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Preparation, properties and behaviour of protein microparticles in salt containing dispersions

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Abstract

This review focuses on the preparation of protein microparticles, considering especially whey proteins. Different production methods, such as heat-induced and cold-induced, could yield different characteristics of particles from each other. Behavior of microparticles discussed at different sizes of particles, at different temperature of the system, at different pH and salt conditions. Therefore functional, colloidal and rheological behaviors of dispersions including protein microparticles were also addressed. Finally, possible use areas of protein microparticles were explained.

Introduction

Microparticulation is a way of structuring different types of proteins in terms of their size, pH, concentration or surface properties. Protein microparticles can be used for emulsifying, foaming, texturizing, adding functionality or controlled delivery systems [1]. Although there are many different types of proteins that can be used in microparticulation, dairy proteins such as caseins and whey proteins were commonly used and investigated in literature [2,3]. This review mainly focuses on the properties of milk proteins at different environmental conditions such as pH or salt concentration.

Microparticulation includes mainly the gelation of proteins in a confined geometry via different routes. In the first part of this review, different ways of protein particle formation will be discussed. In the second part mechanisms of particle formation will be explained considering the water content of particles. For example, physical and chemical differences at a pH value of close to pI or away from the pI during the production of protein particles will be explained. Colloidal behavior of protein particles in dispersions or at interfaces, especially when the salt is present will be explained in third part. Similarly, rheological behavior of protein particles in the presence of salt at different conditions will be the subject of fourth part. In the last part, current situation of microparticulated proteins in food industry and possible applications will be explained.

Preparation of microparticulated proteins

Aggregation or gelation of proteins is used to make protein particles to have a better control over the physicochemical properties. The gelation of whey proteins mainly includes an unfolding step, which allows the reactive groups of amino acids to rearrange. After this step, peptide chains interact through hydrophobic interactions, hydrogen bonding, electrostatic interactions, disulfide bridges and/or van der Waals interactions depending on the applied process [4]. As a result, rearrangement of proteins occurs, and the gel is formed.

Heat-induced gelation

Heat-induced gelation of proteins is the most commonly used method to make particles from globular proteins, especially from whey proteins [5-8]. Whey protein, a mixture of beta-lactoglobulin, alpha-lactalbumin, bovine serum albumin and immunoglobulins, has denaturation temperature around 60°C, above which the reactive groups of amino acids become free and make the reformation possible via hydrophobic interactions, hydrogen bonding and disulfide bridges [6]. As disulfide bonds are mostly responsible for irreversible aggregation; stable aggregates can be formed. The size of aggregates, which can indeed be called as particles, depends on the heating time and temperature [9].

A protein-based fat substitute, known as Simplesse, was explained to be produced by an internationally patented process called "microparticulation", including heating and blending process at the same time. Milk and/or egg proteins were used to create 0.1-2.0 μm sized particle gels. This size range of particles does not create grittiness perception after processing or swelling of the particles. Simplesse was used in several deserts and dairy products as a fat substitute, so the perceived creaminess was an important parameter [10].

In another study, Zhang and Zhong [3] used native whey proteins to make nm-sized particles which have better heat stability than the native protein. In their method, a native protein solution dispersed in an oil phase including surfactant, by adding the protein solution drop wise into the oil phase. After stirring for a while on a magnetic stirrer, emulsion was heated to 90°C, above the denaturation temperature of whey proteins (60°C), for 20 min. At this step, proteins created a network inside the water phase in emulsion; irreversible aggregation occurred, and therefore hard particles were being formed. Heat treatment was followed by a cooling step and then particles were obtained using centrifuge. To remove the remnants of oil, washing step with ethanol was applied before further analyzing the particles. The

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average diameter of produced particles was below 100 nm; therefore, a transparent dispersion could be obtained.

Cold-induced gelation

Cold-induced gelation mostly include electrostatic interactions between the proteins and generally used as a combination of heating step. In cold set gelation, aggregate formation can be achieved via different methods such as acidification [2,11,12], salt addition [9,13-16], or enzymatic crosslinking [17-20]. Heating step may be done before or after the aggregate formation to gel the particles and make them insoluble [8]. For instance, Schmitt and co-workers [8] induced the aggregate formation from whey proteins by changing the pH and then these aggregates were gelled by heating. Alternatively, Alting and co-workers [21] used a preheating step to create soluble β-lactoglobulin aggregates, and then gelled these aggregates by acidification.

Proteins have a net charge, which keeps them separate via electrostatic repulsion, unless they are at the iso-electric point (pI). At the pI, overall charge of the proteins is zero, therefore there is no repulsion between proteins. When the repulsive force is decreased or totally inhibited by bringing the pH close to the pI, proteins start to form aggregates. For most of the proteins, pI is reached by acidification. Acidification can be done using either acid solutions [2,11] or acidifying agents, such as glucono-δ-lactone (GDL) [22,23]. For instance, sodium caseinate particles were obtained in a two-step emulsification process using GDL as acidifying agent [24]. However, as the electrostatic forces were the main forces for particle formation, NaCas particles were stable only in a narrow range of pH. For whey protein particle formation at lower pH values (pH 3-5) than pI, rod-like particles were obtained [25], whereas at higher pH values it was possible to form spherical particles [26].

Enzymatic cross-linking of proteins is an alternative method for cold-set gelation of proteins. Most commonly used cross-linking enzyme is transglutaminase that functions through an acyl transfer mechanism in proteins involving a glutamine residue acid as an acyl donor and a lysine residue as an acyl acceptor [17,19,27].

Factors affecting the functional properties of microparticulated proteins

Particle formation depends on many different parameters, such as protein source or gelation mechanism. Therefore, the stability of particles changes against swelling, hydration, or falling apart with respect to different physical and environmental factors will be different. Here, size of the particles, temperature, pH and salt effect on particles are discussed as examples.

Size of particles

In literature, there are many researches on the properties of different sized-particles of biological origin, such as proteins or starch [28,29]. In these studies, particles from a few nm to a few hundred μm were produced via different methods. For instance, in a study on the gelling behavior of yogurts, small protein particles were found to participate in gel network structure more efficiently than the larger ones. The reason was explained as the higher surface reactivity of small protein particles, thereby increasing the interaction potential of the native proteins present. When larger protein particles were used in yogurt formation, a higher syneresis was observed upon storage, which was taken as an indication of no participation of larger particles in network formation [29]. In another study stability of foams and emulsions with different particles was widely studied [28] and reported that the polydispersity and shape of particles had more importance instead of the size of the particles. This phenomenon is actually related to the Pickering effect on the stabilization, which is about the particle-stabilized emulsions or foams.

In Pickering emulsions or foams, the hydrophobicity, contact angle and wettability of particles, which are affected from the chemistry and shape of particles, are important. In addition, irregularity of particle shape was reported to have a positive contribution to stabilize foams and emulsions, most probably due to a good surface coverage [28].

Functional properties of particles such as solubility, gel strength or foaming ability were found to be affected from the particle size; however, particle size was not the only dominant parameter playing role [30]. As most of the time density, shape and/or the surface structure of particles are changing together with their size, interpretation of results as a combination of parameters would be more reliable.

Heat stability of particles

Heat stability of whey protein particles was found to be higher than the native proteins in different studies [3,31]. Native protein solution and particle dispersion were compared to each other by heating them above the denaturation temperature of proteins. Native protein solution formed a gel upon heating, whereas particle dispersion stayed as liquid [31]. This result indicated that protein particles can be good candidates in food products undergoing thermal treatment and in functional high protein containing foods.

Although protein particles have increased heat stability, Sağlam and co-workers [26] showed that the WPI particles produced at pH 6.8 showed swelling upon heating, which indicated the heat instability. At the pH of 6.8, proteins had a net charge during gel formation; therefore, the gel had an open structure. Although the exact mechanism is still not known, these particles were shown to swell upon heating. The reason was attributed to the incomplete denaturation or shuffling of network strands [26], however these were not confirmed.

In another study, water holding capacity (WHC) of whey protein particles about 80 μm in diameter produced via heating a protein solution were investigated [1]. They reported that an increase in WHC of particles upon heating the dispersions at higher temperatures. For instance, heating at 60°C and 90°C yielded higher water holding compared to heating at 30°C. However, heating at 90°C for prolonged times was found not to have a significant effect on WHC of particles. This finding could be related to the degree of denaturation of native proteins, which could also affect the WHC of proteins.

pH of the medium and the pH of the particles

Changing pH of the dispersion medium changes the electrostatic interactions between proteins. Proteins can form denser aggregates at their pI values, as there is no repulsion between them; whereas if the gelation of particles is done away from the pI, then the network structure may be loose due to the repulsive forces between the proteins.

When particle formation was done only by changing the electrostatic interactions, then the particles would be slightly stable over a pH range. For instance, NaCas particles were produced at pH 3.5, which was close to the pI (4.6), and they were only stable between pH 3 and pH 5. The dispersion of these particles was stable even a narrower pH range, as the pI was reached at 4.6. Outside pH 3 and pH 5, particles were falling apart due to strong electrostatic repulsion [24].

In another study, whey protein particles produced at pH 5.5, close to the pI of the proteins (5.1), by heat-induced gelation, were found

to have a better stability over a wider pH range compared to the particles produced at pH 6.8 [26]. As the protein particles produced at the pI had a denser network structure, stability of them against pH change and also heating was higher than the particles produced away from the pI. The gelation mechanism of whey proteins includes hydrophobic interactions and disulfide bonds via heating, which were not taken place in the gelation mechanism of NaCas particles. So, here the stability of WPI particles against changing pH and heating should only be compared between WPI particles prepared at pH 5.5 and pH 6.8.

Salt type and concentration in the medium

Addition of salt changes the net charge of proteins, which is a similar effect with acids; however, the mechanism is different from the pH-induced aggregation. Salt ions screen the charges of proteins, which are responsible for the repulsive forces. As the repulsive forces decrease between proteins, they start to aggregate.

Different salt ions may affect the proteins at different strength. For instance, if the salt ions are monovalent, the screening of charges occurs; whereas if the salt ions are multivalent besides screening, there are additional effects, such as salt bridge formation or specific ion effect [7,32]. Therefore, multivalent ions are more effective in the aggregation of proteins and thus the minimum concentration to induce aggregation is lower than that of monovalent ions [33]. Stability and integrity of the protein particles thus depends not only on the concentration but also on the valence of the ions [34].

In the study of Zhang and Zhong [3], the size of whey protein particles was reported not to be affected by the NaCl concentration (0-400 mM). However, larger WPI particles, a few μm in diameter, were reported to show shrinking with increasing concentrations of NaCl up to 500 mM [24]. The reason was attributed to the screening of charges by salt ions as a result of decreased electrostatic repulsion at increasing ion concentrations. It was also noted that, changing pH of the medium using an acid solution had a stronger influence on the electrostatic interactions between the proteins compared to the changing the ionic strength with monovalent ions.

Another study investigating the hydration properties of casein micelles in salt solutions showed that increasing concentration of salt ions increased the hydration of casein micelles [35]. Casein micelles, without any salt addition, were spherical and around 150 nm in diameter. When salt is added, depending on the valence and concentration of the salt; shape, size and integrity of micelles changed. For instance, addition of $CaCl_2$ increased the micelle size up to 500 nm, probably because of coagulation. As a result of binding of ions at the surface of casein micelles, water molecules also reoriented according to the bound ions, thereby changing the hydration properties. If the coagulation is not extensive and micelles or particles could stay as separate entities, ions may increase the hydration of particles. However, if there is coagulation that decreases the surface area contacting with water, then hydration of particles may not be possible.

Interaction of salt ions with proteins were widely studied, especially in the case of preparation of protein particles via cold gelation [34,36-37], however the study on interaction of salt ions with particulated proteins is limited [38]. Therefore, in this sense the investigation of interaction of salt ions with protein particles, assuming that they behave as hard or soft colloidal particles, may still be an open area.

Colloidal behavior of microparticulated proteins in salt solutions

Most of the time, protein particles behave like colloidal particles and they can be stabilized against coagulation by changing the surface properties. For instance, increasing the electrostatic or steric repulsive forces through changing pH, adding salt, or coating the particles with polymers prevents the approaching of particles to each other, thereby preventing the coagulation [39]. Stability against aggregation or sedimentation and colloidal behavior of non-food grade particles, such as latex or styrene, in the presence of different salts were studied previously [40]. Studies on food grade particles in the presence of salts are still limited due to their unique properties depending on the fabrication method [38]. Food grade particles can be regarded as soft particles, as there is a network structure in a confined geometry. Therefore, density and strength of this network, physical and chemical interactions in the network, surface structure and porosity of the particles are all important for determining the interactions with the environment.

Hard colloidal particles in salt solutions showed different behaviors depending on the salt type and concentration [41]. Here salt type indicates the different valence of cations. For monovalent and divalent salts, screening of charges occurs; for multivalent ions, beyond screening, charge neutralization may occur depending on the ion concentration through adsorption of oppositely charged ions on the surface of the particles. When the concentration of ions is enough to neutralize all the charged patches on the surface of the particles, that is where the critical coagulation concentration is reached; particles start to coagulate as the repulsive forces are diminished.

Soft colloidal particles have different properties than the hard ones. Porous structure and swelling ability of soft particles change the behavior in salt solutions. For example, the maximum volume fraction of the particles for a stable dispersion in the case of soft ones was found to be lower than that in the case of corresponding rigid particles. As a theoretical approach, the reason was attributed to the smaller energy barrier for two interacting colloids, which includes the electrical and van der Waals energy, for the soft particles than that for the rigid particles. Salt ions change the properties of hard particles surfaces, whereas ions may penetrate into the soft particles through the pores on the surface. The strength of gel network inside the particles could vary due to this penetration. Therefore, the colloidal stability of particles changes against swelling, falling apart or shrinking.

Rheological behavior of microparticulated protein dispersions in salt solutions

Soft protein particles are affected from its environment. Salt ions are able to alter the behavior of particles in two different ways. One of them is changing the electrostatic interactions between the particles. In this case protein particles may act as hard colloidal particles and the electrostatic interactions occur at the surface of the particles. In the first case, if the changes in repulsive forces occur at the surface and particles behave as hard particles, a decrease in the repulsive force would yield coagulation of particles. Larger particles are formed, and phase separation can be seen depending on the volume fraction of particles and ionic strength. The other way is changing the interactions inside the particle, which is within the gel network structure. In this case, due to increasing or decreasing repulsive forces, gel network structure is changing. As a result of the change, particles may shrink

or swell, thereby changing the physical properties of particles, such as size, density, or hardness [24]. In both cases, if electrostatic repulsion is high, viscosity of the dispersion tends to increase. When electrostatic repulsion is between the particles, their effective volume fraction would increase in dispersion, which possibly increases the viscosity of the dispersion. Similarly, when electrostatic repulsion is inside the gel network, particle tends to swell, which also increases the viscosity.

The flow behavior of soft hydrogel particles containing NaCas and pectin showed that increasing concentrations of NaCl upto 100 mM decreased the viscosity of dispersions at constant shear rate, whereas NaCl concentrations from 100 mM to 400 mM increased the viscosities [25]. The reason was attributed to the changes in the effective volume fraction of particles, which depended on the porosity and shape of the particles. It was also indicated that higher salt concentrations could lead to thicker sediment layers, which also increased the viscosity of the dispersions. In the same study, hydrogel particle dispersions were reported to show shear-thinning behavior with increasing shear rate values. Alternatively, in another study, whey protein particles at high particle volume fractions were shown to exhibit shear-thickening behavior at high shear rate values, probably because of interaction of particles with co-solvents [24,42].

Use of microparticulated proteins in foods

Structural modifications of protiens, such as microparticulation, can be done either using only protein solutions or as a combination of different ingredients such as proteins and polysaccharides [43]. Such modifications allow particles having different physical or chemical properties, therefore reasons of use such particles in foods vary from increasing protein concentrations to be a fat replacer [38].

One example is the use of whey protein-based particles as a fat replacer in low fat mozzarella cheese [44]. An increase in the melting properties and total moisture content of the mozzarella cheese were also reported when whey protein-based particles were used. Using the same whey protein-based particles as fat replacer, other researchers produced low fat (6%) and fat-free (0.5%) ice creams [45]. According to the sensory panels, low fat ice cream was found to be comparable with the full-fat control sample, whereas fat-free ice cream was scored lower regarding the viscosity, smoothness and mouth coating properties. As a texture modifier whey protein particle were also used in low-fat yogurt manufacture together with native whey proteins [29]. A lower ratio of particles to native proteins resulted in higher creaminess and viscosity values, and lower syneresis.

Whey protein particles prepared via cold set gelation using GDL and via heat set gelation were used in gluten-free bread formulations as a texture enhancer [46]. Particle sizes have a large distribution from 100 nm to 100 μm. Proofing and baking of breads with protein particles indicated promising results in the formulation of gluten-free bread dough.

Another use of proteins is to produce capsules and shell materials for bioactive compounds, thereby creating encapsulated materials for controlled delivery [47]. For instance, at the isoelectric point of proteins, protein shell or protein particles are at their dense form, therefore they can keep the bioactive compound in or inside their structure. When these particles are away from this pH, the repulsive forces in the network of the structure increase depending on the pH and thereby increasing the opening between the strands in network. As a result, the bioactive compound can release from the particle in a controlled way.

As potential applications of protein particles, production of high protein content medical drinks [24] or acidified milk drinks [24] were suggested. As proteins are susceptible to heat applications, such as aggregation, coagulation or gelation, above certain concentrations during manufacture of drinks, it is important to control the texture of the product. Viscosity increase could be detrimental for the selected products. Alternatively, acidification of drinks, milk products like kefir or fruit juices, could contain limited amount of native protein, whereas if proteins are included in particulated form, the concentration of protein in drinks can be increased without changing the textural and sensorial attributes of the product.

Conclusion

In this review, the importance of designed protein particles, their behavior and use areas were addressed. For the preparation of particles, mainly heat-induced or cold-induced methods were used. The combination of both methods was also used for particle preparation. pH, gelling temperature and ionic strength were found to affect the hydration and swelling of particles; whereas size of the soft particles was found less effective on hydration properties compared to the size distribution or shape. The stability of particle dispersions was affected from the ionic strength of the solution. Colloidal and rheological behavior of particle dispersions was affected mostly from the hardness of the particles and the porous structure of the gel network. These physicochemical properties gave flexibility to such particles to be used in foods with different functional properties, such as texturizer, stabilizer or in encapsulation studies. Protein particles were already used in ice cream, yogurt and cheese and have potential to be used in medical and acidified milk drinks in future.

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