Published in final edited form as:

Food Hydrocoll. 2014 March 1; 35: 670–677. doi:10.1016/j.foodhyd.2013.08.022.

High pressure homogenization to improve the stability of casein - hydroxypropyl cellulose aqueous systems

Ran Ye¹ and Federico Harte^{1,*}

¹ Department of Food Science and Technology, University of Tennessee, 2509 River Road, Knoxville, TN 37996-4539, USA

Abstract

The effect of high pressure homogenization on the improvement of the stability hydroxypropyl cellulose (HPC) and micellar casein was investigated. HPC with two molecular weights (80 and 1150 kDa) and micellar casein were mixed in water to a concentration leading to phase separation (0.45% w/v HPC and 3% w/v casein) and immediately subjected to high pressure homogenization ranging from 0 to 300 MPa, in 100 MPa increments. The various dispersions were evaluated for stability, particle size, turbidity, protein content, and viscosity over a period of two weeks and Scanning Transmission Electron Microscopy (STEM) at the end of the storage period. The stability of casein-HPC complexes was enhanced with the increasing homogenization pressure, especially for the complex containing high molecular weight HPC. The apparent particle size of complexes was reduced from ~200nm to ~130nm when using 300 MPa, corresponding to the sharp decrease of absorbance when compared to the non-homogenized controls. High pressure homogenization reduced the viscosity of HPC-casein complexes regardless of the molecular weight of HPC and STEM images revealed aggregates consistent with nano-scale protein polysaccharide interactions.

Keywords

Polysaccharide–protein interactions; casein micelles; high pressure homogenization; hydroxypropyl cellulose; coacervation

1. Introduction

The physicochemical properties of foods depend on interactions of food components including proteins and polysaccharides. For many processed food products, proteins and polysaccharides serve as fundamental ingredients to improve stability, rheological properties, and mouth feel (Doublier, Garnier, Renard, & Sanchez, 2000; Patino & Pilosof, 2011; Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998; Schmitt & Turgeon, 2011; Turgeon, Schmitt, & Sanchez, 2007). When polysaccharides and proteins are mixed in an aqueous phase, a dose-dependent segregation and/or associative phase (complex coacervation) commonly occurs (Bryant & McClements, 2000; Langendorff, et al., 1999;

© 2013 Elsevier Ltd. All rights reserved.

*Corresponding author: Federico Harte Associate Professor Dept. of Food Science and Technology The University of Tennessee 2509 River Drive Knoxville, TN 37996-4539, USA fede@utk.edu; Phone: 865.974.7265; Fax: 865.974.7332.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Maroziene & de Kruif, 2000; Sandra & Dalgleish, 2005). If the protein and polysaccharide carry similar net charge, electrostatic repulsive forces occur, resulting in thermodynamic incompatibility between biopolymers, further leading to separation in protein- and polysaccharide-rich phases. In contrast, associative phase separation electrostatic attraction of opposing net charges on the surface of the protein and polysaccharide results in a coacervate-rich and a solvent-rich phase (Schorsch, Jones, & Norton, 2000; Singh, et al., 2007; A. Q. Ye, 2008). Numerous factors including characteristics of biopolymers (type and distribution of reactive functional groups; charge density; molecular weight) and medium conditions (i.e., temperature, pH, and ionic strength) determine the specific interaction between a proteins (e.g. casein) and polysaccharides (Doublier, et al., 2000; Klemmer, Waldner, Stone, Low, & Nickerson, 2012).

High pressure homogenization is a unit operation where a fluid is forced through a small orifice (valve or nozzle) and several physical factors (shear stress, high hydrostatic pressure, turbulence, cavitation, and impingement) promote dispersion of aggregates. Recent advances in materials science and engineering allowed the manufacturing of ultra-high pressure homogenizers able to deliver pressures in excess of 500 MPa. High pressure homogenization has proven effective in stabilizing emulsions (Cruz, et al., 2007; Desrumaux & Marcand, 2002; Schorsch, et al., 2000), inactivating microorganisms and enzymes (Diels, De Taeye, & Michiels, 2005; Diels, Wuytack, & Michiels, 2003; Hayes, Fox, & Kelly, 2005). High pressure homogenization is known to induce structural changes on casein micelles (Hayes & Kelly, 2003; Huppertz, Fox, de Kruif, & Kelly, 2006; Huppertz, Fox, & Kelly, 2004; Huppertz, Kelly, & de Kruif, 2006; Roach & Harte, 2008), thereby further altering their capacity to interact with other macromolecules. High Pressure homogenization has been also used to reduce the molecular weight of polysaccharides thereby changing their rheological properties when suspended in aqueous phases (Harte & Venegas, 2010; Lyons, 2011). Little has been reported on the effect of high pressure homogenization on the stability protein-polysaccharide mixtures. Sedlmeyer, Brack, Rademacher, and Kulozik (2004) reported the stabilization effect of homogenization at 80 MPa on acidified milk containing high methoxyl pectin.

The objective of this research was to study the effect of high pressure homogenization on the aggregation-separation properties of Hydroxypropyl cellulose (HPC) and casein micelles from bovine milk. HPC, an ether derivative of cellulose, is a water soluble non-ionic polysaccharide. It is commercially available in a wide range of molecular weights and different degrees of substituted hydroxyl groups per glucose unit. HPC is widely used as stabilizer, thickener, fat substitute, lubricant, or filler agent in foods, pharmaceuticals, and cosmetics (Lopez-Velazquez, Bello, & Perez, 2004; Mezdoor, Cuvelier, Cash, & Michon, 2007; Mezdoor, Lepine, Erazo-Majewicz, Ducept, & Michon, 2008; Ramachandran, Chen, & Etzler, 1999). Caseins are the most abundant proteins in milk (*ca.* 80% of proteins in bovine milk) forming a quaternary structure (casein micelle) stabilized via hydrophobic and electrostatic interactions along with calcium phosphate nano-clusters (Dalgleish & Corredig, 2012; de Kruif, Huppertz, Urban, & Petukhov, 2012; Home, 2006; Marchin, Putaux, Pignon, & Leonil, 2007; Schmitt, et al., 2011; Trejo, Dokland, Jurat-Fuentes, & Harte, 2011; R. Ye, Harte, F.M., 2013). The fundamental hypothesis driving this study was that high pressure homogenization induced dissociation of casein micelles in the presence of a non-ionic polysaccharide would lead to the formation of stable casein-polysaccharide aggregates.

2. Materials and methods

2.1 Materials

Micellar casein powder was purchased from American Casein Corporation (Burlington, NJ, USA) and used throughout the study. Micellar casein is a free flowing powder obtained by

ultrafiltration of milk without acidification. Typical composition consists of 86% protein, 1.5% fat, 6.0% ash, 4% carbohydrates, and 5% moisture. Commercial HPC (Klucel, Hercules Inc., Aqualon Division, La Marada, CA) with two average molecular weights were used throughout this study (Klucel E = 80 kDa and Klucel H = 1150 kDa; weight average molecular weight determined by size exclusion chromatography).

2.2 Preparation of the mixture of HPC solution (0.45% w/v) and casein (3% w/v)

4.5 grams of HPC powder were added to 150 ml of distilled water at 30°C and stirred for approximately 4 hours to properly disperse the HPC in water without any clump formation. Subsequently, de-ionized water was added up to a 250 mL using a volumetric flask to the presoaked HPC slurry and let hydrate overnight at 4 °C. After hydration, 250 ml of HPC solution was mixed with 750 ml of casein micelle solution (4.0%) to yield 0.45% (w/v) HPC and 3 % (w/v) casein in water.

2.3 High pressure homogenization

The samples were prepared according to previously published procedures (Hernandez & Harte, 2008). Briefly, the prepared 1L sample was subjected to high pressure homogenization at 0 to 300 MPa at 100 MPa increments using a high pressure valve homogenizer (Model FPG 12500, Stansted Fluid Power, Essex, UK). The homogenizing chamber was cooled to 1°C using a controlled temperature water bath (Isotemp 3016D, Fisher Scientific, USA). The temperature of the sample prior to homogenization was approximately 20°C. Since the temperature increased linearly from 20 to 75°C corresponding to 0 ~300 MPa of homogenization pressure, a tubular heat exchanger was connected immediately after the homogenization valve to cool down the samples to room temperature. After processing, the samples were kept at 4°C for further analysis.

2.4 Particle Size Analysis

Particle size analysis was conducted by photon correlation spectroscopy using a DelsaNano C particle size analyzer (165° angle, 1.34 refractive index, 50 µm pinhole; Beckman Coulter Inc., Atlanta, GA). Calibration was checked using a standard provided by the manufacturer and by measuring native casein micelles from raw skim milk diluted in protein-free milk serum (pH 7.0). Samples continuously flowed through a quartz measuring cell while temperature was controlled by immersion of a heating/cooling coil connected to a controlled temperature water bath (Isotemp 3006S, Fisher Scientific, Pittsburgh, PA). The average particle diameter ($D_{3,4}$) was calculated as the average of 70 autocorrelation functions and two replicates.

2.5 Viscosity

All dispersion behaved as Newtonian fluids. The viscosity of each pressure treated suspension was determined at a shear rate of 100 s^{-1} using an AR-2000 rheometer (TA instruments, New Castle, DE) equipped with a cone and plate geometry (1°, 40 mm diameter; 30 µm truncation) and a Peltier system set at 25°C.

2.6 Stability

Visual observations of complexes were carried at 0, 7 and 14 days of storage at 4°C. The percentage of stability of complexes was calculated by the following formula Eq. (A.1):

$$Stability = \left(\frac{V_p}{V_t} \right) * 100$$

where V_p is the volume of precipitate layer (ml) and V_t is the total volume of the suspension (ml).

2.7 Protein content in supernatants

The total nitrogen and protein content of the samples were measured by the Dumas combustion method (ISO 14891/IDF185) using a TruMac® N analyzer (Leco, St. Joseph, MI).

2.8 Turbidity

Absorbance at 550 nm was used as an indicator of turbidity for each pressure treated sample. Samples were diluted 1:4 using 20 mM imidazole buffer and subsequently measured using a UV-Vis spectrophotometer (Biomate 5, Thermo Electron Corp., Rochester, NY).

2.9 Scanning Transmission Electron Microscopy (STEM)

5 μ L of sample was carefully placed on a freshly glow discharged carbon film on a 400 mesh copper grid and allowed to sit for 1min, then excess sample was carefully removed and the grid was stained with uranyl acetate. After 1 minute excess stain was removed, the grid was allowed to dry then imaged in a Zeiss Auriga operating in STEM (scanning transmission electron microscopy) mode at 30kV.

2.10 Statistical analysis

Data was analyzed as a split-plot completely randomized block experiment with two replications and two sub-plots. The Student's t distribution was used to generate 95% confidence intervals for the mean and for multiple mean comparisons ($P < 0.05$). Data was analyzed using SAS software, version 9.1 (SAS Institute, Cary, NC, USA).

3. Results and Discussion

3.1 Particle size Distribution

The results for apparent particle size of the HPC-casein mixes subjected to high pressure homogenization are shown in Figure 1a and 1b for 80 KDa and 1150 KDa HPC, respectively. The apparent particle diameter of the HPC- casein complexes was significantly reduced ($p < 0.05$) as the homogenization pressure increased from 0 to 300 MPa, regardless of the molecular weight of the polysaccharide. The term “apparent particle size” is used because the assumption of a constant refractive index in samples processed using high pressure homogenization was challenged during these studies and over-estimation of particle diameter and deviations in particle size distribution were expected. Difference in apparent particle size remained significant throughout the study (2 weeks) and roughly a 20 nm reduction in apparent average particle diameter was observed for every 100 MPa increment in processing pressure. The effect of high pressure homogenization was attributed to a combination of physical phenomena leading to a reduction in molecular weight of the polysaccharide (Chen, et al., 2012; Flourey, Desrumaux, Axelos, & Legrand, 2002; Harte, et al., 2010) and the dissociation of casein micelles into smaller sub-micelles (Chevalier-Lucia, Blayo, Gracia-Julia, Picart-Palmade, & Dumay, 2011; Roach, et al., 2008). Previous studies found that high hydrostatic pressure (>250 MPa) itself induced denaturation of α -lactalbumin and β -lactoglobulin in skim milk (Lopez-Fandino & Olano, 1998), and the complexation of denatured α -la and β -lg with casein micelles contributing to increments in particle size (Huppertz, et al., 2004). However, little whey proteins were present in the commercial casein micelle isolate from milk ultrafiltration used in this study, and whey – casein protein interactions were not expected to be a relevant phenomenon in this study due to the short exposure of hydrostatic pressure during homogenization. In addition, the

presence of the hydrocolloid was expected to interfere in the formation of interconnected casein strands and clusters (Dickinson, 2008).

3.2 Appearance and Viscosity

As depicted in Figure 2a-b, high pressure homogenization significantly reduced the initial viscosity (day = 0) of casein-HPC complexes ($P < 0.05$) for systems containing both polysaccharide molecular weights (80 and 1150 kDa). The viscosity of controls (0 MPa) dropped significantly ($P < 0.05$) overtime as the occurrence of complex coacervation between proteins and polysaccharides led to the loss of casein and HPC in the supernatant (solvent-rich phase). In the systems containing low molecular weight HPC (80 kDa; Figure 2a), the viscosity of the systems treated with high pressure homogenization (100 to 300 MPa) remained constant during the period of storage (14 days). However, those systems containing high molecular weight HPC (1150 kDa; Figure 2b) achieved a constant viscosity only when subjected to 300 MPa homogenization pressure. Although the processing at 100 and 200 MPa allowed the reduction of particle size and viscosity, the casein- HPC (1150 kDa) led to phase separation. Homogenization at 100 MPa did not prevent the presence of complex coacervation regardless of the type of HPC. Regardless of the stability and the formation of casein-HPC complexes, smaller casein micelles and reduction in polysaccharide molecular weight itself contributed to a drop of viscosity in the system (Innocente, Biasutti, Venir, Spaziani, & Marchesini, 2009).

Viscosity of sodium caseinate and whey protein concentrate diminished with the increase of high pressure (Venir, Marchesini, Biasutti, & Innocente, 2010) and previous reports showed significant decrease in viscosity of α -tocopherol acetate and casein micelles aqueous systems when processed by homogenization from 0 to 300 MPa (Chevalier-Lucia, et al., 2011). As expected, systems containing higher molecular weight HPC (1150 kDa) exhibited increased initial viscosity and were more affected by high pressure homogenization than those containing 80 kDa HPC. As previously reported, high pressure homogenization is more effective in reducing the molecular weight of relatively large polysaccharides and there is a critical molecular weight below which no further depolymerization occurs due to homogenization (Harte, et al., 2010).

3.3 Stability of HPC- casein mixtures

Visual evidence of stability of the samples after processing and after 2 weeks of storage at 4°C is shown in Figure 3 and stability data (expressed as percent precipitation) is shown Figure 4. All HPC- casein complexes were initially stable after homogenization. Casein low molecular weight HPC (80 kDa) complexes, displayed excellent stability during 2 weeks of storage when processed at or above 200 MPa (Figure 3 ab, 4a). However, the physical appearance (color and turbidity) was different depending on pressure treatment as samples processed at 300 MPa were translucent compared to the white and opaque color of samples processed 200 MPa. Casein-high molecular weight HPC (1150 kDa) samples exhibited pressure dependant precipitation when processed at or below 200 MPa (Figure 3c-d, 4b). However, samples processed at 300 MPa showed exceptional stability with translucent appearance during the two weeks of storage also indicating the formation of very small aggregates. During high pressure homogenization, a shear-induced temperature increase occurs at the homogenization valve as shown by Roach et al. (2008). Although Carotenuto and Grizzuti (2006) demonstrated that HPC experiences reversible sol to gel transitions at temperature $> 50^{\circ}\text{C}$, a heat exchanger was installed immediately after the homogenization valve to keep the system at $< 20^{\circ}\text{C}$ in a fluid state. In general, protein-polysaccharide interactions are classified into two groups: reversible and irreversible (Doublier, et al., 2000). An electrostatic based protein-polysaccharide complex is generally regarded as a reversible process, mainly relying on the overall net charge of the whole system (Doublier,

et al., 2000; A. Q. Ye, 2008). Specifically, the reduction of opposite net charges on the surfaces of protein and polysaccharide facilitate the promotion of both the hydrophobicity and the insolubility of the complex (A. Q. Ye, 2008). In contrast, non-electrostatic macromolecular interactions in protein-polysaccharide complexes, including hydrophobic interactions and hydrogen bonding, can give rise to the formation of an irreversible complex (Doublier, et al., 2000).

3.5 Turbidity

It is well-recognized that that turbidity is highly associated with several factors including size, concentration and refractive index properties of colloids (Banon & Hardy, 1992). Furthermore, changes in the casein micelle size, composition and density affect micellar refractive index, thus affecting turbidity (Martin, Williams, & Dunstan, 2007). The turbidity of samples was slightly reduced right after processing as the pressure increase from 0 to 200 MPa, but a strong reduction in turbidity was observed in samples processed at 300 MPa (Figure 5a-b). Two very distinct phenomena explained the changes in turbidity observed during the storage of the various aqueous casein-HPC dispersions. Unstable samples exhibited reduction in turbidity as the protein rich coacervates precipitated during storage (Figures 3c-d, 4b). However, all samples processed at 300 MPa exhibited a strong reduction in turbidity ($p < 0.0001$) that remained constant throughout the 2 weeks of study (Figure 5a-b) and no precipitation (Figure 4a-b). Samples containing casein and low molecular weight (80 kDa) HPC also exhibited an initial reduction of turbidity that remained constant throughout the study indicating excellent stability. Previous research on phosphocasein dispersions also showed reduction in absorbance as processing pressure increased (Chevalier-Lucia, et al., 2011).

3.4 Protein content

Results on protein content in supernatants showed close agreement with turbidity and stability data. The protein content on supernatants of casein-HPC samples after two weeks storage is shown in Figure 6. Compared to the non-homogenized samples, the protein content in supernatants of casein - low molecular weight HPC (80 kDa) remained constant when samples were processed at or higher than 200 MPa ($P < 0.05$). For the complexes with casein and high molecular weight HPC (1150kDa), protein precipitation was significantly reduced with increasing homogenization pressures. At 300 MPa homogenization pressure, no loss of protein content in supernatants was observed after 2 weeks of storage.

3.6 STEM

Scanning transmission electron microscopy was used as a means of exploring the nanostructure of casein-HPC complexes. Figure 7 a-d shows the morphology aggregates in casein HPC aqueous dispersions two weeks after processed by high pressure homogenization at 0 and 300 MPa. Numerous micro-meter scale protein aggregates ($\sim 1\mu\text{m}$) leading to coacervation / phase separation were found in non-homogenized samples (Figure 7a & b). In contrast, the samples processed at 300 MPa showed nano-scale aggregates with the size of less than 100 nm (Figure 7c & d). Samples processed at 300 MPa also showed variation in staining patterns consistent with protein-polysaccharide nano-aggregates, i.e., inner areas of strong staining (proteins) surrounding areas with low staining (polysaccharide) (Figure 7d). The formation nano-scale aggregates was also consistent with stability, turbidity and proteins content results found in homogenized and non-homogenized samples.

Figure 8 is a schematic representation of the suggested effect of high pressure homogenization on HPC-casein systems. HPC-casein not exposed to sufficient homogenization pressure exhibit expected protein-protein aggregation leading to

coacervation phase separation. Extensive casein micelle dissociation into single casein proteins is achieved when homogenizing at 300 MPa due to hydrostatic pressure. However, little change in the primary structure of caseins is expected due to pressure or shear stress (no proteolysis). It is hypothesized that polysaccharide molecular weight reduction and shear induced increase in temperature at the homogenization valve, followed by a sudden drop in pressure and temperature right after homogenization would promote rapid protein-polysaccharide hydrophobic interactions, leading to stable nano-scale aggregates.

4. Conclusions

In this study, high pressure homogenization was used to improve the stability of aqueous systems containing HPC and casein. The results indicate that high pressure homogenization can block phase separation of Casein-HPC complexes when casein protein quaternary structures are dissociated at homogenization pressure at *ca.* 300 MPa. This case study shows that high pressure homogenization is robust technology to modify protein - polysaccharide interactions and improve stability of mixed biopolymer aqueous system. Further research is needed on the use of high pressure homogenization to stabilize hydrophobic proteins (e.g., zein) and for systems containing negatively charged proteins and cationic polysaccharides (e.g., chitosan).

Acknowledgments

This project was partially supported by the Dairy Research Institute.

References

- Banon S, Hardy J. A colloidal approach of milk acidification by glucono-delta-lactone. *Journal of Dairy Science*. 1992; 75(4):935–941.
- Bryant CM, McClements DJ. Influence of xanthan gum on physical characteristics of heat-denatured whey protein solutions and gels. *Food Hydrocolloids*. 2000; 14(4):383–390.
- Carotenuto C, Grizzuti N. Thermoreversible gelation of hydroxypropylcellulose aqueous solutions. *Rheologica Acta*. 2006; 45(4):468–473.
- Chen J, Liang RH, Liu W, Liu CM, Li T, Tu ZC, Wan J. Degradation of high-methoxyl pectin by dynamic high pressure microfluidization and its mechanism. *Food Hydrocolloids*. 2012; 28(1):121–129.
- Chevalier-Lucia D, Blayo C, Gracia-Julia A, Picart-Palmade L, Dumay E. Processing of phosphocasein dispersions by dynamic high pressure: Effects on the dispersion physico-chemical characteristics and the binding of alpha-tocopherol acetate to casein micelles. *Innovative Food Science & Emerging Technologies*. 2011; 12(4):416–425.
- Cruz N, Capellas M, Hernandez M, Trujillo AJ, Guamis B, Ferragut V. Ultra high pressure homogenization of soymilk: Microbiological, physicochemical and microstructural characteristics. *Food Research International*. 2007; 40(6):725–732.
- Dalgleish, DG.; Corredig, M. The Structure of the Casein Micelle of Milk and Its Changes During Processing.. In: Doyle, MP.; Klaenhammer, TR., editors. *Annual Review of Food Science and Technology*, Vol 3. Vol. 3. Annual Reviews; Palo Alto: 2012. p. 449–467.
- de Kruif CG, Huppertz T, Urban VS, Petukhov AV. Casein micelles and their internal structure. *Advances in Colloid and Interface Science*. 2012; 171:36–52. [PubMed: 22381008]
- Desrumaux A, Marcand J. Formation of sunflower oil emulsions stabilized by whey proteins with high-pressure homogenization (up to 350 MPa): effect of pressure on emulsion characteristics. *International Journal of Food Science and Technology*. 2002; 37(3):263–269.
- Dickinson E. Interfacial structure and stability of food emulsions as affected by protein-polysaccharide interactions. *Soft Matter*. 2008; 4(5):932–942.

- Diels AMJ, De Taeye J, Michiels CW. Sensitisation of *Escherichia coli* to antibacterial peptides and enzymes by high-pressure homogenisation. *International Journal of Food Microbiology*. 2005; 105(2):165–175. [PubMed: 16126294]
- Diels AMJ, Wuytack EY, Michiels CW. Modelling inactivation of *Staphylococcus aureus* and *Yersinia enterocolitica* by high-pressure homogenisation at different temperatures. *International Journal of Food Microbiology*. 2003; 87(1-2):55–62. [PubMed: 12927707]
- Doublier JL, Garnier C, Renard D, Sanchez C. Protein-polysaccharide interactions. *Current Opinion in Colloid & Interface Science*. 2000; 5(3-4):202–214.
- Floury J, Desrumaux A, Axelos MAV, Legrand J. Degradation of methylcellulose during ultra-high pressure homogenisation. *Food Hydrocolloids*. 2002; 16(1):47–53.
- Harte F, Venegas R. A model for viscosity reduction in polysaccharides subjected to high pressure homogenization. *Journal of Texture Studies*. 2010; 41(1):49–61.
- Hayes MG, Fox PF, Kelly AL. Potential applications of high pressure homogenisation in processing of liquid milk. *Journal of Dairy Research*. 2005; 72(1):25–33. [PubMed: 15747728]
- Hayes MG, Kelly AL. High pressure homogenisation of raw whole bovine milk (a) effects on fat globule size and other properties. *Journal of Dairy Research*. 2003; 70(3):297–305. [PubMed: 12916825]
- Hernandez A, Harte FM. Manufacture of acid gels from skim milk using high-pressure homogenization. *Journal of Dairy Science*. 2008; 91(10):3761–3767. [PubMed: 18832197]
- Home DS. Casein micelle structure: Models and muddles. *Current Opinion in Colloid & Interface Science*. 2006; 11(2-3):148–153.
- Huppertz T, Fox PF, de Kruif KG, Kelly AL. High pressure-induced changes in bovine milk proteins: A review. *Biochimica Et Biophysica Acta-Proteins and Proteomics*. 2006; 1764(3):593–598.
- Huppertz T, Fox PF, Kelly AL. High pressure treatment of bovine milk: effects on casein micelles and whey proteins. *Journal of Dairy Research*. 2004; 71(1):97–106. [PubMed: 15068072]
- Huppertz T, Kelly AL, de Kruif CG. Disruption and reassociation of casein micelles under high pressure. *Journal of Dairy Research*. 2006; 73(3):294–298. [PubMed: 16674842]
- Innocente N, Biasutti M, Venir E, Spaziani M, Marchesini G. Effect of high-pressure homogenization on droplet size distribution and rheological properties of ice cream mixes. *Journal of Dairy Science*. 2009; 92(5):1864–1875. [PubMed: 19389944]
- Klemmer KJ, Waldner L, Stone A, Low NH, Nickerson MT. Complex coacervation of pea protein isolate and alginate polysaccharides. *Food Chemistry*. 2012; 130(3):710–715.
- Langendorff V, Cuvelier G, Launay B, Michon C, Parker A, De Kruif CG. Casein micelle iota carrageenan interactions in milk: influence of temperature. *Food Hydrocolloids*. 1999; 13(3):211–218.
- Lopez-Fandino R, Olano A. Effects of high pressures combined with moderate temperatures on the rennet coagulation properties of milk. *International Dairy Journal*. 1998; 8(7):623–627.
- Lopez-Velazquez D, Bello A, Perez E. Preparation and characterisation of hydrophobically modified hydroxypropylcellulose: Side-chain crystallisation. *Macromolecular Chemistry and Physics*. 2004; 205(14):1886–1892.
- Lyons, DS. Depolymerization of Chitosan by High-Pressure Homogenization and the Effect on Antimicrobial Properties. University of Tennessee; Knoxville: 2011.
- Marchin S, Putaux JL, Pignon F, Leonil J. Effects of the environmental factors on the casein micelle structure studied by cryo transmission electron microscopy and small-angle x-ray scattering/ultras-small-angle x-ray scattering. *Journal of Chemical Physics*. 2007; 126(4)
- Marozzene A, de Kruif CG. Interaction of pectin and casein micelles. *Food Hydrocolloids*. 2000; 14(4):391–394.
- Martin GJO, Williams RPW, Dunstan DE. Comparison of casein micelles in raw and reconstituted skim milk. *Journal of Dairy Science*. 2007; 90(10):4543–4551. [PubMed: 17881675]
- Mezdour S, Cuvelier G, Cash MJ, Michon C. Surface rheological properties of hydroxypropyl cellulose at air-water interface. *Food Hydrocolloids*. 2007; 21(5-6):776–781.
- Mezdour S, Lepine A, Erazo-Majewicz P, Ducept F, Michon C. Oil/water surface rheological properties of hydroxypropyl cellulose (HPC) alone and mixed with lecithin: Contribution to

- emulsion stability. *Colloids and Surfaces a-Physicochemical and Engineering Aspects*. 2008; 331(1-2):76–83.
- Patino JMR, Pilosof AMR. Protein-polysaccharide interactions at fluid interfaces. *Food Hydrocolloids*. 2011; 25(8):1925–1937.
- Ramachandran S, Chen S, Etzler F. Rheological characterization of hydroxypropylcellulose gels. *Drug Development and Industrial Pharmacy*. 1999; 25(2):153–161. [PubMed: 10065348]
- Roach A, Harte F. Disruption and sedimentation of casein micelles and casein micelle isolates under high-pressure homogenization. *Innovative Food Science & Emerging Technologies*. 2008; 9(1):1–8.
- Sandra S, Dalgleish DG. Effects of ultra-high-pressure homogenization and heating on structural properties of casein micelles in reconstituted skim milk powder. *International Dairy Journal*. 2005; 15(11):1095–1104.
- Schmitt C, Sanchez C, Desobry-Banon S, Hardy J. Structure and technofunctional properties of protein-polysaccharide complexes: A review. *Critical Reviews in Food Science and Nutrition*. 1998; 38(8):689–753. [PubMed: 9850463]
- Schmitt C, Turgeon SL. Protein/polysaccharide complexes and coacervates in food systems. *Advances in Colloid and Interface Science*. 2011; 167(1-2):63–70. [PubMed: 21056401]
- Schorsch C, Jones MG, Norton IT. Phase behaviour of pure micellar casein/kappa-carrageenan systems in milk salt ultrafiltrate. *Food Hydrocolloids*. 2000; 14(4):347–358.
- Sedlmeyer F, Brack M, Rademacher B, Kulozik U. Effect of protein composition and homogenisation on the stability of acidified milk drinks. *International Dairy Journal*. 2004; 14(4):331–336.
- Singh SS, Siddhanta AK, Meena R, Prasad K, Bandyopadhyay S, Bohidar HB. Intermolecular complexation and phase separation in aqueous solutions of oppositely charged biopolymers. *International Journal of Biological Macromolecules*. 2007; 41(2):185–192. [PubMed: 17367849]
- Trejo R, Dokland T, Jurat-Fuentes J, Harte F. Cryo-transmission electron tomography of native casein micelles from bovine milk. *Journal of Dairy Science*. 2011; 94(12):5770–5775. [PubMed: 22118067]
- Turgeon SL, Schmitt C, Sanchez C. Protein-polysaccharide complexes and coacervates. *Current Opinion in Colloid & Interface Science*. 2007; 12(4-5):166–178.
- Venir E, Marchesini G, Biasutti M, Innocente N. Dynamic high pressure-induced gelation in milk protein model systems. *Journal of Dairy Science*. 2010; 93(2):483–494. [PubMed: 20105520]
- Ye AQ. Complexation between milk proteins and polysaccharides via electrostatic interaction: principles and applications - a review. *International Journal of Food Science and Technology*. 2008; 43(3):406–415.
- Ye R, Harte FM. Casein maps: Effect of ethanol, pH, temperature, and CaCl₂ on the particle size of reconstituted casein micelles. *Journal of Dairy Science*. 2013; 96(2):799–805. [PubMed: 23200467]

Figure 1.
Effect of high pressure homogenization on the particle size of HPC- casein complexes during 2 weeks of storage: (a) 80kDa; (b) 1150kDa

Figure 2.

Viscosity of HPC-Casein complexes as a function of treatment pressure during 2 weeks of storage: (a) 80kDa; (b) 1150kDa

Figure 3.

Visible evidence of HPC- casein complexes consisting of HPC (0.45%, w/v) and casein (3%, w/v): (a) 80kDa of HPC immediately after high pressure homogenization treatment; (b) 80kDa of HPC after 2 weeks of storage; (c) 1150kDa of HPC immediately after high pressure homogenization treatment; (d) 1150kDa of HPC after 2 weeks of storage

Figure 4.
Effect of high pressure homogenization on the percentage of the precipitation of HPC-casein complexes during 2 weeks of storage: (a) 80kDa; (b) 1150kDa

Figure 5.
Effect of high pressure homogenization on the turbidity of HPC- casein complexes during 2 weeks of storage: (a) 80kDa; (b) 1150kDa

Figure 6.
Effect of high pressure homogenization on the protein concentration of HPC-Casein complexes after 2 weeks of storage. Light grey: HPC (80kDa); dark grey: HPC (1150kDa). Bars are 95% confidence intervals for the mean

a

b

C

d **Figure 7.**

STEM images of the complexes consisting of 1150kDa of HPC (0.45%, w/v) and casein (3%, w/v) after 2 weeks of storage: (a) coacervation phase of the control with 1 μ m of the scale bar; (b) coacervation phase of the control with 100nm of the scale bar; (c) the complex treated with high pressure homogenization at~300Mpa with 1 μ m of the scale bar; (d) the complex treated with high pressure homogenization at~300Mpa with 100nm of the scale bar

Figure 8. Schematic representation of suggested mechanism for the formation of stable HPC – casein aggregates by high pressure homogenization