exert several inter-dependent functional properties simultaneously in each food application [24].

The functional properties of proteins are governed by their structural characteristics (e.g., size, charge, and surface hydrophobicity) [14, 25]. These intrinsic properties are themselves affected by many extrinsic or environmental factors, such as pH, ionic strength, and temperature, and also by interactions between the proteins and other materials in the food system [16, 26].

Milk protein can provide desirable viscosity and texture of dairy foods. The ability of protein to hydrate and thus entrap or bind water is important in many food technologies. It is known that, viscosity is related to quality attributes, such as physical appearance and mouthfeel. Water associates with protein via hydrogen bonding to polar groups [18]. Occasionally, non-polar groups are forced into water as a part of a specific protein structure. In addition, water may be held physically in capillaries within the product or trapped within the food structure by surface forces. In general, proteins, that are completely soluble, are less effective at water binding than those that are less soluble [16]. An important aspect of protein hydration is the rate and extent of swelling.

Milk proteins have excellent emulsifying properties and are therefore used in many food formulations as emulsifying agents [27]. Casein possesses high surface hydrophobicity with a well-balanced distribution of hydrophilic and hydrophobic domains as well as a high degree of conformational flexibility, which allows them to interact strongly at the oil-water interface. Moreover, a number of researchers have reported that β-casein is adsorbed in preference to α_{s1}-casein and other proteins in emulsions stabilized by a mixture of purified β- and α_{s1}-caseins, as it is the most surface active and hydrophobic [8, 16]. Whey proteins also adsorb rapidly to, unfold and reorientate at the oil-water interface forming emulsions that are only slightly less stable than those formed with casein under the same conditions. The state of the droplet size distribution reflects the emulsifying capacity of the proteins, the energy input during emulsion formation, as well as the effects of various factors (such as pH, temperature, ionic strength, and ratio of the two phases) on the surface activity of the proteins [8].

As milk proteins are surface active, they have the ability to adsorb to the air-water interface during foam formation [21]. Essential for the formation of protein-based foams is a rapid diffusion of the protein to the air-water interface to reduce surface tension followed by partial unfolding of the protein. Further interactions between protein molecules at the interface lead to the formation of a cohesive film with a certain degree of elasticity, which stabilizes the foams [16]. Caseinates generally give higher foam overruns but produce less stable foams than whey proteins [28]. Whey proteins are capable of forming a cohesive structure surrounding the foam bubbles as well as providing excellent surfactant properties.

It is well known that partial heat denaturation of the proteins improves the foaming characteristics of whey proteins [26]. However, partial hydrolysis of whey proteins with proteolytic enzymes increases the foam volume but reduces its stability. Limited hydrolysis of whey proteins combined with heating at 55-70 °C gives excellent overrun and stability, provided the pH is between 7 and 8 before whipping [26].

Milk proteins have the ability to form rigid, heat-induced irreversible gels that hold water and fat and provide structural support [18]. The ability of milk proteins to undergo gelation upon the addition of acid or rennet to milk is well known. The heat-induced gelation of whey proteins involves a series of steps, starting with the unfolding of protein molecules, followed by their aggregation in aqueous solution [25]. A gel is formed when the extent of aggregation exceeds some critical level; a three-dimensional, self-supporting network that traps the solvent in the system is formed. When the extent of aggregation is below some critical minimum, soluble aggregates or a precipitate will form.

In general, heating a protein solution above the minimum denaturation temperature of the constituent proteins is required for gel formation. The strength of whey protein gels is affected by the concentration and purity of the protein. A protein concentration of 7.5% or greater is needed to form a strong gel at pH 7.0 upon heating for l0 min at 100 °C [25]. Pure solutions of β-lactoglobulin can form gels at 5 and 4% protein, respectively, after heating for 15min at 90 °C. As the protein concentration is increased, the number of potential interactions between molecules is enhanced, resulting in an increasing of gel strength, a reducing of gelling time, and a finer gel network. A gel hardness increases with increasing heating temperature and time when other factors are maintained. Heating rate also affects the gelation process. Slow heating allows the proteins enough time for unfolding and aggregation, resulting in much stronger gels.

The unique properties of caseins and whey proteins, their interactions during processing (e.g., heat treatment), and their interaction with salts, are responsible for the desirable properties of a wide array of popular dairy food products, including cheese, yoghurt, ice-creams, milk desserts and milk shakes.

Gelation of milk proteins is the crucial first step in both cheese and fermented milk manufactures [29, 30]. However, the proteins’ coagulation is different in these two technologies. Cheese production requires a combination of acidification and enzymatic hydrolysis of milk proteins whereas for fermented milk production is necessary only acidification, as the result the milk curd is different (fig. 2). In addition, the way in which milk coagulates is controlled by heating.

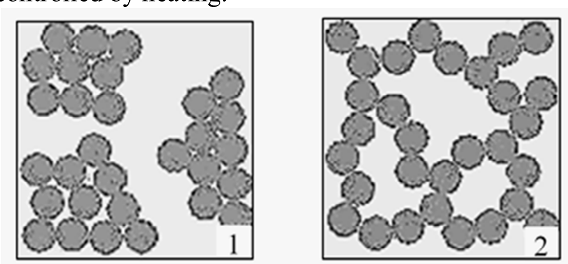

Fig. 2. Casein micelles coagulate: into clusters in the cheese production (1), into branched chains in the fermented milk production (2) [31].

Fermented milks (using the example of yogurt). Fermented milks are obtained by fermentation by suitable microorganisms resulting in reduction of pH with coagulation. There are numerous types of fermented milks manufactured in different parts of the world. Throughout the world, around 400 names are applied to traditional and industrially made fermented milk products. Each type of fermented milk involves specific microorganisms, based primarily on the optimum growth requirements of the starter cultures (i.e., mesophilic and thermophilic microflora). However, there are strong similarities between manufacturing technologies used.

Yogurt has traditionally been made from milk that had been heated almost to boiling. Coagulation of the milk proteins is induced by thermophilic bacteria, such as *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus salivarius subsp. thermophiles*. The milk is coagulated by a slowly increasing concentration of lactic acid as the bacteria metabolize lactose. The proteins do not precipitate (as would happen following an addition of a large amount of lactic acid) but form a gel. Its ability to retain all the water present in the milk is the result of a peculiar microstructure of the protein network. It consists of short branched chains of casein micelles (fig.2) and resembles a sponge with very small pores [31]. In fat-containing products, the presence of (large) fat globules obscures the finer details of pores and strands. The diameter of these pores varies considerably, with larger pores in gels made at a high gelation temperature (usually <30 urn). Lee, W. J. and J. A. Lucey report [32] that yogurt gels made from milk heated at high temperature (>80°C) had a more cross-linked and branched protein structure with small pores compared with milk heated at low temperature.

Heat treatment of milk and the concomitant denaturation of whey proteins affect the characteristics of the acid-gel. When milk is heated at >70°C, the major whey proteins, such as, β-lactoglobulin are denatured [25]. During denaturation β-lactoglobulin interacts with the κ-casein on the casein micelle surface by disulfide bridging [5, 33]. The result is a complex which makes the casein micelle surface markedly coarser. Casein micelles with the κ-casein–β-lactoglobulin complex formed on their surfaces have a limited ability to aggregate. Consequently, short branched micelle chains are formed. Soluble complexes of denatured whey proteins with κ-cascin also associate with the micelles during the acidification process.

The effects of whey protein denaturation on the gel properties can be ascribed to several factors [34].

Firstly, the concentration of gelling protein increases due to the contribution of the denatured whey proteins in the gel structure (2.8% in unheated milk versus 3.3% in heated milk).

Secondly, denatured whey proteins which are associated with the casein micelle could act as a bridging material between the micelles. As denatured whey proteins contain reactive thiol groups, disulfide interactions can also occur. These effects can increase the number and strength of bonds between the protein particles.

Thirdly, whey protein aggregates can act as an additional bridging material.

According to Lee, W. J. and J. A. Lucey, stirred yogurt can have very large clusters of caseins presumably created by the collisions and shearing during the mixing process [35]. Cayot et al. [36] report that the consistency index in stirred yogurts, calculated from the Ostwald model, increased as milk heating temperature increased from 70 to 100°C. An increase in milk heating temperature causes an increasing of apparent viscosity and provides mouth-coating attributes [3, 35]. The characteristic three-dimensional gel matrix of set yogurt is no longer visible in stirred products.

Cheese. Cheese is a concentrated protein gel, which occludes fat and moisture. Its manufacture essentially involves gelation of milk, dehydration of the gel to form a curd and treatment of the curd. Cheese is made from milk that hadn't or had been heated at lower temperature than fermented milks. In result of this treatments milk consists of casein micelles with smooth surfaces [31]. Casein micelle surfaces interact with other casein micelles and form large micellar clusters from which whey separates easily (fig.2.). It is possible to use both rennet-induced and acid-induced coagulations in cheesemaking, however, rennet-induced gelation of milk is used more often [30].

Proteolytic enzymes such chymosin are used to destabilize casein micelles and make them to coagulate [37]. The enzyme is an endopeptidase, which in milk of pH 6.7 cleaves very specifically the Phe105-Met106 bond of κ-casein [38]. K-casein is split into para-κ-casein and caseinomacropeptide (CMP) of which para- κ-casein is insoluble, while CMP is soluble. Para- κ-casein has not the colloid-protective property of κ-casein. Extensive cleavage of the κ-caseins present in the hairy brush results in destabilisation of the micelle. The micelles coagulate and form a gel [11, 39].

Enzyme-induced gelation of milk is hindered by a variety of factors, which either:

• restrict access of the enzyme to (κ-casein), for example complexation of denatured whey protein with κ-casein at the micelle surface, as a result of high heat treatment [39];

• act as obstacles to the aggregation and fusion of rennet-treated casein micelles, for example κ-casein/ β-lactoglobulin appendages at micelle surface, or serum κ-casein/ β-lactoglobulin particles [40].

The milk gel, obtained during enzyme-induced gelation, consists of casein micelle clusters and short chains. They encapsulate fat globules – the natural large corpuscular particles present in milk. Void spaces in the casein matrix are filled with the whey [41]. Following gel formation, the resultant milk gel is subjected to a number of operations that promote the release of whey, an approximate tenfold concentration of the casein, fat and micellar calcium phosphate components, and a transformation to a curd with much higher dry matter content than the original milk gel [42].

During the dehydration process of the gel, protein concentration and aggregation continue via various types of intra- and intermolecular interactions [43], including calcium bridging, hydrophobic interactions between lipophilic domains and electrostatic interactions (other than calcium bridging). The strength of these interactions is modulated by ionic strength, pH, calcium and temperature, and hydrolysis of proteins to peptides, which alters the hydrophile/lipophile balance of the proteinaceous fraction.

Of particular interest in relation to milk composition and cheese quality is the impact of the proportion of intact α_{s1}-casein content in milk on casein aggregation, strength of the enzime-induced milk gel and texture of the final cheese [37]. The sequence of residues 14-24 is a strongly hydrophobic domain and confers intact α_{s1}-casein with strong self-association and aggregation tendencies in the cheese environment. This domain also has 3 mol of glutamate, which are expected to contribute to intra- and intermolecular calcium bridges. It has been suggested that self-association of α_{s1} -casein in cheese via these hydrophobic 'patches', leads to extensive cross-linking of *para*-casein molecules and thus contributes to the overall continuity and integrity of the casein matrix in the cheese curd. The early hydrolysis of α_{s1} -casein at the phenylalanine23-phenylalanine24 peptide bond results in a marked weakening of the *para*-casein matrix and reductions in fracture stress and firmness of the cheese during maturation [4]. This hydrolysis is a key step in mediating the conversion from a fresh rubbery curd to a mature cheese with the desired textural and properties [42].

Lydia Ong et. al highlight the potential of using milk with increasing concentrations of protein to increase the total cheese yield [41]. The increased protein concentration decreases the volume of the sweet and salty whey, potentially reducing the cost associated with processing this by-product. Protein addition could also be used as a tool when cheese with lower moisture content is required and led to only subtle changes in the microstructure, with denser gels, denser milled curds and larger fat globules in the pressed cheese. The cheeses made with higher milk protein are harder than cheeses made with unstandardised milk.

Ice cream and aerated desserts. Other widely popular products in which milk proteins play a crucial role are ice cream and aerated desserts. The structure of ice cream and frozen aerated desserts can be described as a complex colloid consisting of three internal phases (fat globules, some partially coalesced, and their adsorbed interfacial material; air bubbles and their adsorbed interfacial material; and ice crystals) surrounded by a freeze-concentrated aqueous serum or matrix phase that contains the sugars, proteins, polysaccharides and salts [12].

An ice cream mix is first prepared which contains fat, milk solids non-fat (MSNF), sweeteners, emulsifiers and stabilizer. Initial processing of this mix typically involves formulation, homogenization and pasteurization, following which it is aged for at least four hours at 4°C or lower. This last step induces sufficient crystallization of the fat and restructuring of the emulsion droplet surface to facilitate sufficient partial coalescence of the fat droplets during freezing. The aged mix is then simultaneously aerated and frozen, typically using a continuous scraped-surface ice cream freezer.

Milk proteins are added as part of the MSNF component. The protein's content of a mix is usually about 4% [44]. Milk proteins contribute strongly to emulsification of the fat and to partial coalescence of the fat globules and fat structure formation during ice cream manufacture [45]. It is critical that the emulsion droplets are stable during mix preparation, but yet susceptible to partial coalescence during the freezing stage in manufacture. This can happen because the fat droplets are primarily covered by milk protein immediately after emulsification, but are gradually displaced by low molecular mass emulsifiers (e.g., monoglycerides) during aging of the ice cream mix [46].

Milk proteins play a crucial role in the stabilization of air bubbles during the initial stages of ice cream manufacture [15]. During the manufacture of ice cream, air is incorporated in ice cream to approximately 50 % of the volume phase of the final product. It needs to be rapidly covered by surface active compounds to stabilize this expanding air-liquid interface [12]. In ice cream, milk proteins dominate the air-water interface (fig 3).

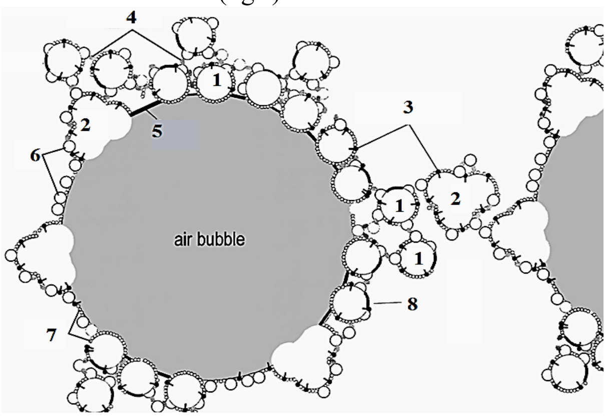

Figure 3. Model for a stabilized air bubble in ice cream and frozen aerated desserts [47]:
1 – intact fat globule attached to the air bubble via calcium bridges; 2 – partial destabilized fat agglomerate; 3 – emulsifier; 4 – calcium bridges; 5 – β-casein; 6 – casein micelle; 7 – casein submicelle; 8 – whey protein.

In addition to emulsification and aeration, milk proteins play a crucial role in structuring the external phase of ice cream. The hydration of milk proteins is important for the rheological properties of the ice cream. Milk proteins are partially incompatible with the added polysaccharides. The resulting networks of polysaccharide and aggregated protein may be partially responsible for controlling recrystallization of the ice phase during storage and temperature fluctuations [48].

In a recent study T. Huppertz shows that, high pressure processing (HPP) of ice cream mixes strong effects on the rheological properties of the mixes and ice cream prepared from these mixes [49]. For instance, viscosity of the ice cream mixes can be increased more than 25-fold by treatment at pressures exceeding 400 MPa. These effects of HPP on ice cream mix can also be largely related to changes in the milk proteins in ice cream mix (the casein micelles have been disrupted) [49]. Like heat treatment, HPP treatment can result in the denaturation of whey proteins, which results in the formation of whey protein aggregates and the association of denatured whey proteins with the casein micelles [50, 51]. Structuring of milk proteins in ice cream by HPP or other means thus offers opportunities for the replacement of fat and stabilizers in ice cream without compromising on hedonic quality parameters.

Moreover, milk proteins can provide the same functional properties (aeration, emulsification) in the structure formation of other dairy dessert products such as milk shakes, whipped cream, frozen aired desserts [13, 52, 53].

Conclusions

Milk proteins provide the structural elements responsible for the textural and melting properties of dairy products. Their ready hydration and strong interactions with each other and with various other components make them valued ingredients in food applications.

The technological properties of milk are strongly influenced by the stability of the casein micelles. Deliberate destabilization of the casein micelles resulting in coagulation or gelation is exploited in the production of a range of dairy products, including all cheese varieties and fermented milks. Another important feature of the milk proteins is their strong interactions with one another. Heat temperature treatments have marked effect on their interaction and solubility at neutral pH. This characteristic is reflected in the high viscosity of proteins' solutions and their ready ability to form foams and emulsions.

It is expedient to use results of review into a further study of the structure formation mechanism of dairy products and the development of recommendations in order to an efficient production.

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