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Colloids in Milk Products

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Abstract: Food, especially milk, is such a familiar material to all of us that it is easy to overlook the scientific input required to assure the quality of existing milk products and/or to come up with new and better processed dairy foods. A major input comes from the research fields of Colloid and Interface science and Soft Condensed Matter Physics, since it becomes more and more evident that relevant properties of foods, such as texture, taste, color, viscosity, stability, mouth-feel or nutritional functionality, are not simply the result of the presence of the ingredients mixed together during processing, but are also the result of the created three-dimensional structure. Recognizing that food materials can be described as colloidal systems allows food technologists to better control the quality of the end product. In the present work we discuss how colloidal concepts can be used to describe the behavior of milk products. We will consider the colloidal properties of milk characterized by the properties of its colloidal entities, *i.e*. the fat globules, the casein micelles and the whey protein aggregates.

Keywords: Casein micelles · Food colloids · Milk · Milk globules · Whey protein aggregates

Introduction

For centuries mankind has hunted and farmed its food supply. However, apart from fruits and nuts, most biological food materials are not easily eaten and digested by *Homo sapiens*. In order to convert these materials to an edible state, physical and chemical transformations are required. Such transformations are achieved by applying some simple or more complicated processing steps. A good example illustrating this is cooking, during which batch processes, such as cutting, heating, cooling, mixing, pressing *etc.*, are used on selected materials during the preparation of a dish. Originally, almost all food was prepared in this way. As civilization advanced and societies became urbanized, mass food preparation became necessary[1] leading to what we today define as the food processing industry. Fundamental and applied research in the area of food colloids and consumer science helps to deliver a consistent quality of food product like milk or its derivative products.

Milk products or other foodstuffs are best described when considering different length scales of observation.[1] Like fabricated composite materials or naturally occurring matter, food products contain structural entities or components that are larger than the dimension of molecules, ranging from the supramolecular (also denoted as the nanoscale), the microscopic up to the macroscopic length scale. Supramolecular structural entities are formed spontaneously from molecular assembly processes.[2] Their properties are determined both by the present molecular species and the conditions under which the assembly structures are formed (thermodynamic control). Prominent examples of such structures are association colloids made of surfactants, for instance phospholipids or monoglycerides, forming structures such as micelles or bilayers in liposomes, cubosomes or hexosomes.[2] Other nanoscale structures are the tertiary structure of polymers, soluble protein aggregates,[3,4] protein–polysaccharide complexes[5] and physical entanglement structures in gel networks.[5] Larger structural entities, *i.e.* microscopic or macroscopic structures, are usually formed *via* energy input, such as shear, provided by the unit operations used during processing. Prominent structural entities in the micrometer length scale (called microstructures, although they can be significantly larger than a micrometer) are oil or water droplets in emulsions, such as milk or margarine, gas bubbles in mousses and fat, sugar or ice crystals in milk, chocolate or ice cream, respectively.[6] These structures have to be stabilized kinetically in order to achieve the desired shelf-life quality of the product. They are thermodynamically unstable.

Mechanical energy input is still today the main route to form structures in food products.[1] However, more and more food colloid scientists are trying to understand the underlying structuring phenomena occurring on the supramolecular scale in relation to structure formation occurring on the microscopic length scale.[7] Examples are colloidal structures like casein micelles (see below) or cubosomes. Cubosomes are particles which have an internal reversed bicontinuous cubic phase structure.[8,9] Their overall dimension is of the order of several hundreds of nanometers (diameter size), and the size of their internal structure is of the order of several nanometers.[8,9]

One principal strategy to better understand the technical problems of unprocessed and processed food materials, such as milk, is a systematic investigation of its colloidal state. Cow's milk, for instance, is a nutritive food with respect to human health and an important functional food from a technological viewpoint. The range of colloidal structures present in milk significantly determines its nutritional, biological and

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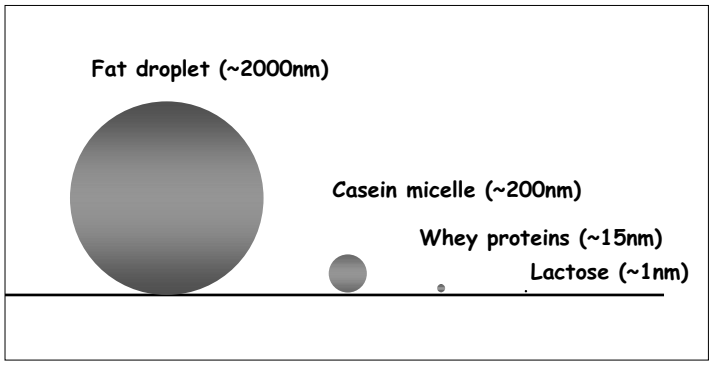

Fig. 1. Schematic illustration of the relative size of the different colloidal entities present in raw milk: the fat globule, the casein micelle, and the whey protein: Copyright © 2008 Nestec Ltd.

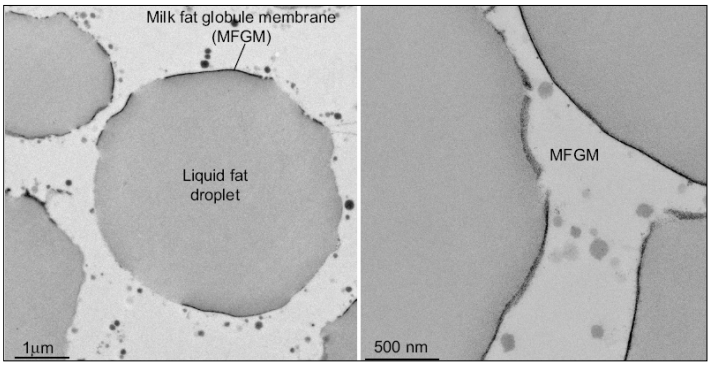

Fig. 2. Transmission electron microscopy picture of fat globules in raw milk. The staining technology allows the milk fat globule membrane (MFGM) surrounding the oil droplets to be visualized; Copyright © 2008 Nestec Ltd.

technological properties. Fig. 1 schematically summaries the three main structural entities present in milk. Each structure has its specific dimension. The largest structures in raw milk are the fat droplets (3–5 µm) which are coated (stabilized) by a mixture of lipoproteins and bilayer structures. The second colloidal structures are the casein micelles (~0.2 µm) consisting of aggregated casein proteins that are sterically stabilized at neutral pH by a 'hairy layer' (brush). The smallest colloidal structures present in milk are the whey proteins and their oligomers (~0.015 µm).

The virtual image of milk, which is constructed by most people, is that of a creamy, opaque and white fluid. It owes its white color to multiple light scattering mainly from the colloidal fat globules and the casein micelles. Raw milk shows colloidal stability,[1] *i.e.* stability against aggregation or flocculation and coalescence. However, it is unstable against gravitational effects, *e.g.* creaming of the oil droplets to the top of the container.[1] If we centrifuge milk to separate the cream from the milk, we create two kinetically stable phases, one rich in oil droplets (concentrated oil-in-water emulsion), and the other one rich in protein molecules and their aggregates. If we take the cream and whip it, *i.e.* incorporate gas bubbles, we create a foam.[1] If even more energy is added to the formed whipped cream, the foam collapses, some water separates out and butter is formed, which is a kinetically stabilized colloidal system of water droplets in fat (water-in-oil emulsion). We can keep butter for quite a long time in the refrigerator, but if we heat it up, the partially crystallized fat melts and water separates out. All these properties of milk can be understood when using scientific concepts developed in the field of colloid and interface science.[1]

In the next sections we will show in more detail how food colloid science can help to investigate and link the empirical description of milk[10] and its transformations with scientific concepts developed in the field of colloid and interface science. We will discuss the colloidal aspects of the three main structural components, *i.e.* milk fat globules, casein micelles and whey protein aggregates.

The Colloidal Behavior of Milk Fat Globules

The composition of milk varies widely across species, with stage of lactation, and in response to diet.[11] For the sake of comparison, the compositions of goat, cow and human milk are given in the Table. The total solids contents of the three milks are roughly the same. The major differences between the two ruminant milks and human milk arise from changes in the protein and lactose contents. The lower casein content in human milk is also reflected in the lower ash content. Bovine milk contains approximately 3–5% fat, which is distributed in the form of microscopic, spherical droplets or globules. The diameter of the fat globule ranges from 0.2 to 15 µm, with an average of around 4 µm. Fig. 2 shows a transmission electron microscopy picture of an oil droplet in raw milk.

Not only the chemical composition but also structural milk properties, such as the fat globule size distribution, depend on the breed of cow, the stage of lactation, and other biological factors. For instance, fat globules from the milk of Jersey cows (average size approximately 4.5 µm) are normally larger than fat globules from the milk of Friesian cows (average size approximately 3.5 µm). However, it has also been observed that there are considerable variations in the fat globule mean size between cows of the same breed.[12] These variations are related to the mechanism of how the globules are formed (see below).

The processes involved in the formation and secretion of fat globules have attracted extensive research attention over the last 50 years.[11] Fat globules are formed in the secretory cells of the mammary gland. The triglycerides are synthesized in or on the surfaces of the rough endoplasmic reticulum membrane and accumulate in the form of micro-lipid droplets in the cytoplasm. These intracellular droplets are covered by a diffuse interfacial layer, which consists of phospholipids, glycosphingolipids, cholesterol and proteins. The micro-lipid droplets grow in volume by fusion with each other to form cytoplasmic lipid droplets of various sizes, which are then transported to the apical pole of the cell through the cytoplasm by unknown mechanisms, and are then secreted from the epithelial cell. During secretion, the droplets are coated with the outer plasma membrane and are budded from the cell. It seems that nature has chosen a droplet-by-droplet production strategy, similar to what is achieved by modern microfluidics techniques, which deal with the control of fluids that are geometrically constrained to a small, typically sub-millimeter, scale. Following secretion, a fraction of the membrane surrounding the globules may be shed into the skim milk, although the extent to which this occurs has been difficult to ascertain. Hence, the fat globules in milk are not a simple oil-in-water emulsion; the globules are surrounded by a complicated membrane, which cannot be considered as a simple monomolecular film of surface-active material.[12] Instead, the membrane has several distinct layers (see also Fig. 2)

Table. Weight percent composition of goats', cows' and human milk, adapted from ref. [10]

	Total solids	Fat	Casein	Whey protein	Lactose	Ash
Goat	13.2	4.5	2.5	0.4	4.1	0.8
Cow	12.7	3.7	2.8	0.6	4.8	0.7
Human	12.4	3.8	0.4	0.6	7.0	0.1

that are laid down during its synthesis in the mammary secretory cell. This membrane is commonly referred to as the milk fat globule membrane (MFGM) and its composition and properties are different from those of either homogenized and pasteurized milk or plasma. MFGM acts as a natural emulsifying agent, preventing flocculation and coalescence of fat globules in milk and protecting the fat against enzyme action. Many properties of dairy products are directly influenced by this unique membrane system.[12]

Because of its original function in stabilizing the fat globules in whole milk, MFGM material isolated from buttermilk or cream is considered to be an efficient natural surface-active material, with high emulsifying capacity.[13] However, method of separation, type of raw material and pre-treatment of cream or buttermilk significantly affect the composition of MFGM isolates and hence their adsorption layer formation and emulsification capabilities. In contrast to MFGM material isolated from fresh cream, MFGM derived from industrial buttermilk has been found to be a poor emulsifier of *e.g.* soy oil-in-water emulsions.[14] This is considered to be due to extensive denaturation of the membrane proteins and the association of the MFGM proteins with the β-lactoglobulin during the heat treatment and churning processes used in the manufacture of buttermilk. Obviously the surface active properties of the MFGM membrane proteins are physically changed during buttermilk manufacture so that they are no longer suitable for the stabilization of the oil droplets. Such physically induced changes are quite common in the food processing industry. Since their impact on the functionality of the present ingredients and the final product quality can be detrimental, they have to be carefully investigated and followed along the respective production process.

Milk products are manufactured from raw milk and subjected to various processes, such as machine milking, cooling, cold storage, homogenization, heat treatment, ultrafiltration or packaging.[15,16] Each of these processing steps induces changes in the intrinsic structure of milk. The most profound structural changes are induced during homogenization and heat treatment. Homogenization is defined as the process of subdividing initially relatively large polydisperse oil globules into smaller droplets of narrow size range. It is an effective physical way to prevent cream separation (creaming of the oil droplets) in the milk during storage. Pasteurization consists in heating milk at 72 °C for 15 s with subsequent immediate cooling. In some cases milk is even heated at 85 °C for 20 s. The UHT (ultra high temperature) process allows shortening of the heating step *i.e.* it consists of heating the milk at 140–150 °C for a few seconds.[16]

The result of homogenization is that the oil droplet size is reduced to around 1 μm, resulting in a four- to ten-fold increase of the interfacial area created between the fat droplets and the aqueous medium. This newly created interface cannot be covered anymore only by the MFGM which was originally stabilizing the oil globules in raw milk. Other surface active components, such as casein and whey proteins, adsorb during homogenization from the bulk milk phase to the new o/w interface forming a new membrane. However, part of the native MFGM remains still associated to the homogenized fat droplets.[17] It is important to note that the structure of milk, including the composition and structure of the new oil/water interface greatly depends on the various mechanical and thermal steps of the processing chain. Therefore, studies dealing with the health properties of milk and other dairy products should always use samples whose physico-chemical properties are well characterized and controlled during the study.

The Colloidal Behavior of Casein Micelles

The dominant structural feature of skim milk (*i.e.* fat-free milk) is the casein micelle. The caseins are a class of four phosphoproteins (α_{S1}-, α_{S2}-, β-, and κ-casein) that represent the majority of proteins present in milk from most mammalian species. Casein proteins are 'disordered', *i.e.* flexible polymers. When dissolved into water, their primary structure prevents tight folding of the peptide chain, partly due to the many proline residues. Moreover, the primary structure of *e.g.* β-casein protein looks like a polymeric surfactant molecule. It has an amphiphilic molecular structure, *i.e.* a hydrophilic 'head' and a long and flexible rather hydrophobic 'tail'. Molecules having such a molecular structure tend to spontaneously associate into structures denoted also as 'association colloids'.[7] It is assumed that the amphiphilic character of the casein proteins significantly influences the formation of the casein micelles. Although some caseins are present also in the serum phase of milk, most casein molecules are part of this unique protein aggregate. The diameter of the casein micelles range from 50 to 300 nm. They are highly hydrated, and contain, on a dry matter basis, ~94% casein and ~6% inorganic materials. These inorganic materials are collectively referred to as micellar (or nanoclustered) calcium phosphate (MCP) and consist primarily of calcium and phosphate, with lower levels of magnesium and citrate.[18]

Casein micelles fulfill two main functions: i) they avoid the risk of a significant increase in the milk viscosity due to protein gelification, and ii) they solubilize and transport the calcium and phosphate.[15] In general it can be said that when milk contains more than 2% protein the accompanying inorganic phosphate and calcium levels would by themselves yield the formation of insoluble precipitates, such as apatite or brushite, depending on the pH. On the other hand, in the absence of these salts, the casein components, as a result of their open structures, would significantly increase the viscosity of the skim milk. Therefore, the creation of the colloidal casein complexes solves these two problems in a very elegant way using physical principles.

For many years the most accepted theory describing the structure of the casein micelle was that it was composed of spherical aggregates of casein sub-micelles held together by calcium–phosphate linkages.[19] Several lines of research led to the sub-micelle model hypothesis,[16] which could also qualitatively be supported by microscopy. Fig. 3a shows a typical freeze fracture microscopic picture of a casein micelle showing the internal structural micellar heterogeneity. However, in recent years the sub-micelle theory has been challenged by concepts arising from the study of the casein–calcium–phosphate interactions, the micelles themselves and physical chemical studies of the individual proteins at interfaces.[11] Two new models have emerged that refute the notion of discrete sub-micellar structures within the casein micelle.[20,21] Especially Horne proposed[21] that the state of association of the caseins inside the micelle is governed by a balance of attractive hydrophobic interactions and electrostatic repulsion. The effects of temperature, pH and ionic strength on the self-association and calcium-induced aggregation of the individual caseins are rationalized in terms of their influence on this balance of forces.[21] This model is denoted as the 'dual-binding' model of the casein micelle reflecting the need for the two different forms of bonding in the network. Fig. 3b shows a sketch of the micellar structure at a pH of 6.7 according to the dual-binding model. The micellar matrix is closely interlinked through a combination of nanocluster bridging bonds (the circles) and hydrophobic interactions, occurring randomly along any selected polymer chain. The hydrophobic interactions at this pH are many but relatively weak being counterbalanced by the negative charges present on ionized carboxyl groups, dispersed along the chains and throughout the network. The micellar outer reaches are mainly κ-casein molecules which have terminated polymer extension and limited micellar growth in the dual-binding model. The negative charges from

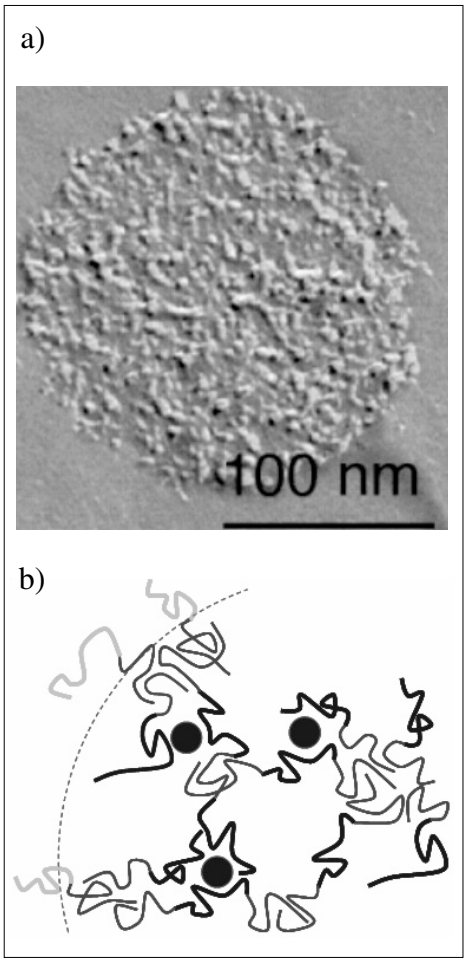

Fig. 3. A) Freeze fracture electron microscopy image of a casein micelle; Copyright © 2006 Nestec Ltd. B) Sketch of the casein micellar structure according to the 'dual binding model'; inside the micelles are the α and β caseins; at the micellar surface κ-casein in located. The points stand for the colloidal calcium phosphate clusters; adapted from ref. [21].

the ionized carboxyls and sialic acid groups on the κ-casein macropeptides provide the electrostatic repulsion component in the inter-micellar interaction potential which inhibits micellar aggregation at a pH of 6.7. Its longer range prevents close approach of the hydrophobic regions buried beneath the micellar surface and sufficiently fulfils the requirements of a hard-sphere model colloid at this pH 6.7 (see below).

In spite of the fact that the exact internal structure of native casein micelles is still not fully known, it could be shown using small angle neutron scattering, viscosity, diffusivity and other measurements that the properties of these particles can be reasonably well described by adopting the adhesive hard sphere model.[22,23] In that model, a steep repulsive interaction of two micelles is preceded by a short-range van der Waals attraction. De Kruif postulated that by relating the strength of the attraction to the degree of technological treatment (*e.g.* renneting time or pH changes) the colloidal properties of the micelles can be described simply by using the adhesive hard sphere theory. Alexander *et al.*[24] investigated the physical and dynamic changes that appear in skim milk at neutral pH as a function of increasing casein micelle fraction (sugar and salt are also increased with respect to the dispersing medium) using diffusing wave spectroscopy (DWS). It was shown that in these static measurements the casein micelle dispersion behaves like a monomodal hard sphere system up to volume fractions of at least 45%.

Recently, however, Horne[25] put forward that under acidification conditions the adhesive hard sphere model no longer properly describes the colloidal behavior of casein micelles. Inadequacies in the adhesive hard sphere model appear when the rate of acidification of the milk employed to gel skim milk is changed either by raising the incubation temperature or increasing the concentration of acidulant. This observations led to the conclusion that the decrease in the pH induces not only changes in the interaction potential between the micelles, but also dominant internal changes in the casein micellar structure occur. This means that under these conditions casein micelles cannot be simply described anymore as 'internally inert' hard sphere particles. In a recent publication[26] Horne *et al.* propose that there is a higher level of complexity to the interactions of the caseins in the casein micelle than an application of any soft condensed matter theory would allow. The difficulty arises from the postulation that casein molecules inside the micelles might interact with calcium phosphate nanoclusters and that the phosphate (the caseins are multi-phosphorylated) plays an active role in the precipitation of casein- (especially α_{S1}-casein)-Ca^{2+}-phosphate mixtures.[26] This interaction forms the basis of the cross-linking mechanism in Holt's model of micellar assembly.[20] The dual-binding model of Horne,[21] however, postulates that beside the calcium phosphate nanocluster formation, there is another way to link casein molecules, *i.e. via* interaction of segregated hydrophobic regions found in all of the caseins. Both kinds of linkages allow the development of a three-dimensional network extending through space. The chains are terminated in the dual-binding model by κ-casein, which thus controls the casein micellar size[26] (see also Fig. 3b). In conclusion one can say that casein chemistry seems to significantly influence the overall casein micellar structure and thereby the colloidal properties of casein micelles.

Thus, the stability of casein micelles may be divided into two categories – intermicellar and intramicellar stability. The intermicellar, or colloidal, stability of casein micelles, denotes the stability of casein micelles against aggregation under, for example, the influence of heat, ethanol, acid, or rennet. Such stabilities are well characterized and form the basis of the conversion of milk into dairy products like cheese or yogurt. They are also used to explain the casein micelle stability during drying, freezing, and addition of salts. However, the intramicellar stability, that is the ability of the casein micelle to maintain its internal structural integrity under the influence of environmental changes, also significantly influences the properties of products derived from milk. As described above, MCP and hydrophobic interactions are primary features in maintaining micellar integrity. Solubilization of MCP, most easily achieved through addition of a calcium-chelating agent, results in the disintegration of the casein micelles,[20] probably into small, hydrophobically bound, casein aggregates. Furthermore, treatment of milk at high hydrostatic pressure can result in a considerable disruption of casein micelles.[27] Disruption of casein micelles can also be achieved through disruption of hydrophobic interactions; for instance, through addition of urea,[20] or sodium docecyl sulfate (SDS),[28] or heating milk to >60 °C in the presence of >30% ethanol.[29]

As a result of the dissociation of casein micelles, the average micelle particle size in skimmed milk is considerably reduced, often to a size no longer detectable by traditionally used particle size analysis techniques. A consequence of the reduction of the mean particle size is the loss of the ability of milk to scatter light and impart turbidity. Therefore, increasing the stability of casein micelles against disruption may positively affect the functional properties of milk. For example, reducing the extent of heat-induced dissociation of κ-casein from the micelle can enhance the stability of milk against heat-induced coagulation[30] increasing the heat or high-pressure stability of milk. Principally this can be achieved by cross-linking casein proteins inside the micelles using glutaraldehyde[31] or transglutaminase.[32]

Aggregation Phenomena of Whey Proteins

Whey proteins are obtained from cheese and casein manufacturing. They make up approximately 20% of all proteins present in milk. Whey proteins are widely used as an ingredient in processed food since they exhibit a high nutritional profile and show a wide range of functional properties.[33] Whey protein isolates (WPI) are obtained by microfiltration/ultrafiltration of milk or ionic chromatography/ultrafiltration of liquid whey. They usually contain more than 90% protein. Their main protein components are β-lactoglobulin (makes up more than 50% of the total whey proteins in milk)

and α-lactalbumin (10–15% of total whey proteins). Bovine serum albumin (BSA) is a minor component in the WPI.[34]

The variety of functional groups present in whey proteins (especially the alkoyl and thiol groups) confers on them a high level of secondary structure (α-helix, β-sheet). These secondary structural elements give the proteins a tightly folded organization in which hydrophilic amino acid residues are predominantly present at the protein surface and the hydrophobic ones in the core of the polymer structure. A way to exclude hydrophilic residues from the protein core is the formation of an elongational shape. Therefore, on the basis of the outer shape, proteins can be classified into three groups, namely globular, fibrous, and disordered proteins.[34] Whereas the caseins do show a range of different conformations rather than one (they are classified as 'disordered' proteins, see above), in whey proteins the peptide chain is tightly folded into a roughly spherical shape. Note that 'disordered' does not mean that no secondary or other ordinary structure exists, but that the protein conformation is much closer to a flexible random coil than to a rigid globular configuration. The conformation of globular proteins is often further stabilized by covalent bonds that are formed during protein synthesis after the creation of the primary structure.[34] Examples are glycosylation reactions or intramolecular S–S bridge formation (oxidation of two –SH groups of two cysteines). Moreover, the conformation of globular proteins is not completely rigid. Limited conformational changes often occur upon changing conditions, such as binding of a ligand or adsorption at the water–air or water–oil interface.[35] These physical properties make whey proteins suitable for forming water-based gels and for generating and stabilizing oil-in-water emulsions and foams.[33]

Whey proteins in their native state are small nanometer-sized particles consisting either of one (monomer), two (dimer), or four protein molecules, depending on the experimental conditions. In milk the whey proteins are dispersed in a continuous aqueous phase containing various salt ions and lactose. The native whey protein particles are much smaller than the casein micelles. For instance, β-lactoglobulin has a molar mass of 18000 g/mol and a radius of about 2 nm.[36] However, since most food products are submitted to heat during processing to ensure microbiological safety, it is very likely that after heat treatment a fraction of the whey proteins is no longer present in its native state but in a denatured conformation. Denatured whey protein molecules are more hydrophobic and, thus, form larger protein aggregates leading to gelation above a critical concentration.[37] Aggregation can occur either among the protein molecules themselves or with other food particles *e.g.* casein micelles or emulsion droplets.

It has long been known that when raising the temperature beyond a critical temperature, native β-lactoglobulin dissociates from a dimer to a monomer, exposing its thiol group and interior hydrophobic residues enabling thiol/disulfide exchange reactions.[38] This process, but also the result of further heat treatment above the protein's denaturation temperature, has been widely examined under various conditions.[18,39,40] For instance, it has been reported that when heating β-lactoglobulin in the presence of α-lactalbumin, the proteins form heterogeneous aggregates.[41] It is also known that changes in β-lactoglobulin and α-lactalbumin ratios in WPI result in aggregates with different protein composition.[41] The mass of the obtained aggregates is dependent on protein concentration, pH, ionic strength and temperature of heating.[42]

When WPI is heated to high temperatures, the aggregation of the whey proteins results in the formation of a gel.[39,43] The structure and properties of the formed gel depend on the medium composition, heating conditions and mechanism of aggregation.[40] At neutral pH, *i.e.* at a pH far from the isoelectric point of the proteins, and low ionic strength, WPI proteins are exposed to strong electrostatic repulsion forces leading to the formation of transparent gels with a fine stranded structure.[44] However, at higher salt concentrations and close to the isoelectric point of the proteins, *i.e.* under conditions of weak electrostatic repulsions, opaque gels with a coarse particulate structure are formed.[44] In spite of the interest in the rheological and microstructural characteristics of these WPI gels, there is still only limited knowledge on the mechanism of association of β-lactoglobulin and α-lactalbumin upon heating. A better understanding of the WPI aggregation behavior upon heating would be of great significance to the food industry because WPI is used more often than isolated proteins due to cost and availability. This includes i) a better characterization of the structural and physico-chemical properties of the intermediate aggregates formed at the early stages of β-lactoglobulin aggregation in the presence and absence of the α-lactalbumin and ii) the understanding of the mechanism of protein aggregation prior to gel formation (see below).

So far, it has been shown that at pH 7 heat induced aggregation of β-lactoglobulin occurs in two steps:[40,45] During the first step well-defined primary aggregates are formed which contain, in the absence of added salt, about 100 protein molecules, *i.e.* with a mass of $M_w \sim 2 \times 10^6$g/mol. Their mass increases with increasing salt concentration.[46] Note that the proteins do not aggregate at all below a critical concentration which decreases with increasing ionic strength.[46] The subsequent second step consists of the aggregation of the first primary globular particles into large aggregates forming the gel.[40,45] The aggregates made out of primary globular particles could be shown by means of atomic force microscopy.[44] The authors showed that a 11% solution of WPI, heated at neutral pH to 80 °C forms aggregates that have a diversity of size and shape though their elemental units are primarily globular in shape. Moreover, they could show that not all soluble primary protein structures are incorporated into the larger aggregates.[44] Another efficient way to investigate the structure of these aggregation phenomena is using scattering techniques.[43] It was found that the aggregates had a self-similar structure characterized by a fractal dimension close to two, independent of the ionic strength (adding salt did not qualitatively change the fractal behavior; it only slightly changed the measured number of d_f from 1.7 to 2). Mahmoudi *et al.*[43] also showed that WPI aggregates have the same self-similar structure as pure β-lactoglobulin aggregates. Slight differences were found only in the quantitative influence of adding salt on the heterogeneity of the formed gels, characterized by the correlation length: the correlation length increased more strongly for WPI gels reaching 1 μm in the presence of 0.1 M NaCl than for pure β-lactoglobulin gels which reached 1 μm in the presence of 0.2 M NaCl.[43]

Recently Schmitt *et al.*[4] showed that the formation of the soluble primary WPI aggregates (heating at 85 °C for 15 min) can be controlled by adjusting both the pH and NaCl content in the WPI system. In this way it is possible to produce soluble aggregates with very specific physico-chemical properties and morphologies. This is not possible when adjusting pH or salt alone. Remarkable is the fact that up to 95% of the initially added native WPI molecules were incorporated into the soluble aggregates. Concerning the regulation of the shape of the formed aggregates it was shown[4] that when using a pH >6.6 in the presence of salt, fibrillar structures are formed, whereas when using a pH <6.6 more compact soluble aggregates are created. Interestingly, the foaming and foam stabilizing properties of these soluble aggregates depend on their morphology. Whereas the fibrillar structures allow quite stable foams to form, the more globular structures did not exhibit high foaming and foam-stabilizing properties.[4] This difference could be related to the large size and compact structure of the globular aggregates which seem to be less suitable to form a stable viscoelastic film around the created foam bubbles as the fibrillar structures seem to be.

The size of aggregates that arise from the assembly of whey proteins can be up to several micrometers or even larger and their structure can be from compact to open.[3] In Fig. 4, a schematic picture is given as a

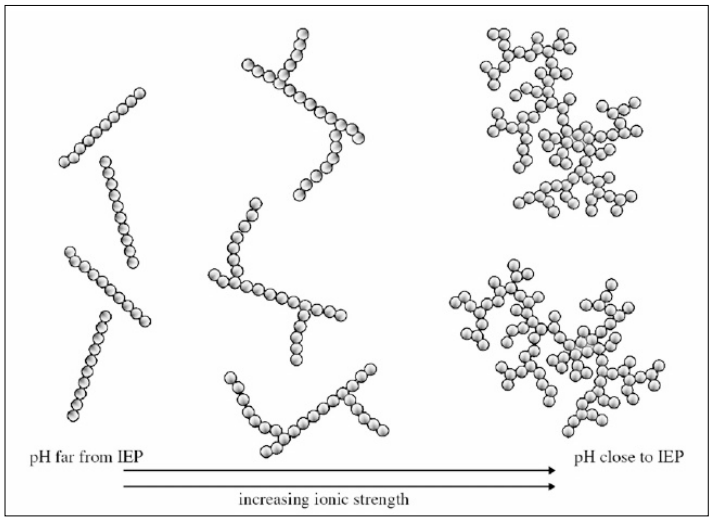

Fig. 4. Schematic representation of protein structures formed by heat-denatured globular whey protein solutions as a function of the ionic strength and difference between the isoelectric point (IEP) of the protein and the pH of the solution; adapted from [3].

summary showing the structures formed when β-lactoglobulin is heated to 80 °C and the pH and/or ionic strength in the system is varied. For a pH near the isoelectric point of the protein, where the overall charge of the protein is zero, spherical and open aggregates are formed, while for a pH far from the isoelectric point linear aggregates, *i.e.* fibrils, are formed. Apart from the fact that (amyloid) fibrils are related to disease, they form gels at extremely low weight fractions or can be used as clotting material. More systematic research is needed in order to fully understand the mechanism of formation of the different aggregate morphologies and in order to find new appropriate applications.

Some Concluding Remarks

Almost all food materials are of colloidal nature. Gel, fiber, emulsion, foam and many more structures are formed during food processing and preparation. These colloidal structures contribute to the stability, shape, texture, organoleptic and nutritional quality of the final foods. They are an indispensable part of the value of all food products. Understanding foods, such as milk, as colloidal or soft materials, *i.e.* their different aggregation states and the multitude of relevant characteristic time and length scales, will give the food processing industry in the future a tool to create more tasty and nutritionally equilibrated products. In this way the food industry might be able to help the society to overcome some of the main problems of the 21st century related to the dramatic increase in public health issues, *i.e.* in the number of people suffering from metabolic disorders.

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