

Effect of high hydrostatic pressure and whey proteins on the disruption of casein micelle isolates

Federico M Harte¹, Subba Rao Gurram¹, Lloyd O Luedecke², Barry G Swanson² and Gustavo V Barbosa-Cánovas^{1*}

¹ Biological Systems Engineering Department, Washington State University, Pullman, WA 99164-6120 USA

² Department of Food Science and Human Nutrition, Washington State University, Pullman, WA 99164-6376 USA

Received 31 August 2005 and accepted for publication 18 June 2007

High hydrostatic pressure disruption of casein micelle isolates was studied by analytical ultracentrifugation and transmission electron microscopy. Casein micelles were isolated from skim milk and subjected to combinations of thermal treatment (85 °C, 20 min) and high hydrostatic pressure (up to 676 MPa) with and without whey protein added. High hydrostatic pressure promoted extensive disruption of the casein micelles in the 250 to 310 MPa pressure range. At pressures greater than 310 MPa no further disruption was observed. The addition of whey protein to casein micelle isolates protected the micelles from high hydrostatic pressure induced disruption only when the mix was thermally processed before pressure treatment. The more whey protein was added (up to 5 g/l) the more the protection against high hydrostatic pressure induced micelle disruption was observed in thermally treated samples subjected to 310 MPa.

Keywords: High hydrostatic pressure, casein, micelle, analytical ultracentrifugation.

Several studies have been conducted on the processing of milk using high hydrostatic pressure (HHP) for the manufacture of cheese (López-Fandiño, 1996; Drake et al. 1997; Trujillo et al. 2000) and yogurt (Needs et al. 2000a; Harte et al. 2002a). HHP technology has been mainly targeted at food safety improvement (Hoover, 2002) and to the change of textural and yield properties of dairy products through whey protein denaturation (Panick et al. 1999; Yang et al. 2001; Huppertz, 2002) and casein micelle disruption (Needs et al. 2000a,b; Gebhardt et al. 2005; Huppertz & De Kruijff, 2006).

Extensive research has been conducted on the denaturing effect of HHP on whey protein isolates in order to alter the functional properties of this by-product of the cheese-making industry (Schrader & Buchheim, 1998; Panick et al. 1999; Scollard et al. 2000; Yang et al. 2001; Huppertz et al. 2002, 2004a). Anema & Li (2003a) and Gulbrandsen et al. (2000) reported increase (15%) in casein micelle size for thermally heated skim milk without high pressure. The level of association of whey proteins with the casein micelles depends on the conditions under which milk was heated, pH of milk (Anema & Li, 2003a), and calcium ion activity (Gulbrandsen et al. 2000).

The other important protein components of milk, i.e. the caseins, have recently received as much attention as the whey proteins and most research on the effect of HHP on casein micelles has been conducted with skim milk using methods such as absorbance and lightness (Buchheim et al. 1996; Gaucheron et al. 1997), or by direct observation of micelles under transmission electron microscopy (TEM; Gaucheron et al. 1997; Needs et al. 2000b; Keenan et al. 2001). The change in size induced by HHP has been measured by light scattering (Kelly et al. 2002) and analytical ultracentrifugation (Harte et al. 2002b). Most recently, the effect of HHP on casein micelles was studied by dynamic light scattering (Gebhardt et al. 2005) and photon correlation spectroscopy (Anema & Li, 2003a; Huppertz et al. 2004a,b,c; Regnault et al. 2004; Anema et al. 2005).

Sedimentation velocity methods are particularly suitable for casein micelle size determination since casein micelles are considered as nearly spherical and non-interactive aggregates (Dalgleish, 1998). Different mechanisms have been suggested to explain the HHP induced increase in casein micelle size. Interaction of denatured β -lactoglobulin (β -lg) with the casein micelles by high pressure increased the casein micelle size (Schrader & Buchheim, 1998; Huppertz et al. 2004a). The thermal or HHP denaturation of whey proteins and their subsequent interaction with κ -casein may protect the micelle from

*For correspondence; e-mail: barbosa@wsu.edu

being disrupted by high pressure (Harte et al. 2002b; Kelly et al. 2002) or may aggregate the casein micelles (Huppertz et al. 2004a). Treatments below 250 MPa increased micelle size but size was decreased above 300 MPa by 50% (Needs et al. 2000b; Huppertz et al. 2004a,b).

Understanding the factors affecting the disruption of casein micelles is important because the tailoring of casein micelles size may improve textural, rheological, and whey retention properties of dairy products. Furthermore, the reversible dissociation of casein micelles is a promising functional property for the stabilization of hydrophobic compounds (such as flavours and drugs) in foods.

The objective of this research was to study the disruption effect of HHP on heated and non-heated casein micelle isolates with and without the presence of whey proteins.

Materials and methods

Milk supply and preparation of casein micelle suspension

Raw whole milk was purchased from the WSU creamery and skimmed by centrifugation at $20\,000 \times g$ for 15 min at 15 °C. The skim milk samples were centrifuged at $100\,000 \times g$ for 1 h 40 min at 25 °C using an L8-70 ultracentrifuge and a 70Ti rotor (Beckman Instruments, Inc., Palo Alto, California, USA) to separate the whey and casein fractions. After centrifugation, the supernatant was discarded and the casein micelle clumps were suspended by stirring overnight at 5 °C in milk permeate obtained from ultra-filtered raw milk using a 10 KDa molecular weight cut-off ultra filter (Romicon, model HF-LAB-5, Koch membrane systems, Inc., Wilmington, Massachusetts, USA).

An absorbance pattern master curve (not shown in this manuscript) was created and used to adjust the casein micelles concentration to keep a constant volume fraction of casein micelles. After the micelle fraction was re-suspended in milk permeate, the separation of the casein fraction from the whey proteins was confirmed by SDS-PAGE electrophoresis using an 8–16% acrylamide Tris-HCl ready gel (Bio-Rad, Hercules, California, USA).

High hydrostatic pressure treatment

The casein micelle isolates, with or without thermal treatment (85 °C, 20 min), were subjected to HHP from 0 to 676 MPa (come-up time only, at room temperature) using an isostatic pressing system (Engineered Pressure Systems, Inc., Haverhill, Massachusetts, USA) having a cylindrical pressure chamber of 125 mm³ effective volume. The same experiments were conducted but with the addition of 5 g whey protein isolate/l (BiPRO®, Davisco Foods International, Inc., Le Sueur, Minnesota, USA) before any thermal or pressure treatment to study the effect of whey proteins on the disruption of casein micelles. To determine the effect of different levels of denatured whey proteins

on the HHP induced disruption of casein micelles, 1 to 5 g whey protein isolate/l was added to the casein micelle isolate, which was then subjected to thermal treatment (85 °C, 20 min) and HHP. The experiments were analysed as a randomized complete block design with two replicates.

Micelle size determination by sedimentation velocity

Casein micelle size determination was done by sedimentation velocity analytical ultracentrifugation using a Beckman ultracentrifuge (Beckman Coulter, Fullerton, California, USA). Three main forces act upon a micelle subjected to a strong centrifugal field (van Holde et al. 1998). The three force vectors are the buoyancy force (F_B), centrifugal force (F_C), and drag force (F_D). These vectors add to zero in order to reach a constant terminal sedimentation velocity,

$$\vec{F}_C + \vec{F}_B + \vec{F}_D = 0 \quad (1)$$

The centrifugal field is given by $\vec{F}_C = V_m \rho_m \omega^2 r$, where the mass of the casein micelle is the product of its volume (V_m) times its density (ρ_m) and the acceleration is given by the product of the centrifugal rotational speed (ω^2) times the position (r) of the particle at a given time relative to the center of the spinning element. Similarly, the buoyancy force is given by $\vec{F}_B = V_m \rho_f \omega^2 r$, where the mass of fluid displaced by the micelle is the volume of the micelle times the fluid's density (ρ_f). If we assume the casein micelle is a solid sphere and laminar flow sedimentation in a Newtonian fluid, the drag force (F_D) is expressed as $\vec{F}_D = -6\pi v_t \eta R$, where v_t is the terminal velocity of the micelle, η is the viscosity of the medium, and R is the radius of the micelle. In this way, eqn (1) can be expressed as:

$$V_m \omega^2 r (\rho_m - \rho_f) - 6\pi v_t \eta R = 0 \quad (2)$$

Expressing the Volume of the micelle in terms of its radius and rearranging eqn (2), then

$$\frac{R^2 2(\rho_m - \rho_f)}{9\eta} = \frac{v_t}{\omega^2 r} = s \quad (3)$$

where s is the Svedberg coefficient, which can be determined experimentally by:

$$\frac{v_t}{\omega^2 r} = \frac{dr}{dt} \cdot \frac{1}{\omega^2 r} = s \Rightarrow s \omega^2 \int_{t_0}^{t_1} dt = \int_{r_0}^{r_1} \frac{1}{r} dr \quad (4)$$

Solving the integral from time t_0 to t_1 ,

$$\text{Ln} \left[\frac{r_1}{r_0} \right] = s \omega^2 (t_1 - t_0) \quad (5)$$

In this way, by knowing the position of the casein micelles at each time, the Svedberg coefficient can be found as the

slope of the $\ln(r)$ vs. t line (Van Holde et al. 1998). The position of the micelles is obtained from the mid point of successive absorbance curves during centrifugation of the sample containing the micelles Vs the reference, in this case the permeate from ultrafiltrated (10 kDa MWCO) milk. The samples were spun in a 4-place rotor at 25 °C. Since the size of the micelles was greatly affected by the high pressure treatments, sedimentation velocity was done at various rotational speeds (2500 to 3000 rpm) and two wavelengths (400 and 500 nm).

Micelle size determination by Transmission Electron Microscopy

Casein micelles from selected treatments were observed under TEM. A mix of casein micelles suspension (1 : 1 (v/v) and 3% (w/v) agar in water at 43 °C was prepared. After solidification by cooling to ~5 °C, 1 mm³ cubes were cut and submerged in 0.05 M-PIPES buffer (pH 7.2) containing 12.5 g glutaraldehyde/l and 20 g paraformaldehyde/l for 24 h at 4 °C for fixation. After three washes (10 min each) in PIPES buffer, the cubes were dehydrated through serial 10 min washes in 30, 50, 70, and 95% ethanol in distilled water. Dehydration was completed with three 10 min washes in 100% ethanol. The fixed samples were infiltrated using a 1 : 1 (v/v) medium grade LR White resin (London Resin Company, Ltd., Reading, England) in 100% ethanol for 12 h at 4 °C. The cubes were left in 100% LR White for 12 h at 4 °C (three times) and cured in 100% LR White for 12 h at 60 °C. Ninety nanometer sections of the fixed and cured samples were obtained using a Reichert ultracut microtome (Leica Microsystems Inc., Chicago, Illinois, USA). The grids with samples were stained with 4% uranyl acetate and Sato's lead stain and examined with a transmission electron microscope, Joel 1200 EX JEM (Joel Ltd., Akishima, Japan) operating at 80 kV.

Results and Discussion

Heat and high pressure induced changes are increasingly becoming important in understanding the functional properties of dairy products for the commercialization of HHP processing technology. The results of this paper add to the current knowledge about the effects of high pressure on casein micelles in the presence of whey proteins. Significant differences in the Svedberg coefficient were not observed for casein micelle isolates after treatment with HHP up to 200 MPa (Fig. 1a). The observation that treatment at pressures up to 200 MPa had no significant effect on casein micelle size was in accordance with the results reported by Needs et al. (2000b) and by Huppertz et al. (2004a). However, a 10-fold reduction in the Svedberg coefficient was observed when casein micelle isolates were subjected to pressure above 310 MPa, with no further reduction even at maximum applied pressure (676 MPa). When the casein micelle isolates were subjected to thermal

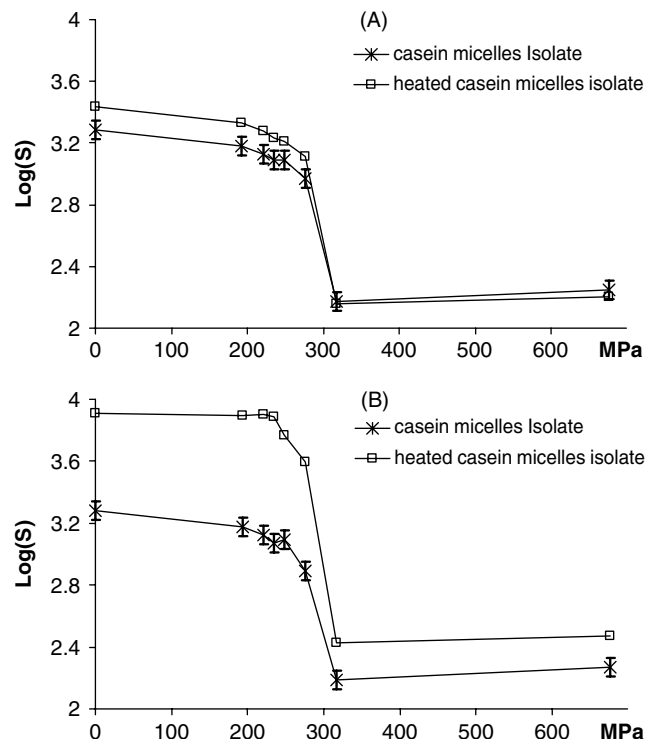


Fig. 1. Effect of different high hydrostatic pressure treatments on casein micelle's Svedberg coefficient (S). (A) No whey protein added, (B) 5 g whey protein/l added. Bars are 95% confidence intervals for the mean.

treatment (85 °C, 20 min) before HHP, a small initial increase in size was observed, but the disruption pattern induced by the HHP treatment was similar to that of casein micelle isolates with no previous thermal treatment. Furthermore, Svedberg coefficient values were the same when the casein micelle isolates were subjected to pressures greater than 300 MPa, regardless of previously applied thermal treatment. Preliminary experiments showed no difference in Svedberg coefficient values for casein micelle isolates subjected to 400, 500, and 600 MPa, compared with those subjected to 676 MPa. These results were in good agreement with turbidity patterns in skim milk reported by Buchheim et al. (1996). The addition of 5 g whey protein/l to the casein micelle isolate did not affect the disruption pattern induced by the HHP treatment (Fig. 1b) when no previous thermal treatment was applied to the mixture. However, when the casein micelle isolates and whey protein mix was thermally treated (85 °C, 20 min), an initial 3-fold increase in the Svedberg coefficient was observed and no significant reduction in the Svedberg coefficient was observed for pressure up to 250 MPa. Furthermore, in the 310 to 676 MPa range, the thermally treated casein micelle isolates containing whey proteins exhibited higher Svedberg coefficient values than the casein micelle isolates, with or without thermal treatment, or the casein micelle samples containing whey proteins but without thermal treatment.

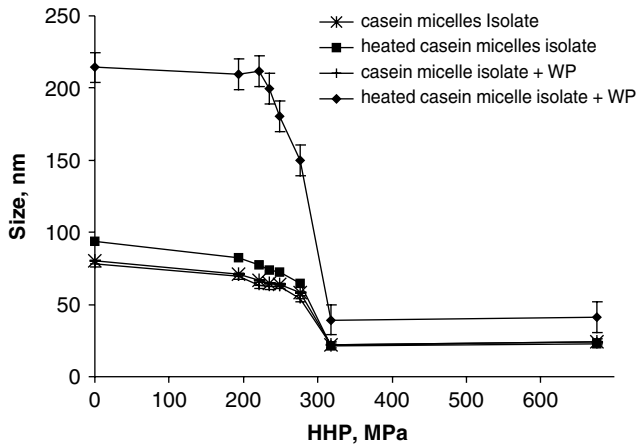


Fig. 2. Effect of different high hydrostatic pressure treatments on casein micelle calculated diameter. WP: whey protein. Bars are 95% confidence intervals for the mean.

Contrary to reports by Gaucheron et al. (1997) for skim milk at 20 to 40 °C, no increase in size of the casein micelles was observed in the 200 to 300 MPa range. As reported by Huppertz et al. (2004a), the final size of micelles pressurized in the 200 to 300 MPa range is both time and temperature dependant, and may be the result of whey protein denaturation and interaction with casein micelles occurring simultaneously to micelle disruption. Gaucheron et al. (1997) observed similar patterns in whey-free milk at 40 °C, suggesting that hydrophobic interaction may also play a role in the pressure-induced increase in size of casein micelles. Since our experiments were done at both lower temperature (~25 °C) and shorter pressure times (seconds), we were not able to observe this phenomenon.

Based on several transmission electron micrographs for casein micelle isolates, the average diameter of the casein micelles was determined as ~80 nm. This was smaller than values reported elsewhere, by Needs et al. (2000a,b; by TEM), Fox & McSweeney (1998), and Regnault et al. (2004; by photon correlation microscopy and atomic force microscopy), and may be the result of (1) different milk, since casein micelle size varies depending on the milk source, (2) the high centrifugal force (20 000 × *g*) used when skimming the milk causing a small precipitate of bigger micelles to be discarded, leaving an increased proportion of smaller micelles in suspension.

The Svedberg coefficient values were transformed to micelle diameter by solving for the variable radius (*R*) in the sedimentation velocity equation and assuming (1) initial mean diameter of micelles ~80 nm before treatment, (2) no changes in the density of micelles and permeate after treatments, and (3) no changes in viscosity of the permeate in all cases but the thermally treated permeate containing whey proteins. The estimated diameter values obtained based on the sedimentation equation (Fig. 2) was in close agreement with the casein micelle

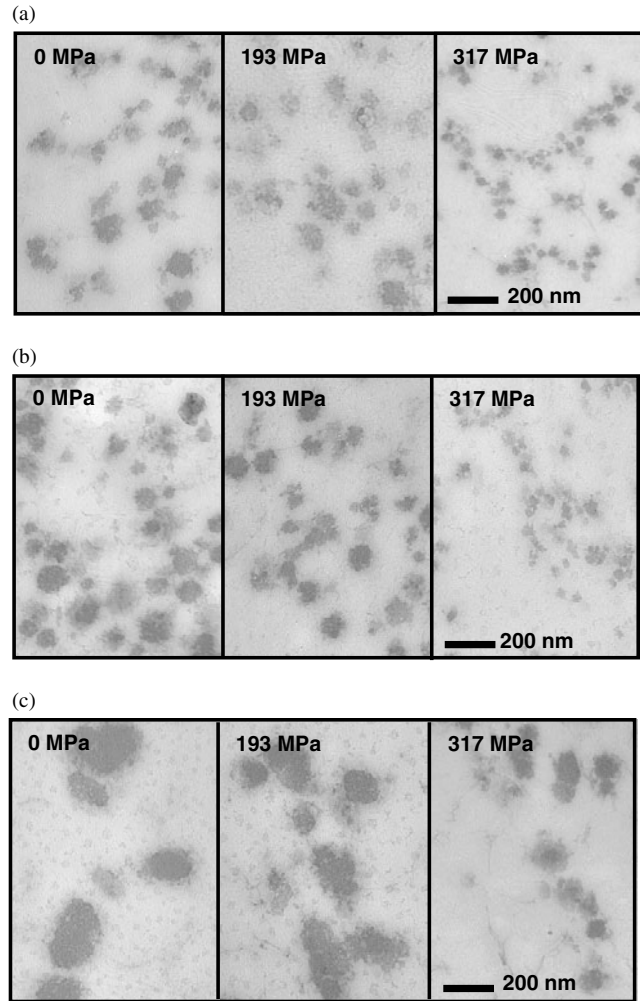


Fig. 3. Transmission electron microscopy of (a) casein micelle isolates subjected to different levels of high hydrostatic pressure; (b) thermally (85 °C, 20 min) treated casein micelle isolates subjected to different levels of high hydrostatic pressure; (c) thermally (85 °C, 20 min) treated casein micelle isolates and whey protein isolate subjected to different levels of high hydrostatic pressure.

diameter observed in the transmission electron micrographs (Fig. 3a & b).

The diameter of casein micelle isolates was not markedly affected by thermal treatment but micelles were disrupted by pressure in the 250 to 310 range. Gulbrandson et al. (2000), and Anema & Li (2003a), observed only small changes (<15%) in casein micelle size when milk samples were heated to 90 °C and above without HHP. The average diameter of casein micelles after HHP of 300 MPa or greater was ~21 nm, regardless of being previously subjected to thermal treatment. These casein micelles are larger than the micelles measured by dynamic light scattering (3 nm at 300 MPa; Gebhardt et al. 2005) and smaller than the micelles measured by atomic force microscopy (40 nm to 80 nm at 300 MPa; Regnault et al.

2004) or by photo correlation spectroscopy (≤ 50 nm at 300 MPa; Regnault et al. 2004). Regnault et al. 2004 also reported that the micellar sub-units formed due to high pressure at 300 MPa are of 30 nm in size and are larger than the sub-micelles measured in unpressurized bovine milk by neutron scattering (13 nm; Stothart & Cebula, 1982) or by TEM of freeze-fractured cow's milk samples (10 nm; Schmidt & Buchheim, 1970). These differences in micelle size are may be due to the separation techniques (centrifugation $20\,000 \times g$ for 15 min and $100\,000 \times g$ for longer times (1 h 40 min), different temperatures, and/or the analysis techniques (TEM: procedure dehydrates the sample). Higher g forces increase the level of whey protein deposited with the pellet without increasing the levels of casein deposited, indicating that larger whey protein aggregates were being deposited (Anema & Li, 2003b). Addition of urea to milk dissociates the casein micelles, disrupting hydrophobic and hydrogen bonds in the micelles without rupturing phosphate linkages according to Huppertz et al. (2004b); they reported considerable changes only in larger casein micelle fractions (220 to 150 nm) with high pressure treatment (250 MPa for 30 min) but not in small casein micelle fractions (118 nm). Huppertz & Kruif (2006) also concluded that micelle stability against HHP induced disruption increases with increasing casein micelle concentration as micelle calcium phosphate increases. In the present study, the addition of whey proteins did not affect the HHP induced disruption provided there was no thermal treatment; however adding whey proteins after a thermal treatment increased the casein micelle size. The increase in casein micelle size (formation of casein micelle aggregates) could be due to (1) Extensive formation of hydrophobic bonds formed between submicellar particles and/or (2) Formation of complexes (hydrophobic, disulphide or covalent bonds) between whey proteins and caseins.

In the case of thermally treated casein micelles containing whey proteins, the diameter of the micelles was calculated correcting eqn 3 to show an increase in viscosity from 1 mPa.s in the medium with no whey proteins to 1.8 mPa.s in the medium containing thermally denatured whey proteins. Viscosity was measured in thermally treated whey containing milk permeate using a rheometer (Model MCR300, Paar-Physica, Ashland, Virginia, USA). Calculated diameter for thermally treated micelles containing whey proteins (Fig. 2) showed good agreement with observations under TEM for pressures up to 250 MPa (Fig. 3c). However, calculated diameters were smaller than observed under TEM for thermally treated micelles containing whey proteins and then disrupted by HHP higher than 310 MPa.

This deviation of calculated versus observed values was caused by three factors: (1) the increase in micelle roughness due to whey protein and κ -casein disulphide interaction promoted by thermal treatment, (2) deviations from sphericity of individual or coalesced micelles and micelles disrupted by HHP (Fig. 3c), and (3) sample

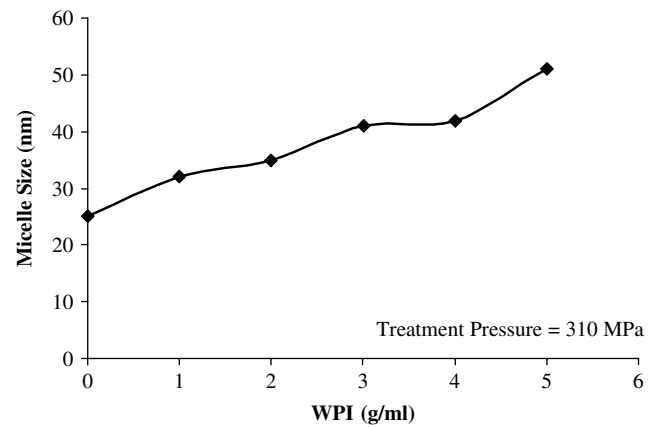


Fig. 4. Disrupting effect of high hydrostatic pressure on heated casein micelles isolate containing various levels of whey protein isolate (WPI).

dehydration during sample preparation for electron microscopy, which causes extensive reduction in size (Pierre et al. 1995). Factors (1) and (2) would decrease the sedimentation velocity of the casein micelles, promoting sub-estimation of the real casein micelle diameter. Other factors, such as individual caseins and CaPO_3 liberation into the serum, might increase the viscosity and thus decrease the sedimentation velocity of the micelles. However, factors (2) and (3) were not considered as important since they would also affect non-heated micelles in which the diameters were well calculated.

In general two possible theories have been suggested to explain HHP induced increase in casein micelle size, the interaction of denatured β -lg with the casein micelles (Schrader & Buchheim, 1998; Huppertz et al. 2004a) or the aggregation of casein micelles (Huppertz et al. 2004a,b). Results from this study support the former theory, i.e., interaction of denatured β -lg with the casein micelles with the application of heat before high pressure. Clearly, further studies on micelle structure and size are necessary to confirm the exact aggregation or disaggregating mechanisms of casein micelles in HHP processing, with or without heat.

An intermediate pressure (310 MPa) was selected to study the protective effect of different levels of thermally denatured whey proteins on the disruption of casein micelles subjected to HHP. The increase in viscosity of the medium induced by increasing levels of denatured whey proteins was taken into consideration for calculation of the diameter based on the sedimentation velocity equation. As seen in Fig. 4, increased levels of denatured whey proteins interacting with the casein micelles, protected the micelles from disruption at intermediate pressure. This is in contrast with the claim that the process is primarily driven by the extent of solubilization of micelle calcium phosphate (Huppertz et al. 2006). Increase in roughness or deviations from sphericity may also affect the calculated diameters in such intermediate pressure. The calculated

diameters should be considered as bottom line estimates of the real diameters of casein micelles since, as discussed before, values from the sedimentation equation may underestimate the real diameter of the micelles in systems containing thermally denatured whey proteins. Furthermore, the studies at different pressures are necessary to confirm the above conclusion, that by adding whey proteins to casein micelle isolate, the micelles are protected from disruption. It is worth investigating at different pressures and temperatures the utilization of whey proteins, which could yield unique product characteristics not achievable by any other means at present.

Conclusions

HHP in the 250 to 310 MPa range promotes a rapid disruption or disaggregation of casein micelles isolates into small casein micelles. The addition of whey proteins and their interaction (promoted by thermal denaturation) with the casein micelles protects the micelle from extensive HHP induced disruption. Furthermore, the higher the concentration of whey proteins, the more the protection to disruption of casein micelles is observed. Therefore the change in functional properties of casein micelles induced by HHP cannot be considered as independent from other factors such as milk composition and temperature.

References

- Anema SG & Li Y 2003a Effect of pH on the association of denatured whey proteins with casein micelles in heated reconstituted skim milk. *Journal of Agricultural Food Chemistry* **51** 1640–1646
- Anema SG & Li Y 2003b Association of denatured whey proteins with casein micelles in heated reconstituted skim milk and its effect on casein micelle size. *International Dairy Journal* **70**(1) 73–83
- Anema SG, Lowe KE & Stockmann R 2005 Particle size changes and casein solubilization in high pressure treated skim milk. *Food Hydrocolloids* **19** 257–267
- Buchheim W, Schrader K, Morr CV, Frede E & Schutt M 1996 Effects of high pressure on the protein, lipid and mineral phase of milk. In *Heat treatments & alternative methods*, pp. 202–213 (Ed. PF Fox). Brussels: International Dairy Federation
- Dalgleish DG 1998 Casein micelles as colloids: surface structures and stabilities. *Journal of Dairy Science* **81**(11) 3013–3018
- Datta N & Deeth HC 1999 High pressure processing of milk and dairy products. *The Australian Journal of Dairy Technology* **54**(1) 41–49
- Drake MA, Harrison SL, Asplund M, Barbosa-Cánovas GV & Swanson BG 1997 High-pressure treatment of milk and effects on microbiological and sensory quality of Cheddar cheese. *Journal of Food Science* **62**(4) 843–860
- Fox PF & McSweeney PLH 1998 *Dairy Chemistry and Biochemistry*. New York: Chapman & Hall
- Gaucheron F, Famelart MH, Mariette F, Raulot K, Michel F & Le Graet Y 1997 Combined effects of temperature and high-pressure treatments on physicochemical characteristics of skim milk. *Food Chemistry* **59**(3) 439–447
- Gebhardt R, Doster W & Kulozik U 2005 Pressure-induced dissociation of casein micelles: size distribution and effect of temperature. *Brazilian Journal of Medical and Biological Research* **38** 1209–1214
- Gulbrandsen TD, Jansen MB, Langsrud T & Elisabeth GV 2000 Size of native and heated casein micelles, content of protein and minerals in milk from Norwegian Red Cattle-effect of milk protein polymorphism and different feeding regimes. *International Dairy Journal* **10** 313–323
- Harte F, Amonte M, Luedecke L, Swanson BG & Barbosa-Cánovas GV 2002a Yield stress and microstructure of set yogurt made from high hydrostatic pressure-treated full fat milk. *Journal of Food Science* **67**(6) 2245–2250
- Harte FM, Luedecke LO, Swanson BG & Barbosa-Cánovas GV 2002b Disrupting effect of high hydrostatic pressure on casein micelle isolates. *IFT Annual Meeting Technical Program Abstracts* 130
- Hoover DG 2002 Microbial inactivation by high pressure. In *Control of Foodborne Pathogens*, pp. 419–449 (Eds VK Juneja & JN Sofos). New York: Marcel Dekker
- Huppertz T, Kelly AL & Fox PF 2002 Effects of high pressure on constituents and properties of milk. *International Dairy Journal* **12**(7) 561–572
- Huppertz T, Fox PF & Kelly AL 2003 High pressure induced changes in the creaming properties of bovine milk. *Innovative Food Science and Emerging Technologies* **4** 349–359
- Huppertz T, Fox PF & Kelly AL 2004a High pressure treatment of bovine milk: effects of casein micelles and whey proteins. *Journal of Dairy Research* **71** 97–106
- Huppertz T, Fox PF & Kelly AL 2004b Properties of casein micelles in high pressure-treated bovine milk. *Food Chemistry* **87** 103–110
- Huppertz T, Grosman S, Fox PF & Kelly AL 2004c Heat and ethanol stabilities of high-pressure-treated bovine milk. *International Dairy Journal* **14** 125–133
- Huppertz T & Kruif CG 2006 Disruption of casein micelles under high pressure: Influence of milk serum composition and casein micelle concentration. *Journal of Agriculture and Food Chemistry* **54** 5903–5909
- Keenan RD, Young DJ, Tier CM, Jones AD & Underdown J 2001 Mechanism of pressure-induced gelation of milk. *Journal of Agricultural and Food Chemistry* **49**(7) 3394–3403
- Kelly AL, Huppertz T & Fox PF 2002 Structural properties of casein micelles in high-pressure-treated milk. *Technical Program Abstract 197 IFT Annual Meeting*
- López-Fandiño R, Carrascosa AV & Olano A 1996 The effects of high pressure on whey protein denaturation and cheese-making properties of raw milk. *Journal of Dairy Science* **79**(6) 929–936
- Needs EC, Capellas M, Bland AP, Manoj P, Macdougall D & Paul G 2000a Comparison of heat and pressure treatments of skim milk, fortified with whey protein concentrate, for set yogurt preparation: effects on milk proteins and gel structure. *Journal of Dairy Research* **67**(3) 329–348
- Needs EC, Stenning RA, Gill AL, Ferragut V & Rich GT 2000b High-pressure treatment of milk: effects on casein micelle structure and on enzymic coagulation. *Journal of Dairy Research* **67**(1) 31–42
- Panick G, Malessa R & Winter R 1999 Differences between the pressure- and temperature-induced denaturation and aggregation of beta-lactoglobulin A, B, and AB monitored by FT-IR spectroscopy and small-angle X-ray. *Biochemistry* **38**(20) 6512–6519
- Pierre A, Michael F & Le Graet Y 1995 Variation in size of goat milk casein micelles related to casein genotype. *Lait* **75** 489–502
- Regnault S, Thiebaut M, Dumay E & Cheftel JC 2004 Pressurization of raw skim milk of a dispersion of phosphocaseinate at 9 °C or 20 °C: effects on casein micelle size distribution. *International Dairy Journal* **14** 55–68
- Schmidt DG & Buchheim W 1970 An electron-microscopy investigation of substructure of the casein micelles in cow's milk. *Milchwissenschaft* **25** 596–600
- Schrader K & Buchheim W 1998 High pressure effects on the colloidal calcium phosphate and the structural integrity of micellar casein in milk II. Kinetics of the casein micelle disintegration and protein

- interactions in milk. *Kieler Milchwirtschaftliche Forschungsberichte* **50** 79–88
- Scollard PG, Beresford TP, Needs EC, Murphy PM & Kelly AL** 2000 Plasmin activity, β -lactoglobulin denaturation and proteolysis in high pressure treated milk. *International Dairy Journal* **10** 835–841
- Stohtart PH & Cebula DJ** 1982 Small-angle neutron scattering study of bovine casein micelles and sub-micelles. *Journal of Molecular Biology* **160**(2) 391–395
- Trujillo AJ, Capellas M, Buffa M, Royo C, Gervilla R, Felipe X, Sendra E, Saldo J, Ferragut V & Guamis B** 2000 Application of high pressure treatment for cheese production. *Food Research International* **33**(3–4) 311–316
- Van Holde KE, Johnson WC & Ho PS** 1998 *Principles of Physical Biochemistry*, pp. 195–198. New York: Prentice Hall
- Yang J, Dunker AK, Powers JR, Clark S & Swanson BG** 2001 β -Lactoglobulin molten globule induced by high pressure. *Journal of Agricultural and Food Chemistry* **49**(7) 3236–3243