

## **Casein polysaccharides interaction – A Review**

**Abdul Manab\***

**Animal Product Technology Department, Animal Husbandry Faculty, Brawijaya University, Jalan Veteran Malang, East Java, Indonesia**

**Abstract :** Casein polysaccharide interaction can be associative or segregative thereby this interaction can improve stability or induce destabilization. The interaction of casein with several anionic, cationic and nonionic polysaccharides such as carrageenan, pectin, carboxymethylcellulose (CMC), gum, chitosan, inulin and glucomannan in aqueous solutions can be interacting to each other, or noninteracting each other. The attractive interactions between casein and polysaccharides mainly contribute to stabilize food emulsion.

Casein micelles especially  $\kappa$ Casein molecules stabilize the casein micelles via electrostatic interaction and steric stabilization. Casein adsorbed into oil-water interface to stabilize oil droplet by the combination of steric stabilization and electrostatic interaction. However, emulsions that stabilized by casein at  $\text{pH} < \text{pI}$  are less stable than those at  $\text{pH} > \text{pI}$ . Destabilisation of emulsion occurs through bridging flocculation or depletion flocculation. If the repulsive and steric stabilization layer is damaged or collapses, Van der Waals interactions appear and the casein micelles form aggregate.

The adsorbing macromolecules such as charged polysaccharides will stabilize oil-water emulsions via steric and electrostatic stabilization. The non-adsorbing macromolecules such as uncharged polysaccharides will stabilize the emulsions via depletion stabilization when the non-adsorbed polymer is in sufficient amount. Casein-polysaccharides layer formation at the oil water interface in continuous aqueous phase, mainly contribute to the emulsion stability of food products.

### **Introduction**

Polysaccharides and proteins in aqueous solutions can be non-interacting to each other, or can interacting each other (attract or repulse)<sup>[1]</sup>. The attractive interactions between proteins and polysaccharides in many ways<sup>[2]</sup> : covalent linking, ionic and hydrogen bonding, hydrophobic and van der Waals interaction, and physical entanglement<sup>[1]</sup>.

Two main types of polysaccharide and proteins interactions: <sup>[2]</sup>: (i) strong association – irreversibly bonding of proteins to polysaccharides or strong electrostatic complexes; (ii) weak association – potentially reversible, includes non-ionic and weak electrostatic complexes<sup>[1]</sup>.

Electrostatic interactions play a predominant role with charged polysaccharides. Strong attractive electrostatic complexes are commonly formed between positively charged proteins ( $\text{pH} < \text{pI}$ ) and anionic polysaccharides. Weaker reversible complexes can be formed between anionic polysaccharides and proteins carrying nearly zero overall charge ( $\text{pH} \approx \text{pI}$ ) or a net negative charge ( $\text{pH} > \text{pI}$ )<sup>[3]</sup>.

Destabilisation of emulsion occurs through bridging flocculation at low polysaccharide concentration or depletion flocculation at extremely high polysaccharide concentration<sup>[4,5,6]</sup>. Depletion flocculation at polysaccharide attributed to the presence of anionic polysaccharides that unadsorbed in the aqueous phase of the emulsion<sup>[4,7]</sup>.

Casein as casein micelles, ie monomer complex over the calcium phosphate bridge<sup>[8]</sup> (Bouزيد et al., 2008), phosphorylated at clusters of serine residues<sup>[9]</sup>. Casein micelles stability due to hydrophobic interactions and Ca-phosphate salt bridges<sup>[10]</sup>. The linkage between Ca-P colloidal and the structure of casein is due to a series of phosphoserine residues<sup>[11]</sup>. Casein will precipitate at low pH, because its solubility is lower in acid conditions<sup>[12]</sup>.

Casein micelles is colloidal structure that contain  $\alpha_1$ ,  $\alpha_2$ ,  $\beta$  and  $\kappa$ casein and roughly 200 nm in diameter<sup>[13]</sup>.  $\kappa$ Casein molecules stabilize the casein micelles from aggregation, it protrude roughly 5 nm out from micelle surface<sup>[14]</sup>.  $\kappa$ -Casein form an entropy-rich brush at the surface of casein micelles ensuring the steric stabilization<sup>[15]</sup>.

Sodium-caseinate adsorbed into oil-water interface to stabilize droplet by the combination of steric stabilization and electrostatic interaction<sup>[16]</sup>. However at pH 4.6 or near pI, the ability of sodium-caseinate to stabilize the emulsion is not stable, it may be attributed to casein aggregation and precipitation and the decreasing of electrostatic repulsion between oil droplet<sup>[17]</sup>.

The distribution and composition of protein charges on the micelles surface appear to generate a repulsive barrier which contributes to the micelles stability in suspension. Thus, if the repulsive and steric stabilization layer is damaged or collapses, Van der Waals interactions appear and the casein micelles aggregate<sup>[18]</sup>.

The adsorbing macromolecules will stabilize oil-water emulsions via steric and electrostatic stabilization. The non-adsorbing macromolecules will stabilize the emulsions via depletion stabilization when the non-adsorbed polymer is in sufficient amount<sup>[1]</sup>.

According to such an approach one must find out for any casein-polysaccharides layer formation at the oil water interface in continuous aqueous phase. Both caseins and polysaccharides contribute to the emulsion stability of food products. It is therefore of interest to review the interaction between casein and polysaccharides.

### Casein polysaccharide interaction

#### Casein-carrageenan interaction

$\kappa$ -Carrageenan have negatively charged, linear and sulfated galactan polysaccharide<sup>[19,20]</sup>. Gelation of  $\kappa$ -carrageenan formed when helical  $\kappa$ -carrageenan become aggregate<sup>[21]</sup>.  $\kappa$ -Carrageenan heating in solution at 50°C or higher, it exists as a random coil, then the temperature is lowered make  $\kappa$ -carrageenan become a helix and subsequent aggregate become gel<sup>[22]</sup>.

$\kappa$ -Carrageenan will interacts with casein micelle surface when  $\kappa$ -carrageenan in the helix form to inhibit separation of phase<sup>[23]</sup>, via electrostatic interaction between positively charged of  $\kappa$ -casein and negatively charged of  $\kappa$ -carrageenan, thus  $\kappa$ -carrageenan adsorb to the surface of casein micelle<sup>[24,25,26]</sup> or via weak gel formation of  $\kappa$ -carrageenan that bind the casein micelles<sup>[27]</sup>.

The casein micelles have 250 nm diameter, the surface rather porous and protrude 10–20 nm into solution<sup>[23]</sup>.  $\kappa$ Carrageenan have 20 nm diameter. If  $\kappa$ carrageenan and casein micelle were mixed  $\kappa$ carrageenan adsorb at the surface of casein micelles. Bridging between casein micelle surface and association among micelles by the strands<sup>[23]</sup>.  $\kappa$ -Carrageenan increase casein micelle diameter, it is may be due to adsorption and interaction between casein micelle with  $\kappa$ -carrageenan<sup>[28]</sup>.

### Casein-CMC interaction

Carboxymethylcellulose (CMC) is a linear  $\beta$ -(1,4)-linked D-glucopyranose chains and anionic carboxylate<sup>[29,30]</sup>. At below 5.2 CMC adsorbed on casein micelles surface formed steric stabilization to prevent  $\text{Ca}^{2+}$ -induced casein aggregation<sup>[31]</sup>. CMC addition on excess amount to neutral Casein-stabilised emulsions could result in depletion flocculation by non-adsorbed polysaccharides<sup>[4]</sup>.

CMC have ability as a stabilising agent to avoid the milk protein flocculation in acidified milk drinks. At pH below 5.2, CMC adsorbed on the layer of casein micelles surface and electrostatic interaction between CMC and casein micelles induced steric stabilisation can prevent acid-induced casein aggregation by steric stabilisation<sup>[31]</sup>. However, depletion flocculation can take place when excess CMC addition to neutral casein-stabilised emulsions and non-adsorbed polysaccharides formed<sup>[4]</sup>.

The CMC binding to casein surface due to the electrostatic attraction between the positively charged domain on casein micelles and anionic groups on the CMC molecules. The emulsion that stabilized by protein if acidified to pH 4 and CMC adding, caused adsorption of negative charged of CMC on the surface of cationic protein-coated droplets. At neutral pH (pH 7), both casein and CMC were negative charge. At pH near casein pI, interfacial layers can be formed by direct adsorption of casein at droplet interfaces as primary layer and secondary layer that formed by CMC molecules that attract to casein layer electrostatic interactions<sup>[32]</sup>.

The formation of casein/CMC complex at oil-water interfaces during acidification can prevent the oil droplets against flocculation and coalescence. This stability was mainly attributed to the increasing of steric repulsion as well as the decreasing of van-der-Waals attraction between oil droplet and the thickening of the interfacial layer<sup>[32]</sup>.

The electrostatic interactions between CMC and casein-stabilised emulsions can be effective when the interaction under optimum condition. At pH 4–5 CMC adsorbed onto the surface casein-coated droplets, but not at pH 6–7. At neutral pH, the excess of nonadsorbed CMC in the emulsion system induced depletion flocculation. However, at acidic pH emulsions containing high concentration of CMC (>0.3 wt.%) the emulsion remained stable during storage may be due to the formation of multilayer structures<sup>[32]</sup>.

### Casein-pectin interaction

Pectin is an anionic polysaccharide<sup>[33]</sup> composed of homogalacturonan (HG), rhamnogalacturonan I (RGI) and rhamnogalacturonan II (RGII)<sup>[34]</sup>. Methylsterified galacturonic acid units and galacturonic acid content ratio is given by the degree of methylesterification (DM) and determines how pectin interacts with other molecules.

Pectin commonly used as a 'stabilizer' in acid milk drinks where it inhibits this precipitation; and as a 'thickener' in other dairy desserts, where it has been assumed to form a gel in the serum aqueous phase<sup>[33]</sup>. At pH < 5.3, charged region of pectin adsorbed on casein micelles surface via electrostatic interactions to prevent from flocculation and stabilize the acid milk drink<sup>[35,36]</sup>, while the other parts of pectin molecule protrude to solution as loop and tails<sup>[37]</sup>.

At pH 6.7, pectin induced a depletion flocculation of the casein micelles may be due to the exclusion of pectin from the space between casein micelles. At pH 5.3, pectin adsorbs onto casein micelles surface; at low content of pectin formed a bridging flocculation. On the increasing of pectin content, casein micelles become fully coated and lowered attraction between casein and pectin, segregative phase separation may be attributed to non-adsorbing pectin. On the increasing of pH from 5.3 to 6.7, pectin desorbs<sup>[35]</sup>.

A sufficient pectin concentration is needed to impart a negative charge of casein surface, to stabilize acidified dairy drinks, and the critical pectin concentration depends on pH, ionic strength and other factors<sup>[38,39]</sup>. If pectin concentration is insufficient, caused bridging flocculation and if pectin concentration is too high caused excessive viscosity or phase separation<sup>[40]</sup>.

Adsorption of pectin onto casein micelles surface by electrostatic interaction creates a net negatively charged of the complex, which is dispersed by electrostatic repulsion and steric stabilization<sup>[36]</sup>.  $\kappa$ -Casein is more sensitive than  $\beta$ ,  $\alpha$ s1,  $\alpha$ s2 casein to charge modified pectin. Pectin adsorption onto the casein micelle

surface has presumptively involved  $\kappa$ -casein, but there is specific affinity between  $\kappa$ -casein and charge modified pectin<sup>[41]</sup>.

### Casein-glucomannan interaction

Glucomannan consist of D- glucose and D-mannose have molar ratio 1 : 1.6 with  $\beta$  -(1-4)glycosidic binding and approximately 1 acetyl group in each 17 residue at C-6 position<sup>[42]</sup>. Glucomannan as neutral polysaccharide and milk protein thermodynamically was incompatible<sup>[43]</sup>, caused segregative phase separation, attributed to segregative interaction between casein and glucomannan<sup>[44,45]</sup>.

Glucomannan-lactic acid complex and  $\kappa$ -casein binding indicated at hydrophobic core of  $\kappa$ -casein. The binding of glucomannan molecule to  $\kappa$ -casein dominated by hydrogen binding and hydrophobic group. Binding energy between glucomannan-lactic acid complex which heated at 90°C and  $\kappa$ -casein higher than glucomannan-lactic acid in water solution without heating, this result attributed with higher hydrogen binding capacity formed<sup>[46]</sup>.

The complex of glucomannan-lactic acid more favorable to bind  $\kappa$ -casein than glucomannan only, it means the glucomannan and lactic acid can make complex easily than glucomannan only. Glucomannan bind to  $\kappa$ -casein using hidrogen bonding and hydrophobic interaction for stabilizing the complex. The interaction is different when lactic acid include in the complex. Glucomannan-lactic acid complex bind to  $\kappa$ -casein and the complex was stabilized by hydrogen bonding and hydrophobic interaction. Lactic acid is important to mediate interaction between glucomannan and  $\kappa$ -casein<sup>[46]</sup>.

### Casein-dextran interaction

Dextran consisting of  $\alpha$ -1.6 d-glucose and  $\alpha$ -1.3 d-glucose unit<sup>[47]</sup>. Maillard reaction was used to modify dextran via grafting of dextran to  $\beta$ -casein to improve casein solubility in acidic pH. During the mixing of dextran with  $\beta$ -casein solutions, the temperature was kept at 0 °C to stabilize casein in monomer state<sup>[48]</sup>. The emulsifying activity of  $\alpha$ s-casein–dextran was 1.5 times higher than t  $\alpha$ s-casein, and the emulsion stability of the  $\alpha$ s-casein–dextran was 10 times higher than that of  $\alpha$ s-casein at pH 7.4<sup>[49,50]</sup>. The solubility of  $\beta$ -casein and dextran mixture is the same as of individual  $\beta$ -casein, implying that dextran does not increase casein solubility.

The Maillard reaction that conjugates protein and polysaccharide by linking the reducing end of the polysaccharide to the amines in the protein (terminus and amino group of lysine), has been made to improve proteins functionality. Copolymer of casein and dextran through the Maillard reaction has a reversible pH-sensitive property<sup>[51]</sup>.

### Casein-chitosan interaction

Chitosan, a positively charged polysaccharide comprising copolymers of glucosamine and N-acetylglucosamine<sup>[52]</sup>, it has been used as stabilizing, emulsifying and thickening agent<sup>[53]</sup>. Caseins contain electronegative hydrophilic and neutral hydrophobic regions. On the other hand, chitosans show positively charged hydrophilic domains containing approximately 80% glucosamine and neutral hydrophobic domains containing 20% N-acetylglucosamine<sup>[54]</sup>.

The electronegative domains in casein micelles made it developing to explore chitosan addition on casein stability, chitosans can promote casein coagulation<sup>[54]</sup>. The mixing milk with the chitosan induced casein aggregation and coagulation without milk pH change<sup>[54]</sup>. Precipitation of milk casein micelles with the polycationic chitosan involved electrostatic and hydrophobic interactions and does not depend on the casein micelles structural integrity<sup>[54]</sup>.

Chitosan is a hydrolyzed derivative of chitin<sup>[55]</sup>, amine groups of chitosan are mostly protonated in acidic solutions<sup>[56]</sup>. Chitosan is only soluble in acidic pH and insoluble in neutral and alkaline pH. The amine groups are protonated at pH < 6 and positively charged and chitosan is soluble in water. At high pH, amine groups become deprotonated and the polymer loses its charge and therefore insoluble in water<sup>[57]</sup>. Chitosan and Oppositely charged electrolytes will interact to form an insoluble precipitate.

At pH 7, casein have negative charge but chitosan is deprotonated whereas, at pH 10, negative charge of casein more than at pH 7 making interaction the hydrogen bonding of casein with alcohol and amine groups of chitosan stronger, leading to better attraction between the polymers<sup>[58]</sup>. At pH 4, the amine groups of chitosan are protonated and acquire positive charge<sup>[57]</sup>. Casein also acquire a net positive charge, since the isoelectric point is 4.6, leading to electrostatic repulsion between the two polymer chains<sup>[59,60]</sup>. Electrostatic interaction of the negatively charged casein protein with positively charged chitosan resulted in the formation of an insoluble phase, which is due to chitosan/casein coacervation formation<sup>[61,62]</sup>.

Chitosan was derived from chitin and abundance in OH and NH<sub>2</sub> groups<sup>[63]</sup>. Chitosan and casein phosphopeptides (CPP) were utilized to produce stable multi-layer coatings. Following chitosan deposition, CPP was sequentially adsorbed on the chitosan tail through bonding CPP to the chitosan free NH<sub>2</sub> groups<sup>[64]</sup>.

### Casein-starch interaction

The casein adsorbed starch via electrostatic attraction, the starch granule surface changed to positive charged. The casein aggregates surface and starch micelles are positive result in electrostatic repulsion. Steric stabilization formed by blocks of low affinity areas on casein may protrude from the starch micelles surface<sup>[65,66,67,68]</sup>. Sodium caseinate adsorb to both normal and waxy rice starch granules (Noisuwan, Bronlund, Wilkinson, and Hemar (2008). Casein absorb on the HPDSP surface via electrostatic forces, the stability of modified starch is the result of “electrostatic repulsion” and “steric stabilization”<sup>[70]</sup>. In yoghurt system (pH 3.8), casein particles absorb on acetylated starch or hydroxypropyl distarch phosphate (HPDSP) surface via electrostatic forces<sup>[67,71,72]</sup>.

### Casein-gum interaction

Portulaca oleracea gum (POG) is a charged hydrocolloid with low protein content (3.1 wt%) and relatively low molecular weight<sup>[1]</sup>. POG consists of d-galactose : l-arabinose : d-galacturonic acid : d-xylose : l-rhamnose at 40 : 20 : 5 : 1 : 31 ratio with O-CH<sub>3</sub> groups attached to its sugar moieties. The POG has a structural possibility to form both hybrids and complexes with proteins<sup>[73,74,75]</sup>.

POG adsorb onto the oil-water interface by itself or together with casein. Displacement of POG by casein from the interface, will take place at pH values that will increase repulsion forces between POG and casein<sup>[1]</sup>. Both POG and casein can be anionic (pH > pI) or noncharged (pH < pI) water-soluble biopolymers, therefore, pH determined both strong and weak associative structures. POG is weakly adsorbing biopolymer while casein is strongly adsorbing macromolecule<sup>[1]</sup>. If the pH value is increase attraction force resulted complementary adsorption of casein-POG complex will enhance oil-in-water emulsions stability.

Casein have excellent emulsifying ability, at a pH > pI casein will compete with the POG on the interface if added simultaneously or consecutively to the POG. Displacement of the POG more intensive when the casein/POG ratio in the solution increases<sup>[1]</sup>. At low pH (pH < pI), emulsions prepared with POG more stable, indicating that significant steric stabilization occurs at low pH<sup>[1]</sup>.

### Casein Inulin interaction

Inulin is a soluble fraction of fiber<sup>[76]</sup>, composed of fructose units joined by β(2 / 1) glycoside bonds<sup>[77]</sup>. Inulin that belongs to a class of carbohydrate known as fructans<sup>[78,79]</sup>. Inulin is a mixture of chain fructan that widely found in nature as a plant carbohydrate that can be classified as fructo-ligosaccharide<sup>[80]</sup>.

Melting properties of cheese was improved with inulin addition, it may be due to weak network formation between inulin with milk protein especially casein<sup>[81]</sup>. Protein-protein interactions weakening in the casein network during melting which increased the viscosity<sup>[79]</sup>. The higher ratio of casein modified:inulin result in more porosity structure and swollen of Mozzarella cheese<sup>[82]</sup>. Increasing inulin in modified casein:inulin ratio tend to decreased the cavity of microstructure of mozzarella cheese analogue<sup>[83]</sup>.

## Conclusion

The interaction of casein with several anionic, cationic and nonionic polysaccharides such as carrageenan, pectin, carboxymethylcellulose (CMC), gum, chitosan, inulin and glucomannan in aqueous solutions can be interacting to each other, or noninteracting each other.

Casein adsorbed into oil-water interface to stabilize oil droplet by the combination of steric stabilization and electrostatic interaction. However at pH near pI, casein formed aggregate and precipitate and the decreasing of electrostatic repulsion between oil droplet. If the repulsive and steric stabilization layer is damaged or collapses, Van der Waals interactions appear and the casein micelles form aggregate.

The adsorbing charged polysaccharides will stabilize oil-water emulsions via steric and electrostatic stabilization. The non-adsorbing uncharged polysaccharides will stabilize the emulsions via depletion stabilization when the non-adsorbed polymer is in sufficient amount. Destabilisation of emulsion occurs through bridging flocculation or depletion flocculation. Casein-polysaccharides layer formation at the oil water interface in continuous aqueous phase, mainly contribute to the emulsion stability of food products.

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