

Interactions of Native and Denatured Whey Proteins with Caseins and Polysaccharides

Alev Emine İnce Coşkun^{1*}, Semih Ötleş¹

¹Department of Food Engineering, Ege University, Bornova, İzmir

Article History

Received: 19.09.2019

Accepted: 16.12.2019

Published: 22.05.2020

Review Article

Abstract – In this review, interactions of native or denatured whey proteins with other proteins and polysaccharides were addressed. Chemical structures of whey proteins and caseins as representatives of proteins and of gum Arabic and dextran as representatives of polysaccharides were explained. Whey protein, as a mixture of different proteins, such as beta-lactoglobulin, alpha-lactalbumin, or bovine serum albumin, has a highly complex nature, and therefore, the main interaction occurs within these proteins upon processing. Structure of whey protein includes hydrogen bonds, disulfide bridges and free thiol group, all of which allows whey proteins highly reactive with other polymers. With these properties, whey proteins can be denatured via heating or acidification in a controlled way; and therefore, several functional particles with different sizes and shapes could be obtained. Here we explained the interactions of native and denatured whey proteins with caseins, gum Arabic and dextran in terms of their behavior in solutions or dispersions, their functional and rheological properties. Denaturation process includes mainly hydrophobic interactions and is most of the time irreversible, whereas the complex formation of proteins with polysaccharides includes electrostatic and/or steric interactions and complex formation could be reversible or irreversible depending on the type of application. Such interactions are important for the stability of food materials especially during processing and storage, therefore, a deep insight on this subject is important.

Keywords – Aggregation, dextran, gum Arabic, protein particles, whey proteins

1. Introduction

Food systems, containing proteins, polysaccharides, fats and minerals are highly complex. These components have molecular or mesoscopic interactions with each other; and therefore, these interactions could result in new structural forms in food materials (Gulao, Souza, Andrade, & Garcia-Rojas, 2016). In complex food systems, such interactions of components may raise stability problems during processing or storage. Particularly, in the case of heating of liquid food products, many proteins, such as whey proteins or soy proteins denature above a certain temperature and forms aggregates or gels depending on the concentration of protein (Wang, Zhong, & Hu, 2013). In this review, we mainly considered the interactions of whey proteins with other proteins and polysaccharides. Aggregates, being formed due to the interactions between components, affect the functionality of food product; and therefore, it is important to know the driving mechanism for the destabilization. In this respect, we first address the chemical structures of native whey proteins as the main ingredient of the system; sodium caseinate, gum Arabic and dextran as representatives of interacting protein and polysaccharides.

¹  <https://orcid.org/0000-0002-8952-4913> alevince@gmail.com

²  <https://orcid.org/0000-0003-4571-8764> semih.otles@gmail.com

*Corresponding Author

Whey proteins are the collection of different proteins, including beta-lactoglobulin (BLG), alpha-lactalbumin (ALAC), and bovine serum albumin, which can be naturally obtained from the liquid part of by-product in cheese production. BLG constitutes almost 65% of the globular whey proteins and nearly 0.3% (w/v) of skim milk. Its molecular weight is 18.4 kDa and iso-electric point (IEP) is at pH 5.1 (van den Akker, Schleegeer, Bonn, & Koenderink, 2014; Elzoghby, Elgohary, & Kamel, 2015).

In milk proteins, other than whey, caseins are present and form 80% of the total milk proteins. Caseins form micelles in milk, which make milk a colloidal suspension. In bovine milk, caseins are found in the form of α S1-, α S2-, β -, and κ -; and the main function of all is thought to be nutritional (Thorn et al., 2005). The structure of caseins is complex with the presence of many different amino acids with no regular sequence, however, it is known that caseins have a small amount of secondary or tertiary structure (Horne, 2006). They show amphiphilic characteristics and they can play a role in electrostatic and hydrophobic interactions. The IEP of caseins is around pH 4.6 (Ruis, van Gruijthuijsen, Venema, & van der Linden, 2007). Caseins have hairy structures, called κ -caseins at the outer phase of a micelle, which could also play a role in steric interactions. In milk, particularly when heated or acidified, caseins, whey proteins, and their interaction with each other change the physicochemical properties.

Other than proteins, in many food products, proteins and polysaccharides are found together. We have chosen gum Arabic and dextran as representatives of polysaccharides. The reason for choosing these two polysaccharides is the difference in electrostatic interactions between proteins and polysaccharides. Whey proteins are negatively charged at neutral pH and electrostatic interactions could occur between whey proteins and polysaccharides once the polysaccharide is also charged. Gum Arabic is slightly negatively charged and a highly branched polysaccharide containing an almost 2% of the protein in its structure (Weinbreck, Tromp, & de Kruif, 2004a; Yadav, Igartuburu, Yan, & Nothnagel, 2007). Gum Arabic can be charged via carboxyl groups on the polysaccharide side chains and amino groups on the protein side chains (Ghosh & Bandyopadhyay, 2012). Therefore, pH of the medium can affect the charge of gum Arabic, and thereby the interaction with the other components in the medium through electrostatic forces (Klein, Aserin, Ishai, & Garti, 2010).

Dextran is a linear polysaccharide, made up of purely hydrophilic polysaccharide and has no charge in solutions (Lu, Pérez-Gil, & Tausch, 2009); therefore, with these characteristics, dextran is different from gum Arabic. As being a hydrophilic molecule, dextran is not expected to involve in any hydrophobic interactions with proteins, instead dextran can form hydrogen bonds via hydroxyl groups. In addition, as being a neutral molecule, dextran is not expected to involve any electrostatic interactions with proteins, either. In aqueous solutions, dextran generally does not form a gel; however, it is known that above a concentration of 5% (w/w) dextran has the ability of increasing viscosity of the solution (Carrasco, Chornet, Overend, & Costa, 1989). Therefore, dextran can also be used in texturizing food materials. In addition, under interacting conditions, depending on the molecular weight of dextran, the gelation properties of whey proteins could differ from each other. The interaction of whey proteins and dextran, as a purely hydrophilic polysaccharide, upon heating could result in different rheological and mechanical properties via Maillard reaction (Spotti et al., 2014).

Interactions of native forms of whey proteins and polysaccharides, particularly gum Arabic, could be through hydrophobic and electrostatic interactions. Depending on the strength of these interactions, aggregation of proteins may occur. Other than the native forms, when these polymers are heated together in the same medium, additional interactions such as hydrogen bonding or disulfide bridges could stabilize the new form of the aggregates (Doublier, Garnier, Renard, & Sanchez, 2000). On the other hand, as dextran is neutral, no electrostatic interaction between proteins and dextran is expected.

2. Chemical Properties of Whey Proteins and Formation of Whey Protein Particles

Whey proteins mainly include beta-lactoglobulin (BLG) and show the main chemical properties of BLG. Particle formation with whey proteins is often done through the aggregation of proteins, mainly BLG. Charge dependent aggregation of BLG happens mostly at and around the IEP, whereas these aggregates could dissolve at pH values away from the IEP. In its native form, it is globular and has mostly beta-sheet structures including patterns of hydrogen bonding, and also BLG has 2 disulfide bridges and a free thiol

group, which has the ability to bind to other molecules under certain conditions (Elzoghby et al., 2015). Depending on the pH of the medium, structure of BLG can change from dimers to monomers; for instance, at low pH values (such as at pH 2-3) as there is strong repulsion between the subunits, BLG is in the form of monomers (Elzoghby et al., 2015). In addition, due to its amphiphilic nature, BLG has good emulsifying properties (Sneharani, Karakkat, Singh, & Rao, 2010).

When BLG is heated, it denatures and is able to form several structural units, such as fibrils or globules depending on the pH of the aqueous medium (van den Akker et al., 2014). Monomeric structures of BLG can also be obtained by heating as the heat denaturation of BLG starts with unfolding (Hoffmann & Mill, 1999). Around neutral pH, denaturation of BLG can be described by 3-step process; initiation, propagation and termination steps (Roefs & de Kruif, 1994). Initiation includes reversible reactions of dimers dividing into monomers and irreversible first-order reaction of thiol group becoming reactive. In the propagation step, reactive thiol groups undergo an exchange reaction with disulfide bonds and consecutive reactions result in the formation of aggregates. When all reactive thiol groups interact with each other, denaturation process of BLG stops (Hoffmann & Mill, 1999). During heating above denaturation temperature, which is at 65°C for BLG, the pH of the medium determines the shape of aggregates, which is either fibril-like or globular form. When pH value is low and BLG is mostly in the form of monomers, upon heating fibrils are formed (Serfert et al., 2014); whereas when pH value is high, globular aggregates are formed (Lazidis et al., 2016; Mahmoudi, Axelos, & Riaublanc, 2011).

In whey protein, another important component is ALAC, which has similar physical and chemical properties with BLG. ALAC constitutes almost 25% of whey proteins, has a molecular weight of 14.2 kDa and an IEP of 4.2. It is also globular due to the presence of 4 disulfide bridges in the structure (Kelly, Woonton, & Smithers, 2009; de Kruif, 2012). Bovine serum albumin, another component of whey proteins (8%), has a molecular weight of 66.5 kDa and its IEP is at pH 4.7 (Ge, Kojo, Takahara, & Kajiyama, 1998). With these basic data and considering the complexity of whey proteins, the molecular weight of whey proteins can be calculated as nearly 21 kDa and the IEP is around pH 5.

Particle formation with whey proteins in the presence of different biopolymers can also be done via many different ways. The presence of different biopolymers mainly affects the microstructure of particles.

Particles could be formed through heat-set, cold-set gelation, enzymatic crosslinking or combination of these methods (Lee & Lucey, 2010; Mehalebi, Nicolai, & Durand, 2008; Nivala, Mäkinen, Kruus, Nordlund, & Ercili-Cura, 2017; Zhang & Zhong, 2009). Many globular proteins can be denatured using heat (Mehalebi et al., 2008). As whey proteins contain a high amount of BLG, which is a globular protein, are temperature sensitive and above their denaturation temperature, they form gelled particles. For instance, a well-known fat replacer, Simplesse, is produced through heat set gelation of whey proteins (Singhal, Gupta, & Kulkarni, 1991).

Another gelation and thus particle formation method is cold-set gelation, which mainly includes the change of electrostatic forces between proteins. As proteins have a net charge away from their IEP, they repel each other and could homogeneously disperse in the aqueous phase. Once the repulsive forces between the proteins diminish, they form aggregates, which can be regarded as particles. The change of electrostatic forces can be done through pH change (Lee & Lucey, 2010) or salt addition (Phan-Xuan, Durand, & Nicolai, 2013).

Enzymatic cross-linking of proteins can also be an example of cold-set gelation (Nivala et al., 2017). Transglutaminase is commonly used as a cross-linking enzyme, which transfers the acyl groups between glutamine and lysine residues (Clare & Daubert, 2010; Nivala et al., 2017).

These methods can be combined for a better control over the production of protein particles. Especially, heating is highly common in whey protein studies. In many studies, a preheating step is done for the preparation of soluble aggregate formation (Ako, Nicolai, Durand, & Brotons, 2009; Nicolai, Britten, & Schmitt, 2011) and then the actual gelation can be performed using cold-set gelation or enzymatic cross-linking. Alternatively, when heating is used as the main gelation mechanism, varying electrostatic forces between proteins yield in different shapes of particles. For instance, at low pH values, such as pH 2; whey proteins form fibril-like structures (Serfert et al., 2014) and once they gelled through heating at this pH, hard fibrils could be obtained. Such fibrils had an average diameter from a few nm to a few microns (Rogers, Venema, Sagis, van der Linden, & Donald, 2005; Serfert et al., 2014). The fibrils of whey proteins prepared at pH 2 were used to stabilize and destabilize the colloidal latex dispersions depending on the fibril concentration (Peng,

Kroes-Nijboer, Venema, & van der Linden, 2016). On the other hand, at the IEP of whey proteins, which is around at pH 5, hard and dense whey protein particles could be obtained via different techniques (Lam & Ikeda, 2017; Sağlam, Venema, de Vries, Sagis, & van der Linden, 2011). They generally form spherical aggregates due to the reduced repulsive forces. Increasing the pH above and away from the IEP before gelation of whey proteins may result in softer particle formation due to increased repulsion (Sağlam, Venema, de Vries, van Aelst, & van der Linden, 2012).

As electrostatic interactions are highly important for the structural formation of whey protein aggregates, polymers in the same medium interacting with proteins through electrostatic forces have also an important effect on the structure formation. For instance, in the presence of casein micelles, denaturation kinetics of whey proteins was found to change compared to the absence of caseins (Corredig & Dalglesih, 1996). In addition, in the same study, it was reported that pH and heat treatment duration were found to be important for the aggregation kinetics. Another study showed that the size of casein micelles in the presence of whey proteins changed depending on the pH and heating temperature (Anema & Li, 2003).

In the presence of polysaccharides, proteins and polysaccharide could aggregate together via electrostatic or hydrophobic effects. For instance, when gum Arabic and whey proteins were heated together, it was found that they aggregated together irreversibly and showed precipitation (Loveday, Ye, Anema, & Singh, 2013). Authors reported that the heat stability and dimensions of formed colloidal particles could have varied depending on the pH and heat treatment. Similar gelation of whey proteins together with dextran, in complex or conjugate formation was also reported by others (Spotti et al., 2013). Such complex formation was generally a result of the electrostatic attraction between amine and carboxyl groups (Benichou, Aserin, & Garti, 2002).

When only electrostatic forces were present, particles were often sensitive to pH changes in the medium (Ince Coskun, Sağlam, Venema, van der Linden, & Scholten, 2015). Depending on the pH of the medium, repulsive forces between the proteins could increase, and this situation could lead to falling apart of particles. Therefore, in the cold-set gelation method, where the electrostatic forces are dominant, particles are often stable at and around the IEP of the proteins. On the other hand, when heat-set or enzymatic cross-linking is used, disulfide or hydrogen bonds become dominant, which stabilize the particles over a wider pH-range.

3. Interactions Between Whey Protein Particles and Milk Proteins (Caseins and Native Whey Proteins)

Whey proteins and caseins are found together in many food systems, including milk as the most common one. Therefore, their interactions under different pH and heat treatment conditions were widely studied (Corredig & Dalglesih, 1996). Heating mostly denatured BLG as it has free thiol groups in the structure; however, ALAC and caseins were expected to be more heat stable compared to BLG. As ALAC has 4 disulfide bridges in the structure, it denatures reversibly upon heating around 65°C. In the study of Corredig and Dalglesih (1996), both whey proteins showed similar reactions with caseins upon heating above denaturation temperature.

Heat denaturation of whey proteins includes the aggregate formation via the interaction within whey proteins themselves. Heating of whey proteins alters the solubility characteristics. Below the denaturation temperature, increasing temperatures could increase the solubility of whey proteins, whereas above denaturation temperature, proteins start to aggregate and thereby decreasing the solubility (Pelegri & Gasparetto, 2005). Whey proteins are stabilized by mainly non-covalent interactions, such as hydrogen bonding, hydrophobic and electrostatic interactions; all of which stabilize the secondary and tertiary structure. Upon heating, secondary and tertiary structures of proteins are lost, which means the structures are unfolded. Sulfhydryl groups become available to interact with each other to form disulfide bridges, which increases hydrophobic characteristics of the protein. As a result of the formation of disulfide bridges, hydrophilic groups are reduced, and thereby reducing the water binding and thus aggregation, coagulation and precipitation of proteins happen, consecutively. As disulfide bonds are involved, the aggregation process is accepted as irreversible (Langerdorff et al., 1999). Thus, the formation of aggregates includes the main interaction between whey proteins.

The complex formation between whey proteins and caseins mainly occurs between BLG and κ -casein. When no or mild heating was applied, the interaction between BLG and κ -casein was found to include mainly hydrophobic interactions, whereas when the temperature was increased at and above the denaturation temperature, disulfide bridges were formed (Corredig & Dalglesih, 1996).

In another study, even a small change of pH in the medium was found to be important for the degree of interaction of whey proteins with casein micelles during heating (Anema & Li, 2003). At pH 6.5, whey proteins interaction with casein micelles upon heating was found to be higher than at pH 6.7. They reported that even such a small change of pH during the course of heating affected the size of casein micelles, which altered the association level with whey proteins.

In a different study, the effect of sodium caseinate on the swelling properties of whey protein isolate particles was investigated (Sağlam, Venema, de Vries, Shi, & van der Linden, 2013). In this study, sodium caseinate was added to the medium after the formation of whey protein particles, therefore the interaction of proteins occurred in different states, that is whey proteins were already denatured, whereas sodium caseinate was in its native form. Whey protein particle dispersions at a volume fraction of 0.35 were heated together with sodium caseinate at a concentration of 4% (w/w). Whey protein particles, which were originally in micron-sized in colloidal dispersion, showed an increase in size upon heating above 70°C in the presence of sodium caseinate. In addition, viscosity measurements of the dispersions by using a rheometer showed an increase after heating together with a shear-thickening behavior. These findings indicated lower heat stability of colloidal whey protein particles in interacting conditions with sodium caseinate (Sağlam et al., 2013). The reason of this low stability has still been unclear due to the complex nature of whey proteins and caseins. In addition, denaturation of whey proteins using heat via emulsification method increased the complexity of the system due to the fact that incomplete denaturation might have occurred. In the same study of Sağlam and co-workers (2013), as another protein in the continuous phase of the dispersions, native whey protein isolate was also used. They also found similar results with the case of sodium caseinate upon heating. Particle dispersions showed low heat stability as the size of particles and viscosity of dispersions increased in the presence of native whey protein isolate in the continuous phase. However, as it is known that above denaturation temperature of whey proteins, they form aggregates; it is possible that aggregation could have contributed to the particle size and viscosity increase (Sağlam et al., 2013). The proposed mechanism for the low heat stability of whey protein particles and dispersions was the swelling of particles particularly in the presence of native proteins in the continuous phase. Mechanism of swelling was thought to be either the aggregation of native proteins in the continuous phase upon heating or the reconfiguration of already denatured whey protein particles upon further heating. However, the exact mechanism of swelling has still been unclear. It was also reported that the macro-gels of whey proteins prepared in the same way with micro-particles swelled when there was protein in the continuous phase during heating.

The concentration of sodium caseinate in the continuous phase was also found to affect the swelling ratio of whey protein particles upon heating (Sağlam et al., 2013). When there was a low concentration of sodium caseinate (1% w/w) in the continuous phase, swelling of particles and shear thickening behavior of dispersions were more pronounced compared to when there was high concentration of sodium caseinate (4% w/w). They attributed the reason of this difference to increased osmotic pressure when there was a high concentration of polymer. When considering the osmotic pressure difference, the penetration of biopolymer from the continuous phase into the protein particles should have been taken into account, which requires a further study.

4. Interactions Between Whey Protein Particles and Gum Arabic

Interaction of proteins and polysaccharides may include hydrogen bonding, hydrophobic interactions, solvent interactions and electrostatic forces (Doublier et al., 2000). As a result of these interactions, complex formation could happen. These complexes are generally irreversible and may dissolve or form clusters depending on the pH and molecules present in the medium (Loveday et al., 2013). To stabilize these complexes or aggregates, several gelation methods were used, as already explained in Section 2. It is also known that one of the main factors controlling the aggregate formation is the surface charge density of the formed particles, which is also affected by pH and ionic strength of the medium (Gulao et al., 2016).

Interaction of whey proteins and gum Arabic has extensively studied before (Klein et al., 2010). Gum Arabic could be found in different molecular weights and is composed of mainly arabinogalactan, which has a molecular weight of ~250 kDa, and a small portion of glycoprotein, which has a molecular weight of ~200 kDa. Chemical potentials of interacting whey protein and gum Arabic samples with different molecular weights showed similar values, which was different from the chemical potential of native pure whey protein. This finding indicated the interactions between whey proteins and gum Arabic was dominated by the electrostatic

forces. Furthermore, electrostatic interactions are known to be affected by the pH and ionic strength of the medium, which is important for the complex formation between proteins and gum Arabic. In the same study, it was reported that at pH values, where whey proteins were not completely negatively or positively charged, which means whey proteins had both positive and negative patches in the structure, the interaction with gum Arabic was mainly electrostatic (Klein et al., 2010). As gum Arabic has a pKa value of around pH 2 (Gulao et al., 2016), it is mainly negatively charged in whey protein solutions. Therefore, once whey proteins have positively charged patches, gum Arabic could bind via electrostatic attractions to those patches. In the study of Klein and co-workers (2010), it was reported that zeta-potential values of mixtures of whey protein and gum Arabic were in between the zeta-potential values of components separately. The electrostatic complexes of whey proteins and gum Arabic were used for encapsulation of oils, such as lemon and orange oils (Weinbreck, Minor, & de Kruif, 2004b). Encapsulation of different flavours using whey protein and gum Arabic coacervates was used in controlled release systems in cheese and it was reported that large capsules showed a high flavour release particularly at pH 4. The encapsulation occurred at the oil/water interface and it was found to be highly dependent on pH of the medium. At pH 4, where the zeta-potential of the coacervate system was almost zero, the stability of coacervates was found to be low and thereby yielding a high release of flavour.

Whey protein particles prepared using the emulsification method showed higher heat stability in the presence of gum Arabic compared to the presence of proteins (Sağlam et al., 2013). Particle size and the viscosity of dispersions after heating at 90°C for 30 min did not change when there is gum Arabic in the continuous phase. The reason was attributed to the remaining oil layer around whey protein particles during the preparation steps in the presence of gum Arabic (Sağlam et al., 2013). As the swelling mechanism of whey protein micro-particles could not have been exactly explained, for the interactions of whey proteins with gum Arabic has only indicated the possible heat stability mechanism. Interaction of protein particles with gum Arabic could be from the protein part of the polysaccharide and the other branches of gum Arabic might have given steric stability to the protein particle, thereby reducing the aggregation between particles. Another possible reason could be that the presence of gum Arabic in the continuous phase increased the macromolecular crowding and suppressed the swelling of protein particles. Previously, macromolecular crowding was found to have an important effect on the heat stability of proteins (Stagg, Zhang, Cheung, & Wittung-Stafshede, 2007; Zhu, He, & Li, 2008a). For instance, increasing concentrations of dextran were reported to increase the protein thermal stability (Zhu et al., 2008a). A similar effect could have happened in the case of denatured whey proteins in the form of micro-particles and gum Arabic.

When electrostatic interactions are considered, Sağlam et al. (2013) reported that the zeta potential of particles in the presence of gum Arabic only slightly changed after heating. Therefore, the effect of heating on the charge density was minimal. However, the effect of different polymers (i.e. proteins and polysaccharide) in the continuous phase on the charge density of particles was reported as different from each other. When there was protein in the continuous phase before heating the dispersion, zeta potential value of particles was more negative than the presence of gum Arabic (Sağlam et al., 2013). This finding suggested that there could be an interaction between protein particles and gum Arabic even before heating. In this case, electrostatic or steric interactions were more likely to occur. Electrostatic interactions between proteins and gum Arabic seem not to be affected by heating application, whereas steric interactions could change with heating, as there might be a structural change.

5. Interactions Between Whey Protein Particles and Dextran

Interaction of whey protein and polysaccharides may yield aggregates with good emulsifying properties and texturizing characteristics. In many studies, this interaction was obtained via a cross-linking reaction known as Maillard reaction with different molecular weight of dextran (Spotti et al., 2013; 2014; Turan, Gibis, Gunes, Baier, & Weiss, 2018; Zhu, Damodaran, & Lucey, 2008b). A Maillard reaction basically happens between an amino acid and a reducing sugar upon heating. As a result of this reaction, when there is whey protein as a reactant, it might gain new physicochemical properties, which supply new functionalities to food systems (Spotti et al., 2014). Therefore, the products of the reaction may suggest new scopes to food industry.

In a study, whey protein isolate and dextran conjugates were produced via Maillard reaction at a temperature of 60°C, which is below the denaturation temperature of whey proteins; and it was reported that dextran gave

an additional stability against denaturation and aggregation of proteins (Zhu et al., 2008b). In addition, they confirmed the mechanism of interaction as a covalent attachment of dextran to whey protein isolate using SDS-PAGE method by both staining protein and dextran separately. As expected, the conjugation reaction rate increased with increasing temperatures, which were all below the denaturation temperature of proteins, and with increasing concentrations of reactants (Spotti et al., 2013).

Conjugates of whey proteins and dextran were found to have high heat stability upon heating at 80°C for 30 min (Zhu, Damodaran, & Lucey, 2010). These conjugates were found to be stable over a wide pH range; pH 1 to pH 8 (Dai et al., 2015; Zhu et al., 2010). Additionally, authors compared the emulsifying properties of whey protein-dextran and whey protein-gum Arabic conjugates, and they reported that the whey protein-dextran conjugates showed better emulsifying properties (Zhu et al., 2010). They explained the reason as whey protein-dextran conjugates formed a thicker steric barrier at the interface to stabilize the emulsion. In this case, non-covalent interactions and sulfhydryl interchange were found to play a role. The interface layer constituted by whey protein-dextran conjugates were reported to be 6 times thicker than that constituted by only whey proteins (Zhu et al., 2010).

In another study, rheological properties of whey protein-dextran conjugates that were produced through Maillard reaction were investigated in detail (Spotti et al., 2014). Gel strength of whey protein-dextran conjugates was determined and it was found that due to a change in the aggregation kinetics, the gelling mechanism was also changed. They reported a lower G' value, which indicates a softer structure, for the conjugate system compared to the native whey proteins, as the secondary structure of proteins changed as a result of dextran attachment. The reason was explained as the decreased strength of hydrophobic interactions due to the presence of purely hydrophilic dextran. In addition, they also explained that conjugate gel strength could alter according to the molecular weight of dextran (Sun et al., 2011; Spotti et al., 2014). Dextran molecules with different molecular weights were conjugated with whey proteins and rod-like conjugates were obtained through Maillard reaction using an electrospinning device (Turan et al., 2018). They reported that a new type of glycoprotein was produced with different functionalities, such as emulsifying properties.

6. Conclusion

Structure of whey proteins, caseins, gum Arabic and dextran and their interactions in the aqueous medium were explained. Interactions within whey proteins are important for optimizing the processing conditions particularly in milk beverages. To optimize the properties and processing conditions, heat-set and cold-set gelation methods can be used to form protein particles. These methods had different advantages over the other and can be combined to have a better control over the particle properties. Interaction of whey proteins with other proteins could be through hydrophobic, electrostatic and steric interactions in the aqueous medium. Such interactions have the potential to add new functionalities to the proteins. Whey protein particles in the presence of gum Arabic were shown to have good stability against swelling upon heating and thereby having good heat stability in the aqueous medium. However, when a protein was present in the continuous phase instead of gum Arabic, swelling of whey proteins was pronounced, implying lower heat stability. When dextran interacted with whey proteins, formed conjugates, mainly through Maillard reactions, were found to have an increased emulsifying capacity and increased heat stability. The stability of such conjugates was also found reasonable over a wide pH range, which is convenient to use in milk beverages. Interactions between different biopolymers gave functional properties and could be used to control the stability behaviour of systems. Such systems can be used in controlled delivery systems for food and pharmaceutical industries or in designing stable emulsions and foams in Pickering systems.

Author Contributions

Alev Emine İNCE COŞKUN: Collected data and wrote the paper.

Semih ÖTLEŞ: Conceived the topic and edited the paper.

Conflicts of Interest

The authors declare no conflict of interest.

References

- van den Akker, C. C., Schleegeer, M., Bonn, M., & Koenderink, G. H. (2014). Structural basis for the polymorphism of β -lactoglobulin amyloid-like fibrils (Chapter 31). *Bio-nanoimaging, Protein Misfolding and Aggregation*, 333-343.
- Ako, K., Nicolai, T., Durand, D., & Brotons, G. (2009). Micro-phase separation explains the abrupt structural change of denatured globular protein gels on varying the ionic strength or the pH. *Soft Matter*, 5, 4033-4041.
- Anema, S. G., & Li, Y. (2003). Effect of pH on the association of denatured whey proteins with casein micelles in heated reconstituted skim milk. *Journal of Agricultural and Food Chemistry*, 51, 1640-1646.
- Benichou, A., Aserin, A., & Garti, N. (2002). Protein-polysaccharide interactions for stabilization of food emulsions. *Journal of Dispersion Science and Technology*, 23, 93-123.
- Carrasco, F., Chornet, E., Overend, R. P., & Costa, J. (1989). A generalized correlation for the viscosity of dextrans in aqueous solutions as a function of temperature, concentration, and molecular weight at low shear rates. *Journal of Applied Polymer Science*, 37, 2087-2098.
- Clare, D. A., & Daubert, C. R. (2010). Transglutaminase catalysis of modified whey protein dispersions. *Journal of Food Science*, 75, 369-377.
- Corredig, M., & Dalgleish, D. G. (1996). Effect of temperature and pH on the interactions of whey proteins with casein micelles in skim milk. *Food Research International*, 29, 49-55.
- Dai, Q., Zhu, X., Abbas, S., Karangwa, E., Zhang, X., Xia, S., Feng, B., & Jia, C. (2015). Stable nanoparticles prepared by heating electrostatic complexes of whey protein isolate-dextran conjugate and chondroitin sulfate. *Journal of Agricultural and Food Chemistry*, 63, 4179-4189.
- Doublier, J. L., Garnier, C., Renard, D., & Sanchez, C., 2000. Protein-polysaccharide interactions. *Current Opinion in Colloid & Interface Science*, 5, 202-214.
- Elzoghby, A. O., Elgohary, M. M., & Kamel, N. M. (2015). Implications of protein- and peptide-based nanoparticles as potential vehicles for anticancer drugs (Chapter 6). *Advances in Protein Chemistry and Structural Biology*, 98, 169-221.
- Ge, S., Kojio, K., Takahara, A., & Kajiyama, T. (1998). Bovine serum albumin adsorption onto immobilized organotrichlorosilane surface: influence of the phase separation on protein adsorption patterns. *Journal of Biomaterials Science, Polymer Edition*, 9, 131-50.
- Ghosh, A. K., & Bandyopadhyay, P. (2012). Polysaccharide-protein interactions and their relevance in food colloids. In D. N. Karunaratne (Ed.), *The Complex World of Polysaccharides* (pp. 395-408).
- Gulao, E. S., Souza, C. J. F., Andrade, C. T., & Garcia-Rojas, E. E. (2016). Complex coacervates obtained from peptide leucine and gum Arabic: Formation and characterization. *Food Chemistry*, 194, 680-686.
- Hoffmann, M. A., & van Mill, P. J. J. M. (1999). Heat-induced aggregation of β -lactoglobulin as function of pH. *Journal of Agricultural and Food Chemistry*, 47, 1898-1905.
- Horne, D. S. (2006). Casein micelle structure: Models and muddles. *Current Opinion in Colloid & Interface Science*, 11, 148-153.
- Ince Coskun, A. E., Saglam, D., Venema, P., van der Linden, E., & Scholten, E. (2015). Preparation, structure and stability of sodium caseinate and gelatin micro-particles. *Food Hydrocolloids*, 45, 291-300.
- Kelly, P., Woonton, B. W., & Smithers, G. W. (2009). Improving the sensory quality, shelf-life and functionality of milk (Chapter 8). *Functional and Speciality Beverage Technology, Woodhead Publishing Series in Food Science, Technology and Nutrition*, 170-231.
- Klein, M., Aserin, A., Ishai, P. B., & Garti, N. (2010). Interactions between whey protein isolate and gum arabic. *Colloids and Surfaces B: Biointerfaces*, 79, 377-383.
- de Kruif, C. G. (2012). Milk nanotubes: technology and potential applications (Chapter 14). *Nanotechnology in the Food, Beverage and Nutraceutical Industries, Woodhead Publishing Series in Food Science, Technology and Nutrition*, 398-412.
- Lam, C. W. Y., & Ikeda, S. (2017). Physical properties of heat-induced whey protein aggregates formed at pH 5.5 and 7.0. *Food Science and Technology Research*, 23, 595-601.
- Langerdorff, V., Cuvelier, G., Launay, B., Michin, C., Parker, A., & Kruif, C. G. (1999). Casein micelle/iota

- carrageenan interactions in milk: Influence of temperature. *Food Hydrocolloids*, 13, 211-218.
- Lazidis, A., Hancocks, R. D., Spyropoulos, F., Kreuß, M., Berrocal, R., & Norton, I. T. (2016). Whey protein fluid gels for the stabilisation of foams. *Food Hydrocolloids*, 53, 209-217.
- Lee, W. J., & Lucey, J. A. (2010). Formation and physical properties of yogurt. *Asian-Australian Journal of Animal Science*, 23, 1127-1136.
- Loveday, S. M., Ye, A., Anema, S. G., & Singh, H. (2013). Heat-induced colloidal interactions of whey proteins, sodium caseinate and gum arabic in binary and ternary mixtures. *Food Research International*, 54, 111-117.
- Lu, K. W., Pérez-Gil, J., & Taeusch, H. W. (2009). Kinematic viscosity of therapeutic pulmonary surfactants with added polymers. *Biochimica et Biophysica Acta*, 1788, 632-637.
- Mahmoudi, N., Axelos, M. A. V., & Riaublanc, A. (2011). Interfacial properties of fractal and spherical whey protein aggregates. *Soft Matter*, 7, 7643-7654.
- Mehalebi, S., Nicolai, T., & Durand, D. (2008). Light scattering study of heat- denatured globular protein aggregates. *International Journal of Biological Macromolecules*, 43, 129-135.
- Nicolai, T., Britten, M., & Schmitt, C. (2011). β -lactoglobulin and WPI aggregates: Formation, structure and applications. *Food Hydrocolloids*, 25, 1945-1962.
- Nivala, O., Mäkinen, O. E., Kruus, K., Nordlund, E., & Ercili-Cura, D. (2017). Structuring colloidal oat and faba bean protein particles via enzymatic modification. *Food Chemistry*, 231, 87-95.
- Pelegrine, D. H. G., & Gasparetto, C. A. (2005). Whey proteins solubility as a function of temperature. *LWT-Research note*, 38, 77-80.
- Peng, J., Kroes-Nijboer, A., Venema, P., & van der Linden, E. (2016). Stability of colloidal dispersions in the presence of protein fibrils. *Soft Matter*, 12, 3514-3526.
- Phan-Xuan, T., Durand, D., & Nicolai, T. (2013). Tuning the structure of protein particles and gels with calcium or sodium ions. *Biomacromolecules*, 14, 1980-1989.
- Roefs, S. P. F. M., & de Kruif, C. G. (1994). A model for the denaturation and aggregation of β -lactoglobulin. *European Journal of Biochemistry*, 226, 883-889.
- Rogers, S. S., Venema, P., Sagis, L. M. C., van der Linden, E., & Donald, A. M. (2005). Measuring length distribution of a fibril system: A flow birefringence technique applied to amyloid fibrils. *Macromolecules*, 38, 2948-2958.
- Ruis, H. G. M., van Gruijthuijsen, K., Venema, P., & van der Linden, E. (2007). Transitions in structure in o/w emulsions as studied by diffusing wave spectroscopy. *Langmuir*, 23, 1007-1013.
- Sağlam, D., Venema, P., de Vries, R., Sagis, L. M. C., & van der Linden, E. (2011). Preparation of high protein micro-particles using two-step emulsification. *Food Hydrocolloids*, 25, 1139-1148.
- Sağlam, D., Venema, P., de Vries, R., van Aelst, A., & van der Linden, E. (2012). Relation between gelation conditions and the physical properties of whey protein particles. *Langmuir*, 28, 6551-6560.
- Sağlam, D., Venema, P., de Vries, R., Shi, J., & van der Linden, E. (2013). Concentrated whey protein particle dispersions: Heat stability and rheological properties. *Food Hydrocolloids*, 30, 100-109.
- Serfert, Y., Lamprecht, C., Tan, C. P., Keppler, J. K., Appel, E., Rossier-Miranda, F. J., Schroen, K., Boom, R. M., Gorb, S., Selhuber-Unkel, C., Drusch, S., & Schwarz, K. (2014). Characterisation and use of β -lactoglobulin fibrils for microencapsulation of lipophilic ingredients and oxidative stability thereof. *Journal of Food Engineering*, 143, 53-61.
- Singhal, R. S., Gupta, A. K., & Kulkarni, P. R. (1991). Low-calorie fat substitute. *Trends in Food Science and Technology*, 2, 241-244.
- Sneharani, A. H., Karakkat, J. V., Singh, S. A., & Rao, A. G. A. (2010). Interaction of curcumin with β -lactoglobulin-Stability, spectroscopic analysis, and molecular modeling of the complex. *Journal of Agricultural and Food Chemistry*, 58, 11130-11139.
- Spotti, M. J., Perduca, M. J., Piagentini, A., Santiago, L. G., Rubiolo, A. C., & Carrara, C. R. (2013). Gel mechanical properties of milk whey protein-dextran conjugates obtained by Maillard reaction. *Food Hydrocolloids*, 31, 26-32.
- Spotti, M. J., Martinez, M. J., Pilosof, A. M. R., Candiotti, M., Rubiolo, A. C., & Carrara, C. R. (2014). Rheological properties of whey protein and dextran conjugates at different reaction times. *Food Hydrocolloids*, 38, 76-84.

- Stagg, L., Zhang, S. Q., Cheung, M. S., & Wittung-Stafshede, P. (2007). Molecular crowding enhances native structure and stability of α/β protein flavodoxin. *Proceedings of the National Academy of Sciences*, *104*, 18976-18981.
- Sun, W., Yu, S., Yang, X., Wang, J., Zhang, J., & Zhang, Y. (2011). Study on the rheological properties of heat-induced whey protein isolate-dextran conjugate gel. *Food Research International*, *44*, 3259-3263.
- Thorn, D. C., Meehan, S., Sunde, M., Rekas, A., Gras, S. L., MacPhee, C. E., Dobson, C. M., Wilson, M. R., Carver, J. A., & MacPhee, C. (2005). Amyloid fibril formation by bovine milk kappa-casein and its inhibition by the molecular chaperones alpha(s-) and beta-casein. *Biochemistry*, *44*, 17027-17036.
- Turan, D., Gibis, M., Gunes, G., Baier, S. K., & Weiss, J. (2018). The impact of the molecular weight of dextran on formation of whey protein isolate (WPI)-dextran conjugates in fibers produced by needleless electrospinning after annealing. *Food&Function*, *9*, 2193-2200.
- Wang, W., Zhong, Q., & Hu, Z. (2013). Nanoscale understanding of thermal aggregation of whey protein pretreated by transglutaminase. *Journal of Agricultural and Food Chemistry*, *61*, 435-446.
- Weinbreck, F., Tromp, R. H., & de Kruif, C. G. (2004a). Composition and structure of whey protein/gum arabic coacervates. *Biomacromolecules*, *5*, 1437-1445.
- Weinbreck, F., Minor, M., & de Kruif, C. G. (2004b). Microencapsulation of oils using whey protein/gum Arabic coacervates. *Journal of Microencapsulation*, *21*, 667-679.
- Yadav, M. P., Igartuburu, J. M., Yan, Y., & Nothnagel, E. A. (2007). Chemical investigation of the structural basis of the emulsifying activity of gum arabic. *Food Hydrocolloids*, *21*, 297-308.
- Zhang, W., & Zhong, Q. (2009). Microemulsions as nanoreactors to produce whey protein nanoparticles with enhanced heat stability by sequential enzymatic cross-linking and thermal pretreatment. *Journal of Agricultural and Food Chemistry*, *57*, 9181-9189.
- Zhu, J., He, H., & Li, S. (2008a). Macromolecular crowding enhances thermal stability of rabbit muscle creatine kinase. *Tsinghua Science and Technology*, *13*, 454-459.
- Zhu, D., Damodaran, S., & Lucey, J. A. (2008b). Formation of whey protein isolate (WPI)-dextran conjugates in aqueous solutions. *Journal of Agricultural and Food Chemistry*, *56*, 7113-7118.
- Zhu, D., Damodaran, S., & Lucey, J. A. (2010). Physicochemical and emulsifying properties of whey protein isolate (WPI)-dextran conjugates produced in aqueous solutions. *Journal of Agricultural and Food Chemistry*, *58*, 2988-2994.