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Review

Effect of heat treatment on milk protein functionality at emulsion interfaces. A review

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ABSTRACT

Heat treatment affects the molecular structure of milk proteins at the interfaces of oil-in-water emulsions and in aqueous media. Experimental evidence of the impact of thermal processing on milk protein structure is presented and the contribution of whey proteins and caseins at film formation during emulsification is discussed. Recent advances in understanding the effect of heat treatment in milk protein functionality at emulsion interfaces are reviewed with particular emphasis on the emulsifying ability of whey proteins with or without the presence of the casein fraction. The major findings regarding the destabilizing mechanisms of oil-in-water emulsions brought about by heat-induced denaturation of milk proteins are presented. This paper aims to combine recent knowledge on how thermal processing of milk proteins affects their molecular configurations in bulk and particularly at interfaces, which in turn appear to be important with respect to the physico-chemical properties of milk protein-stabilized emulsions.

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1. Introduction

Milk is a complex food from a molecular composition perspective which constitutes an important part of human's diet, mainly because of its high nutritional value. It is consumed as fluid or it is used for the production of other dairy products such as butter, cheese, ice cream, etc. Milk is an emulsion of fat globules in an aqueous phase. The aqueous phase consists of dissolved and suspended components such as casein micelles, serum proteins, lactose minerals and vitamins (Brans, Schroën, van der Sman, & Boom, 2004). Traditionally milk proteins are classified in two major categories, the concentration of which in bovine milk is presented in Table 1 (Walstra & Jenness, 1984). The first and most abundant is the casein family, which consists of several fractions (mainly alpha s1, alpha s2, beta, kappa) and most of them exist in a colloidal particle known as the casein micelle. The second protein group in milk is the whey proteins which include heat-sensitive, globular, water soluble proteins and enzymes (Goff & Hill, 1993).

Thermal processing of milk is an essential step of milk production adopted by the dairy industry. Heat treatment of milk aims to extend the shelf-life and improve the quality of this complex biological fluid by reducing the microbial load and thus, minimizing the risk of food poisoning (McKinnon, Yap, Augustin, & Hermar, 2009). However, heating milk is not always employed to ensure

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Table 1
Protein composition of bovine milk (from Walstra & Jenness, 1984).

Major milk proteins	Grams/liter	% of total protein
Total protein	33	100
Total caseins	26	79.5
α_{s1}	10	30.6
α_{s2}	2.6	8.0
β	9.3	28.4
κ	3.3	10.1
Total whey proteins	6.3	19.3
α-Lactalbumin	1.2	3.7
β-Lactoglobumin	3.2	9.8
BSA	0.4	1.2
Immunoglobulins	0.7	2.1
Proteose peptone	0.8	2.4

microbiological safety. In other cases, where milk is used as food ingredient in milk-based products, heat treatment is employed to improve the organoleptic properties of such dairy formulations by manipulating the functionality of milk proteins (del Angel & Dalgleish, 2006). For instance, milk proteins and particularly whey proteins are commonly used as emulsifying and foaming agents in diverse food products thanks to their unique interfacial properties (Nicorescu et al., 2008). Heat treatment, depending on the processing conditions, can result in irreversible changes in milk protein structure. When milk is heated at temperatures above 65 °C whey proteins unfold and expose previously hidden hydrophobic groups (Croguennec, Kennedy, & Mehra, 2004). Following unfolding, whey proteins are capable to interact with themselves and κ-casein to form heat-induced protein aggregates (Donato, Guyomarc'h, Amiot, & Dalgleish, 2007; Jang & Swaisgood, 1990; Smits & van Brouwershaven, 1980). These changes at molecular level may have an impact on protein functionality which sometimes is desirable and other times can be detrimental (Singh & Creamer, 1992).

The effectiveness of heat treatment of milk as a tool for modifying the functional properties of its protein components has been extensively documented in the literature and several mechanisms have been proposed to account for the findings depending on the processing conditions of milk or milk/whey systems (Lucey, Munro, & Singh, 1999; Modler & Emmons, 1976; Modler & Harwalker, 1981; Morr, 1985; Singh & Newstead, 1992). This study aims to present current findings on the effect of milk processing on the interfacial properties of milk proteins. The present work focuses on some aspects of milk component functionality related to the role of heat-denatured milk protein in the formation and stabilization of emulsions. This article reviews current research and emphasis is placed on the extent of structural disorganization of milk proteins induced by the thermal treatment, which is important in order to improve our understanding of the physical and chemical mechanisms responsible for the interfacial properties of these systems.

2. Milk proteins and heat treatment

When milk proteins are subjected to thermal processing, depending on the heating conditions, whey proteins may undergo a structural change, commonly known as denaturation, which is accompanied by protein unfolding and an exposure of hydrophobic groups. During heat treatment, small aggregates of β-lactoglobulin are formed which, at increasing temperature or heating time enlarge, and larger denatured β-lactoglobulin aggregates are formed (Jang & Swaisgood, 1990). When the heating temperature and/or time is further increased, denaturation of α-lactalbumin begins, which forms complexes with large denatured β-lactoglobulin aggregates, and both proteins bind to the surface of casein micelles (Fox, 1992). Thus, following the denaturation of whey proteins, there is a reaction between the latter and κ-caseins originally present on the surfaces of the casein micelles, to yield complexes between whey protein and κ-caseins (Oldfield, Singh, Taylor, & Pearce, 2000). These complexes are located on the surface of casein micelles (Corredig & Dalgleish, 1996) and in the serum phase of milk in the form of soluble complexes mainly between κ-casein and the whey proteins (Guyomarc'h, Queguiner, Law, Horne, & Dalgleish, 2003). Furthermore, whey protein aggregates alone are also formed (Mahmoudi, Mehalebi, Nicolai, Durand, & Riaublanc, 2007; Vasbinder & de Kruif, 2003). Nevertheless, recent studies indicate that when κ-casein is present, there is preferential hydrophobic interaction and/or disulphide bridging between the casein fraction and the heat-denatured, unfolded whey proteins (Guyomarc'h, Nono, Nicolai, & Durand, 2009). This preferential binding has a protective effect against large scale heat-induced aggregation of whey proteins, which in turn reduces the aggregate size. Other studies showed that α_{s1}- or β-caseins also inhibit large scale heat-induced aggregation of whey proteins or other globular proteins even though neither of these two caseins can exchange disulphide bridges (O'Kennedy & Mounsey, 2006). In any case, the possibility of α_{s1}- or β-caseins competing with κ-casein for the interaction with denatured whey proteins cannot be ruled out.

The kinetics of protein denaturation and aggregation is controlled by the heating conditions and the chemical environment with the heating temperature and pH being probably the most important factors in determining the rate and extent of protein denaturation and the degree of the subsequent interaction of the whey proteins with the casein micelles (Anema & Li, 2003; Corredig & Dalgleish, 1996). The extent of heat treatment affects the level of denaturation of milk proteins, as shown in Fig. 1 (Raikos, Kapolos, Farmakis, Koliadima, & Karaiskakis, 2009). The protein bands indicated by the arrows, which are clearly seen when the samples are heated at 50 °C for 1 h, disappear when the temperature of the heat treatment is 95 °C or higher. This is an indication that at least some of the proteins of full-fat and semi-fat milk unfold due to heat treatment, expose previously hidden hydrophobic groups and may form aggregates of large molecular weights. Recent studies revealed that at low pH values (<6.6) most of the whey aggregates are located at the surface of the casein micelles, whereas at high pH

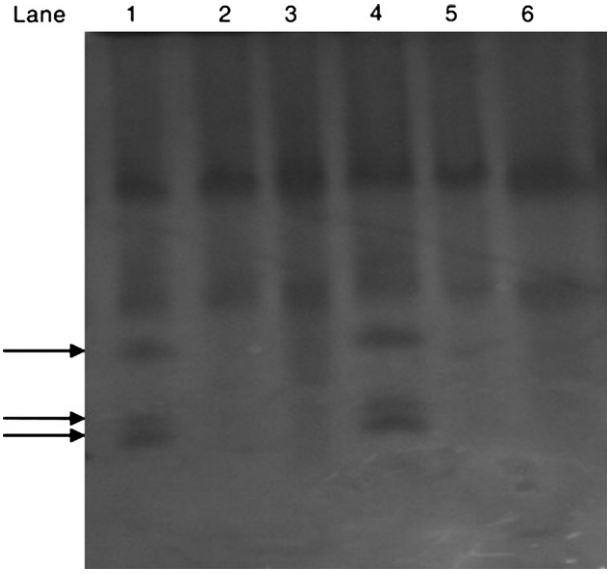

Fig. 1. Native PAGE of heat-treated full-fat and semi-fat milk samples; lane 1: full-fat, 50 °C, lane 2: full-fat, 95 °C, lane 3: full-fat, 125 °C, lane 4: semi-fat, 50 °C, lane 5: semi-fat, 95 °C, lane 6: semi-fat, 125 °C (from Raikos et al., 2009).

values (>6.6) most of the whey aggregates are located in the serum in the form of soluble aggregates (Vasbinder & de Kruif, 2003). Furthermore, previous studies indicate that heating of dispersions at high protein concentration increases the rate of aggregation propagation (but not the rate of the unfolding step) and thus, the formation of large aggregate sizes is favored (Grácia-Juliá et al., 2008). As a result, by controlling the method of heating, the protein concentration and/or the pH of the system under investigation, the degree of heat-induced denaturation of the whey proteins and the level of interaction between the denatured whey proteins and the casein micelles may be manipulated respectively. This in turn may be directly linked to the improvement or impairment of milk protein functionality.

3. Emulsions and milk proteins

3.1. General aspects of emulsions stabilized with proteins

Mixtures of milk proteins are widely used as ingredients in various food products because they are excellent emulsifiers (Dickinson, 1999; Morr & Ha, 1993). Homogenization is an essential step in emulsion formation, in which large deformable drops are broken down by the vigorous application of mechanical energy (Walstra, 1983). During the course of emulsification, milk proteins are capable of rapidly adsorbing at the surface of the newly formed oil droplets, reducing the interfacial tension and forming thick layers which prevent droplets from coalescence or flocculation via steric and electrostatic stabilization mechanisms (Fig. 2, Dickinson, 2008). Protein adsorption at the oil–water interface is thermodynamically favorable because hydrophobic residues of the protein backbone are removed from the bulk aqueous phase and are oriented towards the oil phase, following protein structural rearrangement at the interface (Dickinson, Murray, & Stainsby, 1988). As a result, the adsorbed globular protein structure lies somewhere intermediate between the native state and the fully-denatured state, sometimes referred to as the molten globule state (Dickinson, 1998). Nevertheless, previous studies on the conformation of α-lactalbumin indicate that despite the alteration in the protein conformation following adsorption at the oil-in-water interface, the adsorbed protein still contains a large proportion of well-organized structure (Fang & Dalgleish, 1998). Thus, although the molten globule state has been proposed as a major intermediate of protein folding which may efficiently describe the structural rearrangements occurring during protein adsorption at the interface of an oil-in-water emulsion, it has proven difficult to obtain thermodynamic data characterizing this state.

The behaviour of oil-in-water emulsions in foods is defined by the three parts of the system; the oil phase, the aqueous phase and the interfacial layer between the lipid material and the bulk phase (Fig. 3, Dalgleish, 2006). The stability and rheological properties of emulsions is correlated to a large extent to the interactions between the droplets, which in turn depend on the structure and composition of the adsorbed milk protein layers at the surface of the fat globules (Dalgleish, 1995). Nevertheless, despite the fact that the composition of a milk protein interfacial film can be adequately investigated, the structures of the adsorbed emulsifiers remain unknown with respect to their molecular detail. Furthermore, proteins at the interfacial layer of the emulsion droplets may be capable of interacting with the aqueous phase components. These types of interactions between proteins in adsorbed layers and proteins in solution depend on the chemical environment of the bulk phase. For instance, a general increase in ionic strength can destabilize the emulsion, which is attributed to a masking effect on the charged, adsorbed proteins (Casanova & Dickinson, 1998).

3.2. Milk proteins at the oil–water interface

During homogenization of milk protein emulsions, competitive adsorption between the caseins and the whey proteins occurs, which results to the formation of a thin layer (~10 nm) consisting of both types of proteins (Millqvist-Fureby, Elofsson, & Bergenstål, 2001). This maximum amount of protein adsorbed per surface area of the fat globule (2–3 mg/m^2) is assumed to be reflected by a monolayer of both caseins and serum proteins (Pelan, Watts, Campbell, & Lips, 1997). As a general rule, the protein that arrives first at the interface is the one that predominates (Dickinson, 1997). Nevertheless, the ability of a protein to be the main species at an oil–water interface during or after emulsification depends mainly on its molecular flexibility and its surface hydrophobicity (Dickinson, 1991). This seems to be the main reason for the preferential adsorption of the casein fraction in homogenized dairy emulsions (Tomas, Paquet, Courthaudon, & Lorient, 1994). Caseins predominate at the oil–water interface because of their higher proportion of hydrophobic residues and the more flexible, open molecular

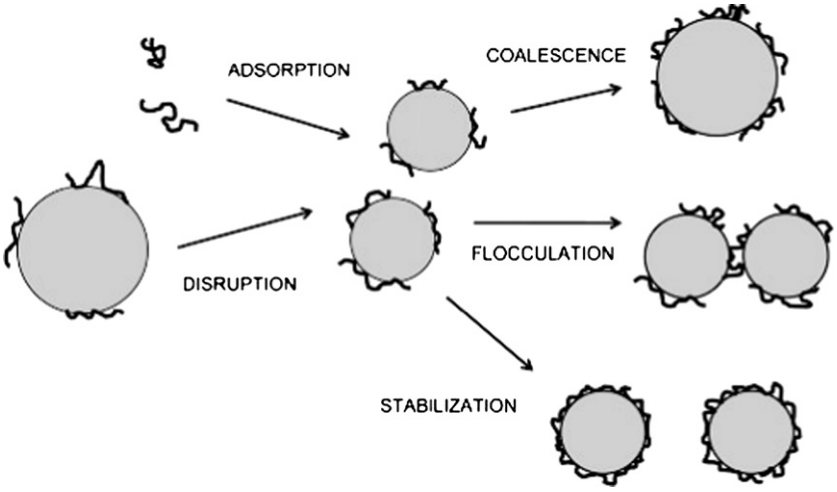

Fig. 2. Illustration of main physico-chemical processes involved in making of emulsions. Stabilization of fine droplets requires mechanical disruption of coarse droplets accompanied by rapid effective adsorption of emulsifier at the new oil–water interface. Collision of droplets with insufficient coverage of emulsifier leads to coalescence and/or flocculation (from Dickinson, 2008).

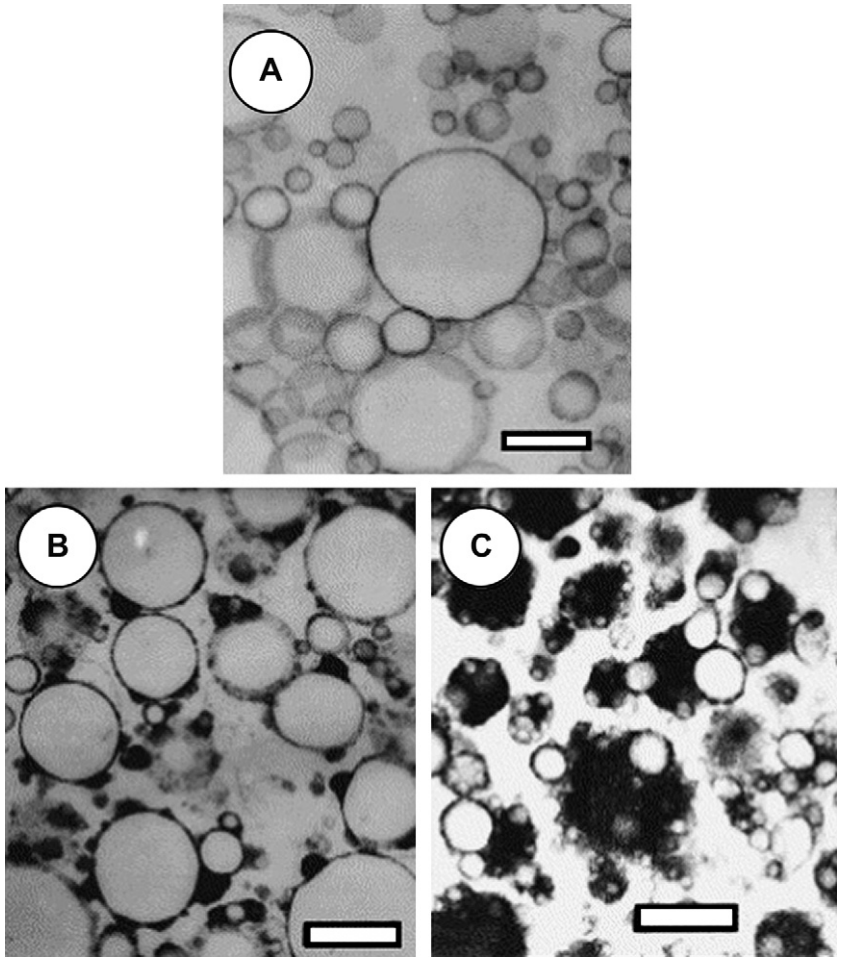

Fig. 3. Transmission electron micrographs of different emulsions. (A) An emulsion of soya oil stabilized by sodium caseinate. The surface is covered by a monolayer of protein. (B) Large fat globules from a sample of homogenized milk, showing the attachment and spreading of casein micellar material (dark clumps) on the oil–water interface. (C) Smaller particles from homogenized milk, showing the extremely high protein load and small size of the fat droplets. Scale bar in A and B represents 300 nm, and in C 200 nm (from Dalgleish, 2006).

structure compared to the whey proteins (Dalgleish, Goff, Brun, & Luan, 2002). The flexible, amphiphilic caseins adsorb fast during homogenization and lower the surface tension. This ability exhibited by caseins makes the latter the dominant species at the stabilizing layer. However, experimental evidence indicates that proteins will adsorb to the oil interfaces in proportion to their concentrations in the aqueous phase (Hunt & Dalgleish, 1994). This statement is further strengthened by recent studies (Ye, 2008) which indicate that the interfacial composition of emulsions made with mixtures of sodium caseinate and whey protein concentrate depend on the protein concentration. Caseins adsorb preferentially at the oil–water interface at high protein concentrations, whereas at low protein concentrations (<3%), whey proteins adsorb in preference to caseins. In any case, as shown in Fig. 4 the interfacial composition of the protein film surrounding the fat globule didn't have an impact on the average droplet size (d_{32}) of the emulsions formed.

Furthermore, once the milk proteins become adsorbed at the oil–water interface, little free reversible exchange occurs between the adsorbed and the proteins located at the aqueous phase (Dickinson, Rolfe, & Dalgleish, 1990). It has been documented (Dickinson & Matsamura, 1994) that when globular proteins such as β-lactoglobulin are adsorbed, displacement from the surface layer by other proteins, even very flexible ones like β-casein or α_{s1}-casein, is rather unlikely to occur. It has been suggested however, that whether one protein will displace another from the oil interface depends not only on their relative molecular flexibilities, but also on the density of interfacial packing and the strength of interfacial protein–protein interactions for the species forming the layer covering the oil droplet (Dickinson, Rolfe, & Dalgleish, 1988). According to previous studies, α-lactalbumin may be displaced from the interface by β-casein, due to the inability of the whey protein to polymerize via sulphydryl exchange reactions (Dickinson, Mauffret, Rolfe, & Woskett, 1989).

4. Effect of heat treatment on milk protein emulsifying ability

Several attempts have been made to investigate the effect of heat treatment on milk protein functionality. Solutions of sodium caseinate and whey proteins were subjected to heat treatment and following completion of this pre-treatment were used for emulsion formation and stabilization. Heat treatment of sodium caseinate near the pI at 50–100 °C for 5 min resulted in enhanced emulsifying ability and capacity (Jahaniaval, Kakuda, Abraham, & Marcone, 2000). This increase in protein functionality of the soluble protein fractions from sodium caseinate heat-treated near the pI, was attributed to the heat-induced exposure of previously hidden hydrophobic domains on the protein backbone. On the other hand, other studies (Millqvist-Fureby et al., 2001) revealed that when

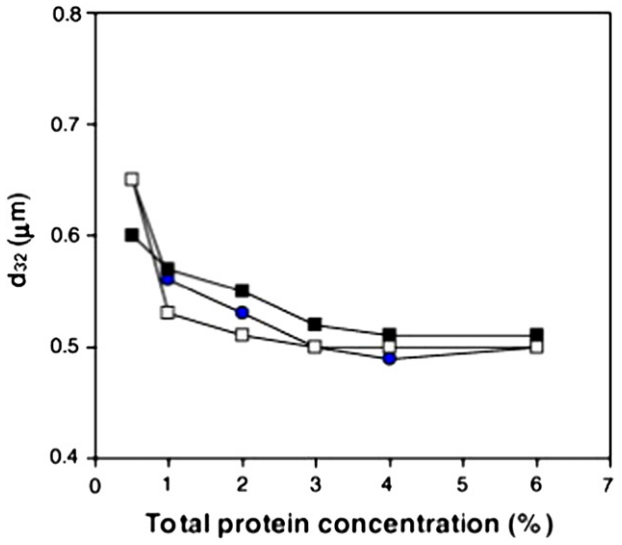

Fig. 4. Average droplet size (d_{32}) of emulsions made with a binary mixture of sodium caseinate and WPC (1:1 by weight) (●), sodium caseinate (■), and WPC (□), in 30% soya oil, pH 7.0, as a function of protein concentration. Each data point is the average of determinations on three separate emulsions (from Ye, 2008).

whey proteins solutions are subjected to thermal processing at temperatures between 60 and 90 °C for up to 1000 s, they exhibit significant loss of emulsifying ability. Following protein denaturation due to heat treatment, large protein aggregates are formed which are unable to cover efficiently the fat droplets, leading to emulsion instability. As shown in Fig. 5, droplet size of the milk protein-stabilized emulsions increases with increasing heating treatment. Furthermore, proteins become less surface active and this has a negative impact on their interfacial properties as compared to the untreated proteins. Thus, pre-heat treatment of proteins at the specified conditions resulted in reduced emulsifying efficiency which most likely is correlated to the degree of protein unfolding.

Thermal processing affects the functionality of the ingredients used for emulsion formation, which among others is protein emulsifying ability. The findings of the previous studies indicate that the effect of heat treatment on whey protein functionality is different to the one exhibited by caseins. The application of heat treatment in milk protein solutions which contain whey proteins and caseins in varying proportions and their utilization for emulsion formation and stabilization needs to be further exploited in the future.

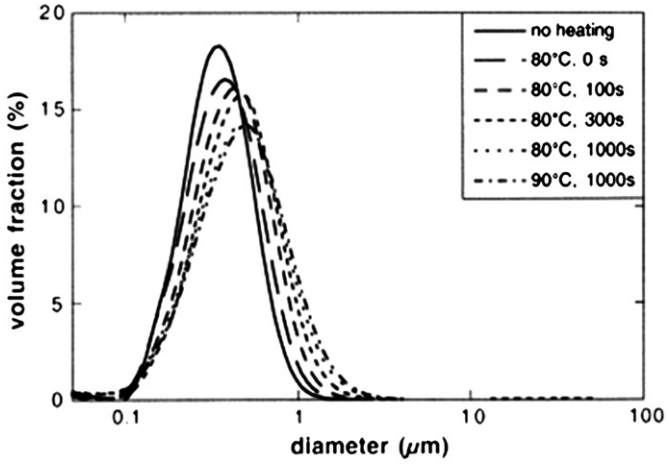

Fig. 5. Emulsion droplet size distribution in fresh emulsions stabilized with pre-heated whey proteins (from Millqvist-Fureby et al., 2001).

5. Impact of heat treatment on milk protein emulsions

Heat treatment, an essential step in dairy product processing, affects the rheology and structure of milk protein emulsions and determines to a large extent the consumer acceptability of such products. It has been documented that heating has an impact on the particle size of model emulsions stabilized with milk proteins (McSweeney, Mulhivill, & O'Callaghan, 2004). The particle size distribution profile of model emulsions (pH 6.8) shifted from below 1 μm prior to heating to 1–10 μm, when heated at 140 °C for 80 s. This increase in particle size distribution was attributed to fat globules aggregation, which resulted from interactions between non-adsorbed protein molecules in the serum phase and proteins adsorbed at the interface of fat globules. The same type of protein–protein interactions was suggested to be responsible for the enhanced creaming rates observed when whey protein-stabilized emulsions were heated (Euston, Finnigan, & Hirst, 2000). In this case, the non-adsorbed whey protein fraction acted as "glue" which entangled the emulsion droplets in large aggregates. Furthermore, Monahan, McClements, and German (1996) hypothesized that emulsion droplet aggregation was enhanced by unfolding of both the unadsorbed whey proteins in the continuous phase and the adsorbed proteins at the oil–water interface (30–90 °C, 30 min). In this case, disulfide-mediated polymerization between milk proteins upon heating resulted in increase in particle size due to droplet flocculation.

Similar findings were revealed by other studies, in which whey protein-stabilized oil-in-water emulsions were subjected to heat treatment at holding temperatures between 50 and 90 °C for 15 min (Keowmaneechai & McClements, 2006). The mean particle diameter increased with increasing holding temperature and this effect was attributed to droplet aggregation, which resulted in increased emulsion viscosity and creaming instability. In this case, droplet flocculation due to increased surface hydrophobicity following heat-induced denaturation of whey proteins at the interface was the proposed mechanism for the shift of particle size distribution to larger sizes. Furthermore, an increase in the volume-weighted mean particle diameter (d_{43}) was observed when conventional and nutritionally-modified whey–protein concentrates were used to stabilize emulsions which were held at temperatures from 30 to 90 °C for 30 min (Surh, Ward, & McClements, 2006). Once again, it was postulated that droplet aggregation was induced by the increased intermolecular interactions between the protein molecules adsorbed onto different droplets. Following thermal processing, hydrophobic attractions and thiol-disulfide interchange reactions facilitated the interactions of the denatured protein molecules.

Colloidal interactions between droplets resulting from heat-induced unfolding of protein molecules adsorbed at the oil–water interface has been documented in previous studies of whey protein-stabilized emulsions (Demetriades, Coupland, & McClememnts, 1997). However, in this case the increase in particle size distribution followed a different pattern. Flocculation of emulsion droplets (pH 7) led to an increase in particle size, when emulsions were heated between 65 and 80 °C for 30 min. Further heat treatment (80–90 °C) resulted in decrease in particle size. According to this study, the emulsion behaviour in terms of particle size distribution as related to heat treatment was attributed to the extent of protein denaturation at the oil–water interface. Adsorbed whey proteins are only partially unfolded when heated at temperatures as high as 80 °C, which promotes surface

hydrophobicity and droplet flocculation. At higher temperatures, proteins become fully unfolded and are able to rearrange effectively all non-polar amino acids towards the oil phase, thus reducing the tendency for aggregation. Most likely, at higher temperatures proteins at the interface possibly may form a more compact layer covering the oil droplet, which increases the density of the droplet and lowers the susceptibility to creaming (Monahan et al., 1996). It has been documented that the amount of protein adsorbed at the emulsion droplet increases with increasing temperature for whey–protein-stabilized emulsions (Sliwinski, Roubos, Zoet, van Boekel, & Wouters, 2003). In this case, it was concluded that initially oil droplets aggregate and upon further heating deaggregation takes place, leading to the formation of smaller, more compact emulsion droplets. Furthermore, the relationship between the amount of adsorbed protein and droplet aggregation is not clear. A similar pattern was observed in particle size changes relative to heat treatment for chocolate milk (Raikos et al., 2009). In this case, chocolate milk exhibited considerable increase in particle size (104 nm, 12.53%) within a certain temperature range (50–110 °C), followed by a decrease in particle size when heated at 125 °C for 1 h. Heat-induced flocculation due to attractive interactions between hydrophobic sites on denatured protein molecules on different droplets was assumed to be mainly responsible for the increases in particle size observed in this study.

Other studies (Dickinson & Parkinson, 2004) which involved emulsions made with β-lactoglobulin showed a significant increase in particle size distribution, when heated at temperatures higher than 85 °C for a varying period of time (30 min to 48 h). This increase in effective particle size triggered by protein thermal denaturation with consequent droplet flocculation was also accompanied by an expected increase in viscosity. Interestingly, when sodium caseinate was added to the emulsion ingredients, whey protein emulsion was protected against heat-induced flocculation. According to the authors, adsorbed casein molecules, due to their long tangling tails, induce a steric stabilization effect which prevents droplets from flocculation and thus reduces the heat-induced thickening effect (Fig. 6). Further evidence supports the positive effect of sodium caseinate on emulsion stability when emulsions are stored at temperatures above 15 °C (Euston & Mayhill, 2001). According to this study, crosslinking occurs between casein molecules adsorbed on the same droplet, which can inhibit the susceptibility of the emulsion to heat-induced destabilization by increasing the surface viscosity of the adsorbed layer. As a result, emulsions remain relatively stable with respect to the creaming stability and particle size distribution over a period of storage time due to the intradroplet crosslinking of caseins. Nevertheless, although there seems to be a temperature-dependent effect on the emulsion stability, the application of higher temperatures is required to clarify the effect of thermal processing on caseinate-stabilized emulsion droplets.

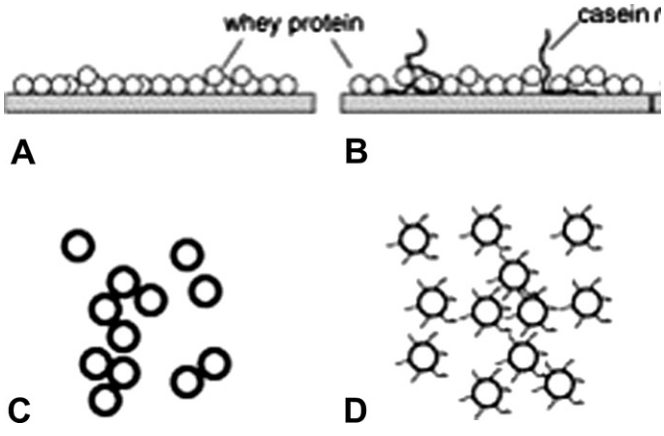

Fig. 6. Schematic representation of the effect of a small addition of sodium caseinate on the colloidal structure of a heat-treated WPI-B emulsion: (A) thin layer of globular whey protein molecules adsorbed at the oil–water interfaces; (B) thicker layer with disordered casein molecules incorporated; (C) reversibly flocculated heat-treated WPI-B emulsion (high viscosity); (D) non-flocculated emulsion (low viscosity) sterically stabilized by low density of dangling casein tails (from Dickinson & Parkinson, 2004).

6. Conclusions

This work aims to describe recent findings in the effect of heating on the interfacial properties of milk proteins. The impact of thermal processing on milk protein structure concerns mainly the whey proteins, whereas caseins seem to have a protective effect against denaturation of the serum proteins. Heat treatment of milk proteins prior to emulsion formation was also proved to reduce the protein ability to form stable coarse particles, when whey proteins were involved in the process. On the other hand, heat treatment of sodium caseinate solutions in controlled chemical environment resulted in significant improvement of emulsifying properties.

In whey protein systems, heating had an adverse effect on the emulsion stability, which possibly arises from droplet flocculation resulting from protein–protein interactions. Despite the fact that heat-stable caseins predominate at the oil-in-water interface during emulsion formation, thermal processing had a negative impact on mixed protein systems as well. However, the addition of small amounts of sodium caseinate can substantially improve the heat stability of a whey protein-based emulsion system. In all cases, the degree of protein denaturation induced by heat treatment, under the given chemical environment, was concluded to be the key factor which determines the interfacial functionality of milk proteins with subsequent effects on the emulsion properties. Although recent work describes the effect of thermal processing on milk protein functionality, little is known about the molecular configurations of the heated proteins adsorbed at the oil-in-water interface. Most of the proposed mechanisms which may account for the altered milk protein functionality as a result of thermal processing are based on hypotheses. This appears to be even more challenging for emulsions stabilized with mixtures of whey and casein proteins. Controlling the heat-induced changes of protein structure at the molecular level may prove to be beneficial for compensating for the negative impact of heat treatment on the organoleptic properties of milk protein-based emulsions.

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