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Chapter

Promising Food Ingredients: Milk Proteins

Roua Lajnaf, Hamadi Attia and Mohamed Ali Ayadi

Abstract

Milk, well known for its nutritional properties, has also good functional properties as foaming, emulsifying and biological activities due to proteins. Milk proteins are then considered as promising food ingredients due to their particular structural characteristics leading to various interesting properties in the industrial field. Thus, the examination of the biological activities and techno-functional properties (foaming and emulsifying properties) of some milk protein fractions revealed interesting ingredients for food industry due to their nutritional value, which is of a great scientific and industrial relevance. This chapter presented an overview of the studied functional properties of some milk proteins.

Keywords: foam, emulsion, biological activities, caseins, whey proteins

1. Introduction

Milk proteins are known by their spatial structure and physical properties which can explain their use in various techno-functional properties (such as water absorption, emulsifying or foaming properties) in their native state or after a suitable treatment (enzymatic, physical or chemical treatments) [1, 2]. Overall, to have interesting foaming or emulsifying properties, proteins should be soluble, amphiphilic and tensioactive with the ability to orient and change the conformation easily at the created interfaces (**Figure 1**) [3].

2. Techno-functional properties of milk proteins

2.1 Foaming properties

Milk is well known by its important foaming properties encountered with many various milk-based aerated foods such as ice cream, cappuccino, whipped cream, chocolate mousse, etc. [4]. Indeed, milk proteins determine the structure and stability of milk foam and emulsions due to their particular physicochemical characteristics as well as their interaction with other milk constituents [4–6].

Foaming properties of milk proteins are attributed to their ability to:

1. absorb at the air-water interface leading to a rapid decrease of surface tension at the air-water interface

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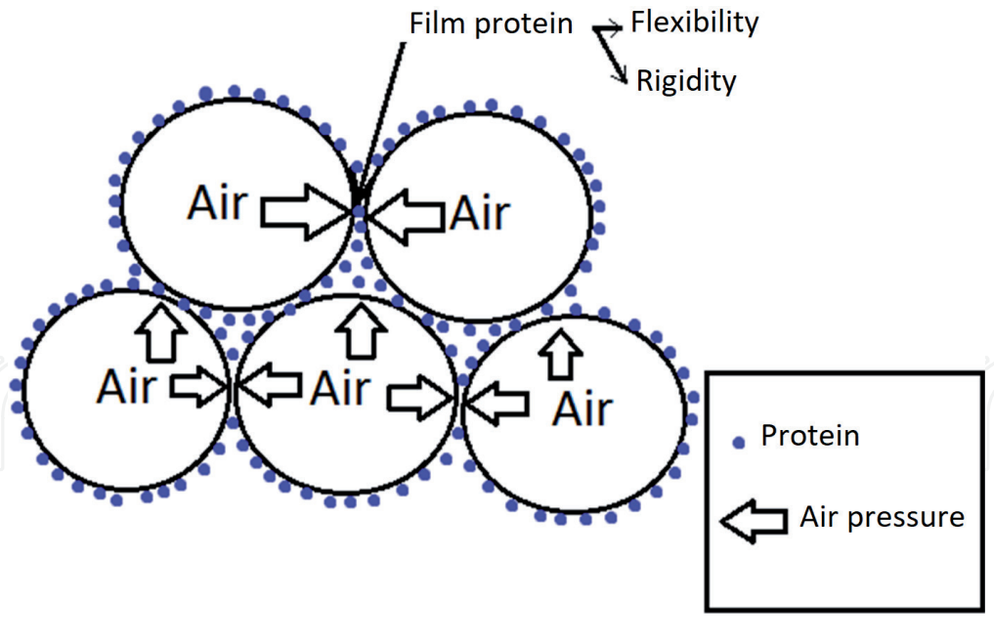


Figure 1.
Schematic presentation of protein based foam.

2. unfold at the interface with orientation of hydrophilic and hydrophobic groups of proteins at the aqueous and non-aqueous phases, respectively

3. form an interfacial film protein by using the interactions of partially denatured proteins to stabilize the created foam [7].

According to their structure and surface rheological properties, milk proteins can be classified in two main groups [8–10] flexible and globular proteins:

- Flexible caseins including proteins α_{S1}-, α_{S2}-, β- and κ-casein as well as the mixtures of calcium caseinates, sodium caseinates and acid caseins. They are flexible and have no tertiary structure.

- Globular proteins including β-lactoglobulin, α-lactalbumin, wheys obtained after cheese making as sweet and acid wheys. Overall, these proteins contain tertiary structure contrary to caseins, they are stabilized by disulfide bridges and preserve their globular molecular shape even after adsorption on the interface.

All milk proteins (β-casein, α-casein, κ-casein, β-lactoglobulin and α-lactalbumin) compete to the interface as follows: proteins with a more flexible structure such as β-casein are quickly adsorbed, whereas, globular proteins adsorb slowly [11]. Hence, the β-casein causes the creation of the foam due to its disordered structure. Indeed, it is considered as a "mobile" protein with an intrinsically unstructured molecular structure [12]. On the other hand, despite the low adsorption of whey globular proteins (β-lactoglobulin and α-lactalbumin), they intensively contribute to the formation of the protein film by improving its rigidity [13]. The order of the foaming efficiency of the insoluble and soluble protein fractions respectively of cow's milk is as follows: β casein> α casein = κ casein> whole casein, β-lactoglobulin> α-lactalbumin> whey [14].

Finally, whey globular proteins are characterized by a lower ability to adsorb at interfaces than those of caseins. On the other hand, their compact structure stabilized by the disulfide bridges, makes them suitable for creating a rigid interfacial protein film and consequently a higher ability to stabilize foams [8, 15].

The foamability of purified whey proteins is higher than that of the whole extracted whey. The β-lactoglobulin is the predominant adsorbed protein on the interface, regardless of its concentration ratio with α-lactalbumin [14]. At pH 6.7, this protein exists as dimers which are maintained by non-covalent interactions. Each monomer is characterized by two intramolecular disulfide bridges and a free thiol group. Upon adsorption at the interface, the β-lactoglobulin is not fully unfolded and its rate of lowering interfacial tension is slower compared to that of β-casein. However, once adsorbed, the created protein film of β-lactoglobulin is distinguished by a high density and an important protein–protein interaction in comparison with the protein layers of caseins. Indeed, the partial unfolding of β-lactoglobulin during its adsorption at the interface leads to the exposure of its free thiol group. Consequently, the adsorbed protein undergoes slow polymerization which is explained by the exchange between free thiol groups and disulfide bridges between the adsorbed β-lactoglobulin dimers [12, 16].

The purified β-lactoglobulin showed a better tensioactivity compared to other whey proteins such as the α-lactalbumin [17]. The β-lactoglobulin is characterized by significant foaming and stabilizing properties due to its high hydrophobicity and its unstructured conformation. On the other hand, the α-lactalbumin has interesting foaming properties but a low foaming stability [18]. This behavior is attributed to the compact globular structure of α-lactalbumin and the presence of four buried disulfide bridges which reduce its flexibility, and therefore its foaming and emulsifying properties [19].

Brooker et al. [20] showed that the main constituents of the milk foam interface are β-casein, β-lactoglobulin and α-lactalbumin. Other studies have shown that the stability of milk froth increases with increasing β-casein content [4, 10, 21, 22]. Indeed, during the creation of dairy foams, the β-casein is first adsorbed protein on the interface with a faster diffusion than that of globular whey proteins [23]. Thus, β-casein, once injected into a casein solution, is even able of moving other caseins such as α_{S1}-casein and β-lactoglobulin from the interface, while the reverse phenomenon is difficult to achieve (**Figure 2**) [25].

Thus, β-casein plays the key role in the stabilization of the foam due to its well-structured molecular conformation. It is even able to dissociate the α_{S1}-β complexes releasing the α_{S1}-casein and β-casein monomers. This behavior can be observed only at pH levels above 6, indeed at a pH close to 4.5, the solubility dominates foaming properties of caseins regardless of pH value [17].

Bovine proteins mixtures (β-casein-β-lactoglobulin and β-casein-α-lactalbumin) at different mixture ratios (100,0, 75:25; 50:50; 25:75; 0:100) presented an intermediate foaming behavior between those of pure β-casein and globular proteins alone (α-lactalbumin or β-lactoglobulin): the added β-casein increased significantly the foaming capacity value of protein solution. For β-casein-α-lactalbumin mixture, an increase of β-casein proportion from 25–75% of total protein amount, significantly increased foamability of 41%. For β-casein-β-lactoglobulin protein mixture, the foamability of the mixed systems was mainly dominated by β-casein. For instance, foaming capacity increased of 46.2% between pure β-lactoglobulin and the mixture

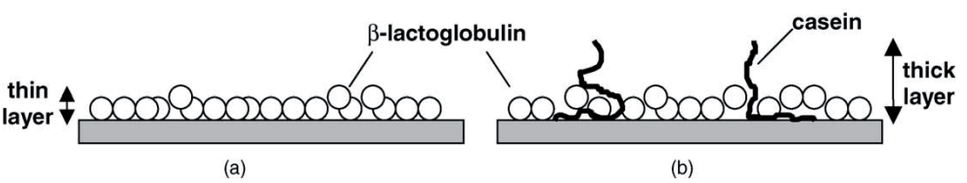


Figure 2.
The incorporation of casein in the structure of the β-lactoglobulin adsorbed layer; (a) monolayer of β-lactoglobulin; (b) incorporation of caseins into the β-lactoglobulin layer [24].

containing 50% of β-casein and 50% of β-lactoglobulin [10]. On the other hand, the foam stability is mainly governed by the β-casein regardless of the other mixed protein (β-lactoglobulin or α-lactalbumin). Indeed, the increase in the stability of foams is attributed to an increase in the diffusion and adsorption of milk proteins at the air-water interface [10, 26]. In the same way, Xiong et al. [27] studied foaming properties of caseins: whey proteins mixture at different ratios (80:20–75:25 and 80:20–40:60). These authors found that proteins at a ratio of 40:60 exhibited the lowest foam stability compared to that of 80: 20 sample because of the adsorption and spreading behavior of micellar caseins at the air-water interface, whereas, samples with ratios 80:20 and 75:25 did not show any significant difference in foaming properties [27].

Laleye et al. [28] reported that the milk origin and consequently the protein composition of whey have a great influence on its foaming and emulsifying properties. For instance, bovine and camel whey presented different foaming properties, which is attributed to the difference in protein composition of both wheys especially the absence of β-lactoglobulin in camel milk.

2.1.1 Effect of pH on foaming properties

Milk proteins molecules change their conformation and surface activity depending on pH level. Hence, foaming and interfacial properties also change depending on the physicochemical parameters of proteins [8]. For instance, foaming properties of skimmed milk decrease considerably at acidic pH (pH 4–5) because of caseins precipitation. However, these properties increase at pH 3 due to the dissociation of the casein micelles and the re-solubilized caseins characterized by a higher tensioactivity [23].

Surface properties of caseinates are predominantly determined by the β-casein regardless of pH value. Furthermore, surface pressure isotherms of caseinates were nearly identical to those of pure β-casein. Hence, caseinates adsorption layers were modeled by treating them as β-casein ones [8, 11]. The β-casein polypeptide is constituted of 209 amino acid residues; the first 50 are mainly hydrophilic, while the remaining 159 residues are mainly hydrophobic [29].

Neutron reflectivity studies [9, 30] have shown that the adsorbed β-casein layer can be represented as a dense inner layer adjacent to the interface with a thickness of 1–2.5 nm and another less dense outer layer released in the aqueous phase 3–7.5 nm in length. The inner layer includes the hydrophobic amino acids in a "train" configuration, while the outer layer is extended as a "tail" or "loop" constituting of hydrophilic amino acids. These data were used by Marinova et al. [8] in order to schematize sodium caseinates adsorption behavior at the air-water interface (**Figure 3a**). By reducing the pH to the pI (Isoelectric pH) of β-casein, the hydrophilic residues are electrically neutral at this pH value resulting a decrease the thickness of the protein layer (**Figure 3a**). Consequently, the decrease in the foaming properties of β-casein is caused by the precipitation proteins leading to a lower protein coverage of interface and a reduced electrostatic repulsion between protein films [8].

Unlike the foaming and interfacial properties of sodium caseinates, whey foams more at a pH levels close to the pI of β-lactoglobulin (pI = 5.2) and α-lactalbumin (pI = 4.1–4.8). At this pH value, the foam created by whey is more stable than that at neutral pH due to the reduced negative charge and electrostatic repulsion of proteins [5, 8, 23]. The modeling of whey protein adsorption layers is not realized by the major protein alone (β-lactoglobulin) as observed for sodium caseinates. Marinova et al. [8] represented the adsorbed layer of the whey protein mixture by an "average" of globular proteins which adsorb almost intact at the interface. At neutral pH, the molecule is negatively charged and electrostatic repulsions prevent the formation of a dense and continuous protein adsorption layer.

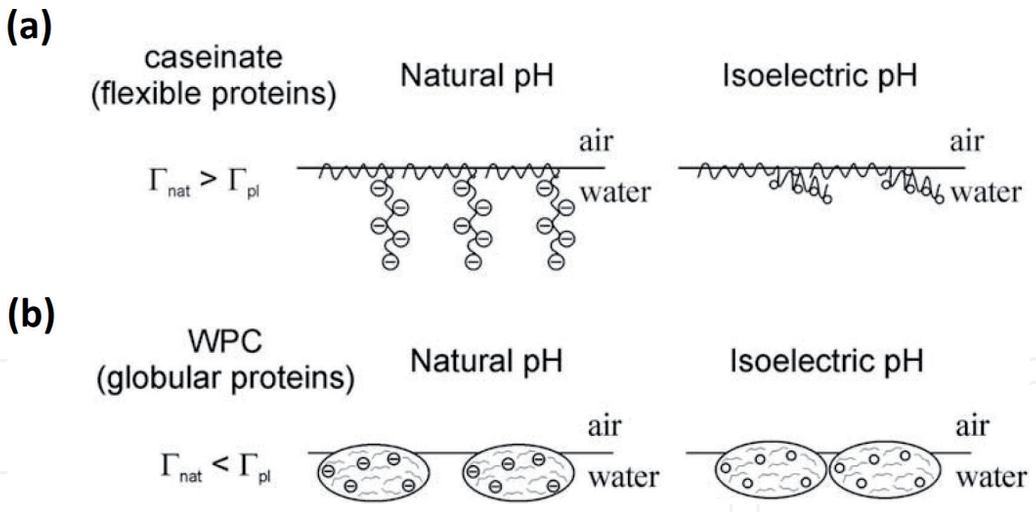


Figure 3.
Schematic presentation of caseinates (a) and WPC (whey protein concentrate) (b) at air-water surface at neutral pH (~7) and isoelectric pH (~4.5) [8].

However, in acidic conditions, the molecules are not charged and their adsorption and interaction are much higher (**Figure 3b**).

At pH 6,7, Lajnaf et al. [15] showed that the adsorbed protein layer of whey at the air-water interface consists of the β-lactoglobulin, while at pH 4.6, the adsorbed protein layer consists of the α-lactalbumin which is the most surface active protein in whey in acidic conditions. Indeed, the α-lactalbumin loses its bound calcium ion at pH values less than 5 and takes on the molten globular state and hence, becomes more surface active. However, the β-lactoglobulin is more rigid and thermodynamically stable at low pH levels leading to a less competitive adsorption of the protein in acidic conditions [23, 30–32].

2.1.2 Effect of temperature on foaming properties

Temperature is a very important parameter which affects the conformation of milk proteins and their distribution between both of whey and the colloidal phases of milk [33]. Therefore, temperature affects the molecular structure and foaming properties of milk proteins [33, 34].

Foaming properties of milk are significantly enhanced by increasing the temperature from 45–85°C, whereas stabilizing foam ability are maximum at 45°C [35]. After heating at 50°C, transmission electron microscopic observations shows that the film protein at the air-water interface consists mainly of the soluble caseins as well as whey proteins [4].

Overall, the denaturation of milk proteins after thermal treatments improves their foaming and interfacial properties due to their increased molecular flexibility, as well as their surface hydrophobicity [36]. However, foaming behavior heated milk proteins usually depends on the rate of protein aggregation. Denaturated and unaggregated proteins adsorb faster at the interface than aggregates, leading to the creation of foam. On the other hand, the adsorption of aggregates is slower, whereas, they contribute to the stability of the created foam (**Figure 4**) [37, 38].

Furthermore, greater foaming and stabilizing properties was measured for bovine milk proteins after increasing the temperature of thermal treatments, (up to 90°C for 30 min). This behavior was linked to the heat denaturation and aggregation of milk proteins especially globular whey proteins (β-lactoglobulin and α-lactalbumin), which led to an increase in the surface hydrophobicity and a decrease in the electronegative charge and interfacial tension [39].

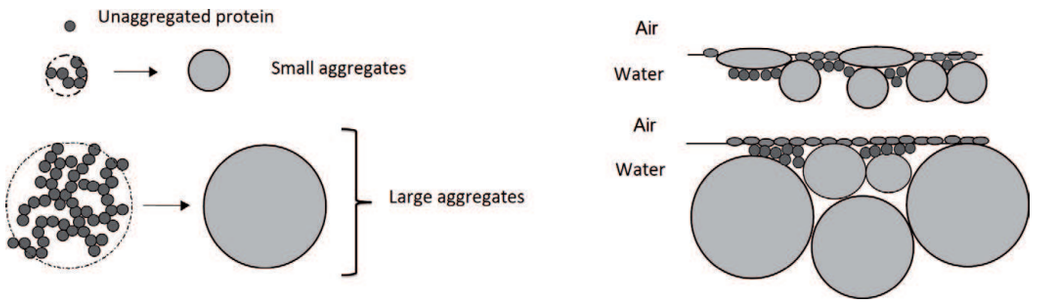


Figure 4.
Schematic representation of milk protein adsorbed layers adsorbed at the air-water interface by mixing unaggregated proteins and aggregates that within a heat treatment [38].

Similarly, whey proteins improve their foaming and stabilizing properties after heating process. However, the excessive heating denaturation of leads to a reduction of the resulted foam volume (for instance: 85°C for 750 s). Heating improves the tensioactive properties of α-lactalbumin and β-lactoglobulin by the exposure of the buried hydrophobic molecular parts of proteins leading to an improvement in their foaming and emulsifying properties [14].

2.2 Emulsifying properties

Emulsification is a common operation in food industry which is encountered with various food products such as mayonnaise sauces, soft drinks, salad dressings, soups, creams, butter and margarine [40]. Overall, an emulsion is obtained by mixing two immiscible liquids in the presence of one or more emulsifiers, where one is finely dispersed as droplets within another as oil in water emulsions (**Figure 5**) [16, 41]. During homogenization, emulsifiers are adsorbed onto the interfaces of freshly formed oil droplets leading to the reduction of the interfacial tension and oil droplets disruption. The most common emulsifiers used in the food industry are proteins which are the most surface-active agents in formulated emulsion systems [42].

During emulsion creation, mechanical shear is induced to create oil droplets within a continuous aqueous phase. Proteins dissolved onto this phase migrate to

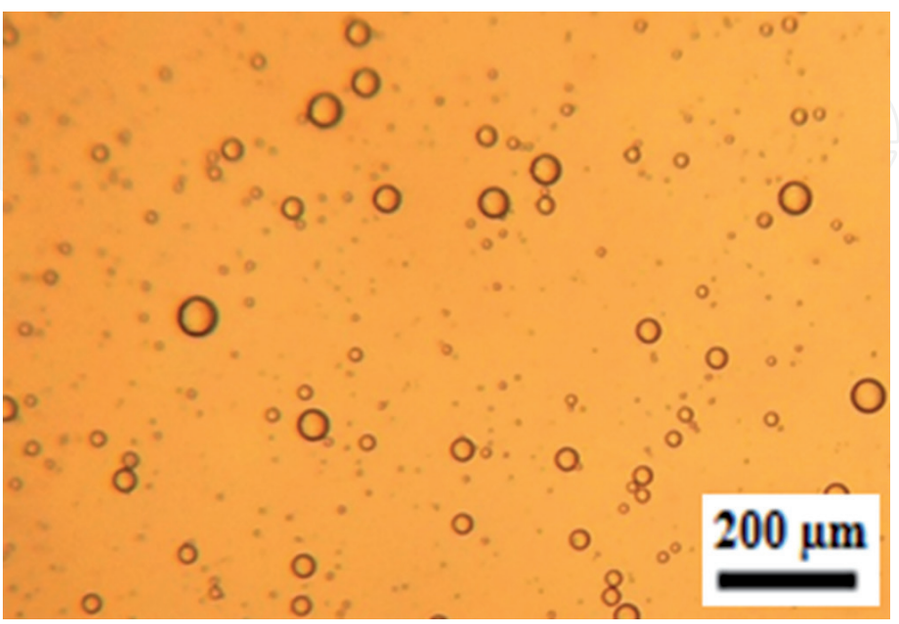


Figure 5.
Microscopic images of oil-in-water emulsions (85%) stabilized by whey protein isolate emulsion. The emulsion is diluted in a solution of SDS 0.1%.

the interface, and then realign to position its hydrophilic and hydrophobic amino acids towards water and oil phases, respectively. Once adsorbed, proteins accumulate to form a viscoelastic film around the created oil droplet and to keep the emulsion stable [41, 43].

Caseins are well known by their ability to adsorb rapidly at the oil–water interface, they are more effective in decreasing the interfacial tension than whey proteins. Furthermore, all casein types are adsorbed at the surface of oil droplet to provide stability to the resultant emulsion against coalescence and flocculation [31, 44]. Previous works evidenced that the diffusion and reorientation of β-casein at the interface occurs more rapidly than β-lactoglobulin and α-lactalbumin due to the low structuring of β-casein. Indeed, the β-casein is a flexible protein characterized by an amphiphilic nature allowing it to be the most effective in reducing surface tension at the oil–water when compared to β-lactoglobulin and even whole milk [12]. The β-casein is considered as a "disordered mobile protein" due to the low structuring molecular conformation and its rapid diffusion at the oil–water interface. It can occupy the majority of interfacial sites leading to a complete or partial replacement of the β-lactoglobulin molecules from the interface [45]. Seta et al. [45] noted that the protein mixtures containing different proportions of β-lactoglobulin and β-casein (1:3, 1:1 and 3:1) at pH 6.8 had an interfacial behavior similar to that of pure β-casein, suggesting the dominance of β-casein at the oil–water interface. Assessment of *in vitro* digestibility of milk protein isolate showed reduced emulsion stability compared with the intact proteins emulsions. Emulsion instability was hydrolytic enzyme preparation dependent and increased with increasing the degree of hydrolysis for a given enzyme [46].

2.2.1 Effect of pH on emulsifying properties

Emulsifying properties of milk proteins change significantly depending on pH level of proteins [41]. Mellema and Isenbart [47] studied the effect of acidification of a solution of reconstituted skim milk powder and whey protein on their interfacial properties (at a concentration of 0.7% (w/w)). These authors found that at pH 4.6, acidified casein micelles lose their colloidal stability, they aggregate and become less amphiphilic and tensioactive. Unlike the foaming and interfacial properties of sodium caseinates, whey proteins improve their flexibility when lowering pH level from 6.7 to 4.6. The dominant whey protein at the oil–water interface in acidic conditions is the α-lactalbumin: this protein adsorbs slowly at the interface but gives a high viscoelastic modulus [47]. The β-casein coated and stabilized the oil-droplets better at pH levels above neutrality when compared to acidic conditions. However, emulsions made camel β-casein at pH ~ 5 were unstable leading to significantly bigger oil droplets. Indeed, the acidification of caseins usually leads to the decrease in emulsion activity and stability because of precipitation and aggregation which alter their amphiphilic nature [42].

For whey proteins, Kilian et al. [48] compared their emulsifying behavior in both pH values 5.7 and 7.0. These authors reported that the emulsion was more stable in pH 5.7 than that at pH 7.0 with lower diameter droplets. However, the interfacial film formed by the proteins presented an essentially elastic behavior in both pH values with no significant differences in the resistance parameters of the oil–water layer interface [48]. Lam and Nickerson [19, 41] found that EAI (Emulsion Activity Index) as well as ESI (Emulsion Stability Index) values of whey protein isolate and the pure α-lactalbumin declined when pH increased from pH ~ 3 to pH ~ 5, before increasing at pH ~ 7. Stability of emulsions depends on the charge of the proteins: a higher stability is observed under conditions where electrostatic repulsion occurs. Indeed, electrostatic repulsion aided in keeping droplets from

flocculating. However, this behavior was less effective for neutrally charged protein near its pI [19]. Emulsifying properties of whey protein aggregates were also investigated. These fabricated aggregates (native, nanoparticles, and nanofibrils) showed significant emulsifying properties at pH 3 especially for whey nanofibrils. However, whey proteins nanoparticles had the highest EAI and ESI values at neutral pH [49].

The results of Lajnaf et al. [50] indicated that the α-lactalbumin molecules at neutral pH coated the oil-droplets better than those in acidic conditions with higher EAI values of apo bovine α-lactalbumin proteins (without calcium). Furthermore, ESI values of both apo and holo (with calcium) states of the α-lactalbumin were higher at pH ~ 7 than those at pH ~ 5. This behavior was explained by the electrostatic repulsive forces of the α-lactalbumin far from its pI which led to a better adsorption of the protein to the oil-droplet surface [50–52].

2.2.2 Effect of heating temperature on emulsifying properties

Structure–function relationships of heated milk proteins has been widely studied in the literature, especially as it relates to their aggregative properties after heating and nature of interactions (thiol-disulfide exchange reactions, hydrophobic interactions, and electrostatic interactions hydrogen bonding) [19, 39, 41, 53, 54]. These interactions can even alter the physicochemical and emulsifying properties of milk proteins molecules by heating the proteins to a partial or complete denaturation of the protein structure and to expose buried hydrophobic moieties [41].

The surface protein coverage of emulsions created with heated calcium caseinates solutions at 121°C for 15 min was higher compared to that of native caseinates. This behavior was attributed to protein aggregation upon heating and to the higher viscosity of the aqueous phase. On the other hand, milk proteins heating induces the increase in emulsion stability due to an increase in the diffusion and adsorption velocity of milk proteins at the interface and a decreased apparent viscosity [26, 44]. On the other hand, the emulsifying properties of whey protein were strongly associated with the size of generated thermo-induced aggregates [41]. For instance, heated whey proteins at 85°C and at pH 7 exhibited lower emulsifying compared to those heated at 55°C and 25°C. The difference in the size of the aggregates as a function of temperature: larger aggregates are usually obtained after heating at a higher temperature. Furthermore, Lam and Nickerson [41] found that EAI values of whey protein isolate were greater at both pH 3 and 7 since protein aggregates are smaller and the

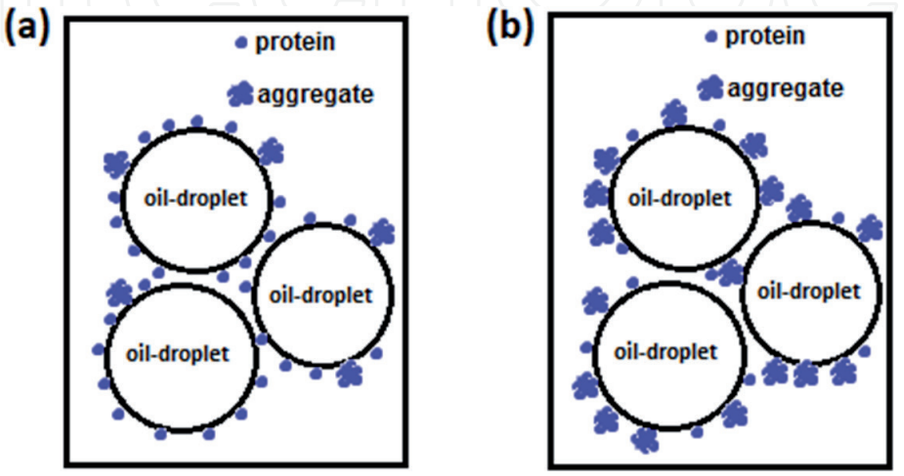


Figure 6.
Schematic presentation of whey protein based emulsion after a thermal treatment of proteins at neutral pH (a) and in acidic conditions (b).

hydrodynamic radii of the generated aggregates are lower leading to a rapid migration and integration of heated proteins into the interface (**Figure 6a**). In contrast, protein–protein aggregation was the highest after heating whey proteins at acid pH resulting a reduction in their EAI values. Indeed, the aggregation and hydrodynamic radii of the whey protein aggregates were highest in these conditions because of the reduction in electrostatic repulsion between heated proteins close to their pI (**Figure 6b**).

For pure whey proteins, the applied heat treatment to the α-lactalbumin at 65°C improves its stability to create and stabilize emulsions when compared to the unheated α-lactalbumin. However, increasing the temperature of the heat treatment from 65–95°C for 30 min leads to in reduction in its emulsifying stability because of the excessive denaturation of this protein [19].

3. Biological activities of milk proteins

3.1 Antioxidant activities

Overall, proteins have antioxidant activity through some amino acid residues such as cysteine, methionine and tryptophan. Indeed, these residues are involved in free radical-scavenging as they possess the highest antioxidant activity compared to the other amino acids [55]. Hence, the amino acid composition of proteins, their positioning and their accessibility are important in scavenging the free radicals [56].

Lactoferrin, representing between 1 and 2% of the total whey proteins, is characterized by its exceptional antioxidant capacity especially its ability to scavenge free radicals due to its sulfur-containing amino acids in its structure and the chelation of transition metals [57]. Native α-lactalbumin also exhibited significant antioxidant activities with respect to Ferric-reducing (FRAP), iron chelating and antiradical activities in both apo and holo forms with higher antioxidant activities for the apo form due to the greater exposure of antioxidant amino acids after calcium depletion [50]. Previous works indicated that caseins exhibited also important antioxidant activities. For instance, the β-casein samples showed significant iron chelating and antiradical activities depending on the protein concentration (0.1, 1 and 5 g/l) which could be explained by the higher content of antioxidant amino-acid residues in the β-casein protein [42].

Peptides generated from the enzymatic digestion of milk proteins are reported to have significant bioactivities such as antioxidant, antihypertensive, antidiabetic, immunomodulatory, antimicrobial, opioid properties. Indeed, peptides can be released through in vitro enzymatic hydrolysis, in vivo digestion approaches and fermentation, alone or in combination [58]. Antioxidant activities of native and hydrolyzed whey protein isolate were studied and compared to those of the major individual whey proteins (β-lactoglobulin, α-lactalbumin, serum albumin and lactoferrin) [59]. Antioxidant activities of whey proteins were significantly increased after enzymatic digestion compared with native proteins. The α-lactalbumin showed the highest FRAP (8.19 ± 1.19 μmol of Trolox equivalent/g) and ABTS free radical-scavenging activity (20.97 ± 1.44%) when compared of the other tested whey proteins with the release of the highest amount of the antioxidant peptides. These results lead to prefer the α-lactalbumin in food formulations to boost antioxidant defenses [59]. Investigations revealed that "Corolase PP", a commercial complex mixture of enzymes is the most appropriate enzyme in obtaining antioxidant hydrolysates from the pure α-lactalbumin [60]. The enzymatic hydrolysis of α-lactalbumin revealed a peptide having an IC50 inhibition value of 143 of superoxide radical-scavenging. This peptide was separated through a Sephadex G-200

column within a size-exclusion chromatography after peptic hydrolysis of whey filtrate [61, 62].

3.2 Antimicrobial activities

Except lactoferrin, the major milk proteins do not exhibit any antimicrobial activity in their native state even at high concentrations [63, 64].

Indeed, lactoferrin belongs to the protein family of transferrin family. It presented an activity against a wide spectrum of pathogenic microorganisms for humans. For instance, lactoferrin exerts a bacteriostatic and bactericidal effect on various Gram-negative bacteria such as *E. coli*, *Salmonella*, *Ps. aeuruginosa*, *H. pylori*, *Enterobacter*, *Yersinia*, *Porphyromonas gingivalis* and *Klebsiella pneumoniae*. Besides, this protein had antibacterial activities against Gram-positive bacteria such as *Listeria monocytogenes*, *Bacillus* and *S. aureus* [65]. Likewise, lactoferrin has been used against different yeasts such as *C. dubliniensis*, *C. albicans*, *C. glabrata*, and *Cryptococcus*, in synergy with different antifungal drugs [66]. In this context, several mechanisms of action of lactoferrin have been demonstrated against bacteria, fungi, parasites and viruses, including possible activity against the novel coronavirus SARS-CoV-2 infection [67].

On the contrary, pure β-casein and α-lactalbumin (apo and holo forms) had no bactericidal activity against *Escherichia coli, Staphylococcus aureus Enterococcus faecalis* and *Pseudomonas aeruginosa*. Furthermore, these proteins had no antifungal activity against, *Aspergillus tamarii, Aspergillus sclerotiorum Aspergillus protuberus* and *Penicillium bilaiae* even at a concentration of 5 g/l [42, 50]. However, pure proteins of milk from other mammalian species as goat and camel exhibited significant antimicrobial activities. For instance, apo camel α-lactalbumin showed moderate antimicrobial activities towards *Pseudomonas aeruginosa, Penicillium bilaiae, Aspergillus tamari* and *Aspergillus sclerotiorum* [50]. Furthermore, camel β-casein had strong antifungal activities against *Aspergillus tamarii* and *Aspergillus sclerotiorum* [42]. Meanwhile, the α_{S2}-casein from goat milk had antimicrobial effects against Gram-positive and Gram-negative bacteria, including *Bacillus cereus Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhi*, *Staphylococcus aureus*, and *Shigella flexneri* [68].

The same trends were reported for native caseins which exhibited no antimicrobial activity: caseins just release bioactive peptides after digestion presenting these activities [69]. Once these peptides are released, they can act as regulatory compounds in the host organism with specific biological activities such as antioxidant and antimicrobial activities [70]. Similarly, four peptide fragments were yielded after a proteolytic digestion of the β-lactoglobulin by trypsin. These peptides exerted bactericidal activity against Gram-positive bacteria only [71]. However, generated peptides of β-lactoglobulin through the action of other enzymes such as alcalase, pepsin or trypsin, have been shown to be bacteriostatic against pathogenic strains of *E. coli*, *Bacillus subtilis* and *Staphylococcus aureus* [63].

On the other hand, previous works noted that the trypsin enzymatic treatment of α-lactalbumin led to the release of peptides with antibacterial activities. However, only one antibacterial peptide was generated after a treatment using the chymotrypsin enzyme. These peptides are known by their activity against Gram-positive bacteria, whereas, weaker effects were detected with Gram-negative bacteria. Overall, the peptides obtained from the α-lactalbumin after pepsin or trypsin treatments inhibited the growth of *E. coli*. However, pepsin treatment did not release any antibacterial peptides from the α-lactalbumin [63, 72].

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