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Pressurisation of raw skim milk and of a dispersion of phosphocaseinate at 9 °C or 20 °C: effects on the distribution of minerals and proteins between colloidal and soluble phases

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The effects of high pressure treatments (100–300 MPa; 15 min; 9 °C or 20 °C) on the distribution of minerals and proteins of raw skim milk (RSM) and of a dispersion of industrial phosphocaseinate (PC) were studied after separation of the micellar and soluble phases by ultracentrifugation (UCF). Whatever the temperature of high pressure treatments, the pressure-induced dissociation of the casein micelles was accompanied by calcium (Ca), phosphorus (P) and casein release from the micelles. The released Ca and P were or became bound to soluble proteins since progressive increases in Ca and P concentrations were observed in the UCF supernatants of RSM and of the PC dispersion but not in the ultrafiltrates from these UCF supernatants (free of soluble proteins). Simultaneously, α_{S1}-, α_{S2}-, β- and κ-caseins were progressively released from the micelles, as seen by electrophoretic analysis. The pressure-induced solubilisation of α_{S1}- and α_{S2}-caseins, essentially located in the core of the micelles, suggests that high pressure destabilised micelles including their internal structure.

Keywords: High pressure, raw skim milk, phosphocaseinate, calcium, phosphorus, caseins, β-lactoglobulin.

Casein micelles contain four types of individual caseins, at a molecular ratio of approximatively $\alpha_{S1}:\alpha_{S2}:\beta:\kappa = 4:1:3{\cdot}7:1{\cdot}3$. These phosphoproteins are able to bind bivalent cations, especially calcium. Two-thirds of calcium (Ca) are in colloidal state, either directly bound to the phosphoseryl, glutamyl and aspartyl residues (Byler & Farrell, 1989) or integrated in the colloidal calcium phosphate complex (CCP). The CCP plays a major role in stabilizing casein micelles and its structure is not exactly known (Schmidt, 1982; Holt et al. 1989; Van Dijk, 1990; Tarodo de la Fuente et al. 1999). The remainder of Ca is in soluble state, either in the form of calcium salts (citrate or phosphate), or in ionised form. Mineral and casein equilibria between the colloidal and soluble phases of milk are influenced by temperature, pH or ionic strength (Pierre & Brulé, 1981; Dalgleish & Law, 1989; Walstra, 1990; Le Graet & Brulé, 1993; Law, 1996).

High pressure (HP) treatments are also able to influence proteins and minerals in milk. The pressure-induced denaturation of whey proteins and dissociation of casein micelles have been most investigated (Cheftel & Dumay, 1998; Huppertz et al. 2002, 2004a, c; Needs, 2003; Regnault et al. 2004). The pressure-induced dissociation of casein micelles is accompanied by mineral release in the aqueous (or soluble) phase of milk. The level of soluble Ca increased after treatment of reconstituted skim milk (Desobry-Banon et al. 1994; Gaucheron et al. 1997) or raw skim milk (RSM) (López-Fandiño et al. 1998) at 200–300 MPa and 20 °C for 10–30 min, then remained constant (Gaucheron et al. 1997) or decreased (Desobry-Banon et al. 1994; López-Fandiño et al. 1998) at higher pressure (400–600 MPa). The pressure-induced increases in soluble Ca in raw or reconstituted skim milk was accompanied by corresponding increases in soluble P (Gaucheron et al. 1997; López-Fandiño et al. 1998). All the above results have been obtained after pressure treatment. Nevertheless, a great variability in published data was observed and probably resulted not only from the milk characteristics (race, breeding conditions and lactation stage of the cows) but also from the thermal history of the milk before and after pressure processing and the characteristics of the pressurisation (pressure level, real temperature of sample during pressurisation). The increases

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in soluble Ca and P observed after HP treatment were generally attributed to the dissolution of CCP.

The protein distribution between the colloidal and soluble phases of milk is also affected by high pressure. The concentrations of individual caseins, as determined by capillary or gel electrophoresis in the soluble phase of RSM pressure-treated at 20 °C for 30 min, increased with the pressure level up to 250–300 MPa, then slightly decreased at higher pressure levels (400–600 MPa) (López-Fandiño et al. 1998; Huppertz et al. 2004b). Recently, Anema et al. (2005) reported that pressurisation of reconstituted skim milk at >200 MPa for 15–30 min induced less release of non-sedimentable caseins in the soluble phase after pressurisation at 10 °C than at 20 °C. However, in previous studies, Gaucheron et al. (1997) did not detect any change in the concentration of soluble casein (as analysed by chromatography) after pressurisation of reconstituted skim milk at 250, 450 or 600 MPa and 4, 20 or 40 °C for 30 min. Other studies have dealt with the effects of high pressure on whey proteins in milk (skim or whole milk). α-Lactalbumin and bovine serum albumin were resistant to pressurisation up to 400 MPa and 20–25 °C, while β-lactoglobulin was pressure-sensitive for treatments at ⩾250 MPa and 20–25 °C (López-Fandiño et al. 1996; López-Fandiño & Olano, 1998; Scollard et al. 2000; Needs et al. 2000; Huppertz et al. 2004a).

In a previous study (Regnault et al. 2004), it was found that treatment of RSM or of a phosphocaseinate (PC) dispersion at 100–150 MPa and 9 °C or 20 °C induced an increase in hydrodynamic volume of the main micelle population, as determined by photon correlation spectroscopy (PCS). At higher pressure levels (200–300 MPa), a progressive micelle dissociation occurred as the pressure level increased at 9 °C, while both micelle dissociation and aggregation phenomena occurred at 20 °C. Formation of large-sized micelles at 250 MPa and 20 °C have been confirmed by Huppertz et al. (2004c). Treatment at 300 MPa induced irreversible changes in casein micelles, with resulting diameters half the size of the original micelles, at 9 °C as at 20 °C (dissociation predominant over aggregation), as observed by PCS and atomic force microscopy. In the present study, soluble Ca, P and protein constituents have been determined in pressurised RSM and PC dispersions in order to obtain further insights into micellar dissociation/re-association mechanisms. Pressure treatments have been carried out for 15 min at different pressure levels (100 to 300 MPa) and 9 °C comparing to 20 °C. Few studies have been carried out on the distribution of minerals and proteins between the colloidal and soluble phases of pressure-treated milk at low temperatures (<10 °C). From a sanitation view-point, it is important to keep raw milk at 4–10 °C during the whole industrial process to avoid microbial growth. The dispersion of industrial PC was also studied because of potential applications as a functional dairy ingredient.

Materials and methods

Raw skim milk and dispersion of phosphocaseinate

Raw bovine milk, stored at 4 °C for 24 h, was obtained from a local dairy farm (Chazalmartin, Nîmes, 30000, France). It was centrifuged at 2000 ***g*** and 4 °C for 20 min (Sorvall centrifuge RC5B plus, SS-34 rotor). The cream layer was removed and the operation was repeated a second time. The resulting RSM (pH 6·79 ± 0·05 at 20 °C) was stored at 4 °C overnight. The industrial PC powder (Promilk 852A, Ingredia, Arras, 62000, France) contained 56 g water/kg and, on dry basis, 831 g protein nitrogen/kg, ~58 g lactose/kg and ~85 g minerals/kg including 24 g Ca/kg. PC was dispersed in simulated milk ultrafiltrate (SMUF) prepared without lactose and adjusted to pH 6·6 with 1 M-KOH (Jenness & Koops, 1962). The PC dispersion (32 g dried powder/l; pH 6·72 ± 0·05 at 20 °C) was stored at 4 °C overnight. Before pressurisation, it was heated in a water bath at 40 °C for 1 h to induce complete hydration and dispersion of the initial powder, and then cooled to ambient temperature.

Chemicals

All chemicals were of analytical grade. Calcium carbonate (Code 433185), sodium dihydrogen orthophosphate (Code 480087), di-sodium hydrogen orthophosphate (Code 480137) came from Carlo Erba (Farmitalia Carlo Erba, Milan, Italy). Lanthanum oxide (L-4000), L-ascorbic acid (A-5960), sodium molybdate (S-6646), sodium desoxycholate (10GM), bicinchoninic acid solution (BCA, B-9643), copper II sulphate pentahydrate solution (C-2284), SDS (L-4509), Tris (T-1503), ammonium persulphate (A-3678), EDTA (ED2SS), glycine (G-7126), urea (U-1250) and N,N′-methylenebisacrylamide (M-7279) came from Sigma (St-Louis, MO, USA). Purified protein, all from bovine milk and in freeze-dried form, also came from Sigma: α_s-casein (C-6780, lot 99H7431), β-casein (C-6905, lot 30K7442), κ-casein (C-0406, lot 19H7836), α-la (L-6010, lot 52F8075), β-lg (L-2506, lot 104H7200), serum albumin (A-2153, lot 58H0451) and a protein kit (SDS-6H, lot 53H9450) containing 6 protein markers (bovine serum albumin, egg albumin, pepsin, trypsinogen, β-lg and egg white lysozyme). Trichloroacetic acid (A4716801) was from Labosi (Elancourt, France), acrylamide (Code 1002) from Gerbu Trading GmbH (Gaiberg, Germany), coomassie blue (Code 17525) from Serva (Heidelberg, Germany). β-mercaptoethanol (MSH, Code 805740) and N,N,N′,N′-tetramethylethylenediamine (Temed, Code K17704332) were from Merck (Darmstadt, Germany).

High pressure treatment

HP treatment was carried out at 100–300 MPa in water as pressure transmitting medium (PTM) at 9 °C or 20 °C. Samples (40 g) of RSM or of PC dispersion were poured into flexible tubings (26 mm diameter, 50 μm thick,

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polyvinylidene chloride, Krehalon, Egalières, France) sealed with a double knot at the bottom and with a silicon stopper at the top. Samples were equilibrated for 1–2 h at the treatment temperature, then processed for 15 min at 100–300 MPa and 9 °C or 20 °C (±1 °C) in a 'high pressure-low temperature' 1 liter stainless steel vessel from ACB (Nantes, France), as already described (Regnault et al. 2004). Temperature and pressure recordings have been already detailed and the temperature and pressure profiles of samples and PTM during HP treatment given (Regnault et al. 2004).

Separation of soluble and colloidal phases of raw skim milk or of the phosphocaseinate dispersion

After pressurisation, RSM and PC dispersions were stored at 20 °C for 1–2 h. Soluble and colloidal phases were then separated by ultracentrifugation (UCF) at 149 000 ***g*** and 20 °C for 55 min (rotor Ti70, Beckman L7-65 ultracentrifuge, Beckman Instruments France S.A., Gagny, France). The UCF supernatant (soluble phase) was delicately removed for determination of soluble Ca, P and proteins.

Determination of calcium content

The Ca content of samples was determined by atomic absorption (Varian Spectra-100 spectrophotometer, Mulgrave, Australia). The detection of Ca was made at 422·7 nm in an air-acetylene flame with a Ca-Mg cathode lamp (25 mA). The calibration curve (from 0 to 10 mg Ca/l) was established from a solution of 25 g dried calcium carbonate/l in ~0·02 M-HCl. Interferences from phosphate ions were avoided by addition of a lanthanum oxide solution (118 g/l in 2·5 M-HCl) at a final concentration of 5·9 g/l. Ca content was determined (i) in control RSM and PC dispersions, (ii) in the UCF supernatant of control or pressurised samples, and (iii) in the ultrafiltrate from UCF supernatants of these samples. Ultrafiltration of UCF supernatants was carried out by centrifugation (swing-out rotor, MSE, London, UK) at 1500 ***g*** and ~20 °C for 30 min. Sample supernatants were placed in concentric tubes with polyethersulfone membrane of 10 kDa molecular weight cut-off (Vivascience, Hannover, Germany). For determining Ca concentration, control RSM and PC dispersions were diluted 500-fold, UCF supernatants and ultrafiltrates were diluted 100-fold, all in ultra-pure water in the presence of 5·9 g lanthanum oxide/l. Each sample was measured in triplicate. Results are the average of 9 measurements from 3 replicate experiments carried out on different days. Statistical analysis was carried out by the Student's test.

Determination of phosphorus content

The P content was determined colorimetrically by the molybdenum blue method according to the International Dairy Federation (1990), after mineralisation of 1 ml samples at 550 °C for 12 h. The calibration curve (from 0 to 1 mg P/l) was established with a solution of potassium dihydrogenphosphate in ultra-pure water. The molybdenum blue absorbance was measured at 820 nm using a Unicam UV2 spectrophotometer (Thermo-Optek, Montigny-le-Bretonneux, 78180, France). Each sample was measured in duplicate. Results are the average of 4 measurements from 2 replicate experiments carried out on different days. Statistical analysis was carried out by the Student's test.

Determination of the soluble protein content

Proteins were determined by the bicinchoninic acid (BCA) method (Smith et al. 1985) after protein precipitation by trichloroacetic acid to overcome interferences due to carbohydrates. The calibration solutions (from 0 to ~1 g protein/l) were prepared with proteins from Sigma (see section 2.2): (i) α_S-casein (mixture of α_{S1}+α_{S2}-caseins) for determining the protein content in RSM and PC dispersions and (ii) β-lg for determining the protein content in UCF supernatants. Control RSM and PC dispersions were diluted 75-fold, UCF supernatants from control or pressurised RSM were diluted 30-fold, and UCF supernatants from control or pressurised PC dispersions were diluted 10-fold in 50 mM-sodium phosphate buffer (pH 7). Proteins of calibration and sample solutions (100 µl), supplemented by 25 µl 1 mM-sodium desoxycholate, were then precipitated by centrifugation at 2200 ***g*** and 20 °C for 30 min (Sorvall centrifuge RC5B plus, SS-34 rotor) after adding 7 ml trichloroacetic acid (120 g/l in final). Precipitated proteins (0 to ~100 µg/pellet) were resuspended in 100 µl of the 50 mM-sodium phosphate buffer (pH 7·0). After addition of 2 ml of freshly prepared BCA reagent followed by incubation at 37 °C for 30 min, then cooling in tap water for 5 min to stop colour development, absorbance of the protein-BCA mixture was measured at 562 nm (Unicam UV2 spectrometer) in triplicate. Results are the average of 9 measurements from 3 replicate experiments. Statistical analysis was carried out by the Student's test.

Polyacrylamide gel electrophoresis

PAGE was carried out in a vertical slab apparatus (Pharmacia, Uppsala, Sweden) in the presence of dissociating and reducing agents, SDS and MSH, according to Funtenberger et al. (1995) with some modifications. The separating gel was prepared at 140 g acrylamide/l [acrylamide (A)/bisacrylamide (B) ratio of 22·5] in a pH 8·7 Tris-HCl buffer (0·41 M-Tris; 1 g SDS/l; 0·7 mM-ammonium persulphate; 1·1 mM-Temed). The stacking gel was prepared at 53 g acrylamide/l (same A/B ratio) in a pH 6·8 Tris-HCl buffer (0·1 M-Tris; 0·1 g SDS/l; 1·5 mM-ammonium persulphate; 2·2 mM-Temed). After adequate dilution in a pH 6·8 Tris-HCl buffer (0·1 M-Tris; 20 g SDS/l; 200 ml glycerol/l; 230 mM-MSH), samples of RSM or PC

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Table 1. Ca and P concentrations (mg/l) in the UCF supernatant of RSM or of the PC dispersion and their ultrafiltrate after HP treatment at 100–300 MPa for 15 min in the pressure transmitting medium at 20 °C or 9 °C

	20 °C				9 °C			
	Soluble calcium[1]		Soluble phosphorus[2]		Soluble calcium[1]		Soluble phosphorus[2]	
Pressure (MPa)	UCF supernatant	Ultrafiltrate	UCF supernatant	Ultrafiltrate	UCF supernatant	Ultrafiltrate	UCF supernatant	Ultrafiltrate
				Raw skim milk (RSM)				
0·1	385 ± 9[a]	267 ± 10[a]	428 ± 15[a]	387 ± 30[a]	389 ± 21[a]	278 ± 20[a]	427 ± 18[a]	383 ± 28[a,b,c]
100	407 ± 10[b]	273 ± 7[a]	421 ± 2[a]	400 ± 8[a]	398 ± 13[a]	281 ± 16[a]	435 ± 8[a,b]	401 ± 2[a]
150	418 ± 18[b]	285 ± 21[a]	431 ± 2[a]	399 ± 15[a]	427 ± 26[b]	294 ± 21[a]	455 ± 3[b,d]	363 ± 7[b]
200	437 ± 7[c]	286 ± 19[a]	481 ± 2[b]	416 ± 15[a]	429 ± 9[b]	275 ± 17[a]	480 ± 8[c]	364 ± 6[b]
250	439 ± 8[c]	287 ± 14[a]	464 ± 4[c]	404 ± 15[a]	426 ± 13[b]	286 ± 27[a]	462 ± 5[d]	382 ± 3[c]
300	468 ± 18[d]	284 ± 11[a]	475 ± 11[b,c]	403 ± 8[a]	454 ± 6[c]	292 ± 14[b]	458 ± 10[d]	379 ± 3[c]
				Phosphocaseinate (PC) dispersion				
0·1	374 ± 5[a]	291 ± 9[a]	392 ± 16[a]	345 ± 11[a]	379 ± 11[a]	302 ± 6[a,b]	397 ± 16[a]	355 ± 14[a]
100	447 ± 22[b]	279 ± 9[a]	418 ± 2[b]	361 ± 14[a]	451 ± 16[b]	303 ± 6[a,b]	440 ± 12[b]	370 ± 25[a]
150	451 ± 18[b]	282 ± 4[a]	416 ± 2[b]	351 ± 22[a]	449 ± 14[b]	298 ± 5[a]	468 ± 20[b,c]	376 ± 25[a]
200	444 ± 4[b]	287 ± 4[a]	444 ± 22[b]	352 ± 18[a]	472 ± 36[b]	309 ± 2[b]	437 ± 38[a,b,c]	351 ± 39[a]
250	449 ± 25[b]	287 ± 8[a]	422 ± 1[b]	364 ± 14[a]	457 ± 12[b]	309 ± 2[b]	453 ± 3[b,c]	369 ± 8[a]
300	450 ± 31[b]	304 ± 6[b]	433 ± 29[b]	347 ± 22[a]	459 ± 13[b]	315 ± 3[c]	466 ± 7[c]	341 ± 6[a]

[1] Mean values ± standard deviation of 9 measurements from 3 independent experiments. The values were determined by atomic absorption spectrometry
[2] Mean values ± standard deviation of 4 measurements from 2 independent experiments. The values were determined colorimetrically by the molybdate method
[1,2] Determination of Ca or P was carried out in the UCF supernatant (soluble phase) of RSM or of the PC dispersion and in soluble phase after ultrafiltration, using polyethersulfone membrane with a 10 kDa molecular weight cut-off, to retain the soluble proteins
Values with the same letter within a column are not significantly different at $P < 0{\cdot}05$

dispersion and UCF supernatants were heated at 100 °C for 5 min, then pipetted (15–33 μg protein/deposit) onto the plates. Electrophoresis was carried out using pH 8·3 Tris-HCl buffer (25 mM-Tris; 192 mM-glycine; 1 g SDS/l), at a constant power of 35 W and 18 ± 1 °C for 5 h.

PAGE was also done in the presence of urea and MSH according to Bastier et al. (1993) with some minor modifications. The separating gel was prepared at 140 g acrylamide/l (A/B ratio of 22,5) in a pH 8·9 Tris buffer (0·3 M-Tris; 5 M-urea; 1·7 mM-ammonium persulphate; 2·6 mM-Temed). The stacking gel was prepared at 40 g acrylamide/l (same A/B ratio) in a pH 7·6 Tris buffer (6 mM-Tris; 5 M-urea; 1·6 mM-ammonium persulfate; 3·3 mM-Temed). After adequate dilution, samples of RSM or PC dispersions and UCF supernatants were heated at 100 °C for 5 min, then pipetted (10–33 μg protein/deposit) onto the plates. Electrophoresis was carried out using pH 8·3 Tris-HCl buffer (62 mM-Tris; 190 mM-glycine), at a constant power of 35 W and 18 ± 1 °C for 5 h.

After staining with R-250 Coomassie blue as already described (Funtenberger et al. 1995), the relative intensity of stained bands was determined by scanning at 590 nm with a GS-300 densitometer (Hoefer Scientific Instruments, San Francisco, CA, USA). The content and relative proportion of each soluble protein, expressed by the ratio × 100 of protein content in UCF supernatant over protein content in total RSM or PC dispersion, were determined from areas (cm^2) of each protein peak. In the case of SDS-MSH-PAGE, results are the means of 2 electrophoretic patterns (from 2 independent experiments). In the case of urea-MSH-PAGE, results are determined from 1 electrophoretic pattern.

For protein identification and quantification, purified proteins from Sigma were used (see *Chemicals*.). Calibration curves (0 to ~10 μg protein/deposit) have been done using α_S-casein, β-casein, κ-casein and β-lg solubilised in a pH 6·8 Tris-HCl buffer (0·1 M-Tris; 20 g SDS/l; 200 ml glycerol/l; 230 mM-MSH) in the case of SDS-MSH-PAGE, or in an urea solution (9·8 M-urea; 287 mM-MSH; 10 mM-EDTA) in the case of urea-MSH-PAGE. The calibration curves were linear between 0 and ~10 μg of protein deposits.

Results and discussion

Effects of high pressure on the distribution of minerals in the colloidal and soluble phases of raw skim milk and of the phosphocaseinate dispersion

The total Ca content of RSM and of the PC dispersion was 1·31 ± 0·05 g/l and 1·22 ± 0·05 g/l (861 mg Ca/l from PC powder and 359 mg/l from SMUF), respectively, and the total P content 1·02 ± 0·05 g/l and 0·92 ± 0·07 g/l (560 mg P/l from PC powder and 360 mg/l from SMUF). The concentrations of soluble Ca and P as measured in the UCF supernatants and the ultrafiltrates from control or pressurised RSM and PC dispersion are given in Table 1. The concentrations of Ca and P in the UCF supernatant

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of the control RSM (0·1 MPa, Table 1) were in good agreement with published values for bovine milk (Gaucheron et al. 1997; López-Fandiño et al. 1998). Calcium and P in the PC powder were mainly in micellar form since about 95% (w/w) Ca and 91% (w/w) P determined in the UCF supernatant of the control PC dispersion came from SMUF and consequently only 5–9% (w/w) came from the powder. About 30% (w/w) Ca and 43% (w/w) P in the control RSM and PC dispersion were in soluble form, i.e. in the UCF supernatants. Besides, about 71 and 79% (w/w) of this soluble Ca, and 90 and 89% (w/w) of this soluble P were present in the ultrafiltrates from the control RSM and the control PC dispersion, respectively. The ultrafiltration membranes retained proteins and peptides of high molecular weight (>10 kDa) present in the soluble phase and consequently P and Ca bound to them, i.e.: (i) the P of phosphoseryl residues, (ii) the P indirectly bound to caseins in the form of phosphate salts, (iii) the Ca directly bound to caseins as Ca^{2+}, and (iv) the Ca indirectly bound to caseins as Ca phosphate salts. Ca from α-la was also retained by ultrafiltration membrane having a 10 kDa molecular weight cut-off. In that way, about 20–30% of the soluble Ca and about 10% of the soluble P may be considered as bound to the soluble proteins.

Pressurisation of RSM from 100 to 300 MPa for 15 min at 20 °C induced a significant ($P<0·05$) and progressive increase in the soluble Ca as measured in the UCF supernatant (Table 1), reaching a 22% increase after treatment at 300 MPa. The soluble P content of UCF supernatants was not significantly modified after pressurisation of RSM at 100–150 MPa and 20 °C for 15 min, then significantly ($P<0·05$) increased at 200–300 MPa by 11–12% (Table 1). The concentrations of Ca and P in the ultrafiltrate from these UCF supernatants did not significantly change with the pressure level (Table 1). The Ca and P released in the UCF supernatant of RSM pressurised at 200–300 MPa and 20 °C for 15 min (+52 and +83 mg/l for Ca and +53 and +47 mg/l for P at 200 and 300 MPa, respectively) are much smaller than those observed by López-Fandiño et al. (1998) with raw skim milk (+177 and +204 mg/l for Ca and +98 and +137 mg/l for P at 200 and 300 MPa, respectively) as measured immediately after pressurisation at 20 °C for 10 min. These investigators reported that the duration of HP treatment (10–30 min) in water at 20 °C did not influence the Ca and P concentrations in the soluble phase of RSM. This great variability of data obtained after similar HP treatments could result from reversibility phenomena taking place after pressure release, as observed by Schrader & Buchheim (1998) through turbidity measurements carried out immediately, 2 h or 4 h (at ∼20 °C) after HP treatment of pasteurised skim milk at 200–400 MPa and 20 °C for 1–10 min. Huppertz et al. (2004b) have reported some reassociation of caseins after storage at 20 °C for 24–48 h of pressure-treated RSM (100–600 MPa; 20 °C; 30 min). However, the reversibility of pressure-induced solubilisation of minerals in milk has not yet been reported.

Effects of HP treatment carried out in PTM at 9 °C (for 15 min) on the Ca and P concentrations in the UCF supernatants of RSM were close to those observed after HP treatment carried out at 20 °C (Table 1). The soluble Ca concentration increased progressively with pressure, reaching a ∼17% increase after treatment at 300 MPa. The soluble P concentration also increased progressively with pressure, reaching a maximal increase of ∼12% at 200 MPa (Table 1). As already observed for RSM at 20 °C, no significant increase of Ca and P concentrations was noticed in the ultrafiltrate of RSM after treatment at 100–300 MPa and 9 °C.

Pressurisation of the PC dispersion for 15 min at 20 or 9 °C induced a significant ($P<0·05$) increase in Ca (by ∼20%) and P (by ∼7–11%) concentrations of the UCF supernatants at 100 MPa, and no significant additional solubilisation at higher pressure levels (150–300 MPa; Table 1). The PC dispersion seems more pressure-sensitive than RSM at both 20 and 9 °C since maximal Ca and P solubilisation was observed as soon as 100 MPa for the PC dispersion, whereas a progressive increase in soluble Ca and P was observed with increasing pressure in the case of RSM (Table 1). Previous results (Regnault et al. 2004) had effectively shown that casein micelles of PC dispersion were more easily dissociated by pressure than those of RSM at 20 °C and especially at 9 °C. These results confirm that prior treatments undergone by the industrial PC, such as membrane filtration and spray-drying, have somewhat weakened the structure of casein micelles (Regnault et al. 2004). The Ca concentration in the ultrafiltrate from the PC dispersion was not significantly modified after HP treatment at 20 or 9 °C, except at 300 MPa, in which case a slight increase by ∼4·5% was observed; P concentration in the same PC ultrafiltrate was not significantly modified at all pressure levels and 9 or 20 °C (Table 1).

Comparing the results obtained after HP treatment, no significant differences in pressure-induced Ca solubilisation were observed to be dependent upon the treatment temperature for most of the results for RSM and PC dispersions (Table 1). Pressure-induced P solubilisation at 9 °C was similar in the case of RSM but significantly higher than at 20 °C in the case of PC dispersion (Table 1). The enhanced solubilisation of phosphate may be due to the temporary acidification of water under pressure and the increased solubility of calcium phosphates at low temperature. Furthermore, the slightly weakened structure of casein micelles in the industrial PC, as compared with RSM, could explain why the higher phosphate solubilisation at 9 °C was observed only in the case of the PC dispersion.

Ca and P concentrations in the ultrafiltrate of RSM or of the PC dispersion were slightly or not at all altered by pressurisation. These results appear to indicate that the Ca and P released out of the casein micelles were or became bound to soluble proteins. To verify if proteins (caseins) are effectively released in the soluble phase,

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Table 2. Protein concentration (g/l) in the UCF supernatant of RSM or of the PC dispersion after HP treatment at 100–300 MPa for 15 min in the pressure transmitting medium at 20 °C or 9 °C

Pressure (MPa)	Raw skim milk[1]		Phosphocaseinate dispersion[1]	
	20 °C	9 °C	20 °C	9 °C
0·1	$6·2 \pm 0·3^{a}$	$6·4 \pm 0·4^{a}$	$2·7 \pm 0·4^{a}$	$2·6 \pm 0·3^{a}$
100	$7·1 \pm 0·5^{b}$	$7·3 \pm 0·5^{b}$	$2·8 \pm 0·5^{a}$	$3·0 \pm 0·6^{a}$
150	$7·6 \pm 0·7^{b,c}$	$7·3 \pm 0·4^{b}$	$2·9 \pm 0·2^{a}$	$2·9 \pm 0·7^{a}$
200	$8·2 \pm 1·1^{c}$	$7·0 \pm 0·7^{b}$	$3·3 \pm 0·4^{b}$	$3·1 \pm 1·1^{a}$
250	$8·1 \pm 1·2^{b,c}$	$7·4 \pm 0·1^{b}$	$3·5 \pm 0·6^{b}$	$3·7 \pm 0·3^{b}$
300	$7·4 \pm 0·9^{b,c}$	$7·7 \pm 0·9^{b}$	$3·2 \pm 0·4^{b}$	$3·6 \pm 0·4^{b}$

[1] Mean values ± standard deviation of 9 measurements from 3 replicate experiments. The values were determined by the bicinchoninic acid method with β-lactoglobulin as calibration protein
Values with the same letter within a column are not significantly different at $P < 0·05$

protein constituents present in the UCF supernatant of RSM and of the PC dispersion were quantified by the BCA method then identified by PAGE after pressurisation.

Effects of high pressure on the distribution of protein constituents in the colloidal and soluble phases of raw skim milk and of the phosphocaseinate dispersion

The total protein content of RSM was 37·6 ± 0·3 g/l and that of the PC dispersion 31·1 ± 0·7 g/l, as measured by the BCA method. The protein concentrations in the UCF supernatants are given in Table 2. Soluble proteins in control RSM (about 6·3 g/l) are mainly composed of whey proteins and, to a lesser extent, of individual caseins. The control PC dispersion contains about half the soluble protein content of control RSM.

Pressurisation of RSM at 20 °C for 15 min induced a significant ($P < 0·05$) and progressive increase in soluble protein up to 200 MPa, followed by a slight (but not significant) decrease at 300 MPa (Table 2). Desobry-Banon et al. (1994) observed the same tendency for the concentration of soluble nitrogen of reconstituted skim milk pressurised between 130 MPa and 430 MPa (20 °C, 22 min). For comparison, pressurisation of RSM at 9 °C for 15 min induced a significant ($P < 0·05$) increase in the soluble protein concentration at all pressure levels (Table 2), but to a lesser extent than at 20 °C.

Pressurisation of the PC dispersion for 15 min induced progressive increase in soluble protein concentration in the same order of magnitude at 20 and 9 °C (Table 2).

The pressure-induced protein solubilisation of RSM or PC dispersion was not significantly ($P < 0·05$) influenced by pressurisation temperature at most pressure levels. However, a slight decrease in the soluble protein content of the RSM or PC dispersion was observed after treatment at 300 MPa and 20 °C, compared with 200–250 MPa. This decrease was not observed for pressurisation at 9 °C (Table 2).

For a clearer understanding of the effects of pressurisation on caseins and whey proteins, the UCF supernatants were analysed by PAGE in the presence of SDS and MSH. Under these conditions, α_{S2}-casein, κ-casein, bovine serum albumin, β-lg and α-la were well separated, while α_{S1}- and β-caseins were not. However, in combination with urea-MSH-PAGE, estimation of all proteins was possible.

A semi-quantitative evaluation of the contents of each protein in control RSM obtained from SDS-MSH-PAGE (data not shown) indicated (i) 2·1 ± 0·1, 21·4 ± 2·7 and 4·6 ± 0·8 g/l for α_{S2}-, (α_{S1}+β)- and κ-caseins, respectively, i.e. ∼28 g total caseins/l, and (ii) 1·8 ± 0·5, 4·5 ± 0·3 and 0·5 ± 0·1 g/l for α-la, β-lg and bovine serum albumin, respectively, i.e. ∼6·8 g total whey proteins/l. The total whey protein content in RSM (∼6·8 g/l) was of the same order of magnitude as that determined in the present study by the BCA method (6·2–6·4 g/l) or those previously published (6·3 g/l, Walstra & Jenness, 1984; 5·5 g/l, Alais, 1984). SDS-MSH-PAGE of the control PC dispersion (data not shown) indicated (i) 1·1 ± 0·1, 16·0 ± 1·2 and 5·5 ± 0·1 g/l for α_{S2}-, (α_{S1}+β)- and κ-caseins, respectively, i.e. ∼22·6 g total caseins/l, and (ii) 0·15 ± 0·04, 1·4 ± 0·2 and 0·3 ± 0·1 g/l for α-la, β-lg and bovine serum albumin, respectively. The PC powder is thus much poorer in whey proteins than RSM, as expected from its manufacturing process and from the results obtained by BCA method (Table 2).

Figure 1 indicates the relative proportions of each casein, expressed as the ratio × 100 of each individual casein in the UCF supernatants of RSM or PC dispersion samples, over that of the same individual casein in the corresponding control samples. After pressurisation of RSM at 20 °C (Fig. 1a), the contents of soluble (α_{S1}+β)- and κ-caseins increased from 0·1 MPa to 200 MPa, but exposure to higher pressure levels (250–300 MPa) brought no additional solubilisation of these caseins. Urea-MSH-PAGE patterns of the same UCF supernatants confirmed this tendency for α_{S1}- and β-caseins separately (Fig. 1a). The α_{S2}-casein (quite negligible at 0·1 MPa) appeared in the soluble phase of RSM after treatment at 100 MPa and progressively increased up to 300 MPa.

According to these results, the order of casein dissociation and release, in quantitative term (content of each individual casein in the UCF supernatant of pressurised sample over the total content of the same individual casein in control sample), is κ-casein > β-casein ∼ α_{S1}-casein > α_{S2}-casein. It seems that this order is inversely correlated to the number of phosphoseryl residues in caseins (i.e. 1, 5, 8 and 11 for κ-, β-, α_{S1}- and α_{S2}-caseins, respectively), as suggested by López-Fandiño et al. (1998; the lower the number of phosphoseryl residues, the higher the level of casein solubilisation). But, if we consider the content ratio of each individual casein in the UCF supernatant of pressurised sample over that of the same individual casein in the UCF supernatant of control sample, we observe that pressure-induced increases in casein

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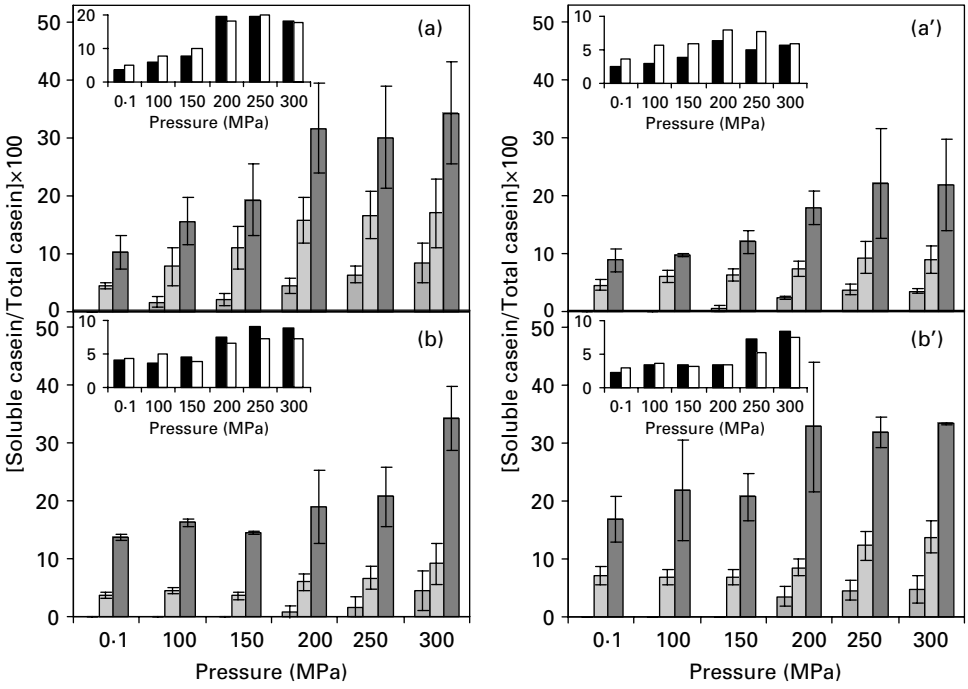

Fig. 1. Effects of treatment at 100–300 MPa for 15 min at 20 °C (a, a′) or 9 °C (b, b′) on the relative proportion (×100) of soluble caseins in the ultracentrifugation supernatants of raw skim milk (a, b) or phosphocaseinate dispersion (a′, b′). Determination of α_{S2}- (▨), α_{S1}+β- (▨) and κ- (▨) caseins from SDS-MSH-PAGE. Determination of α_{S1}- (■) and β- (□) caseins from urea-MSH-PAGE.

Values are the means ± deviation of the mean of 2 SDS-MSH-PAGE patterns (from 2 independent experiments). In the case of urea-MSH-PAGE, values come from 1 experiment

content at the highest pressure levels (250–300 MPa) are quite similar (×4) for α_{S1}-, β- and κ-caseins and much higher for α_{S2}-casein. This does not suggest some preferential release of caseins depending on their number of phosphoseryl residues. The pressure-induced solubilisation of α_{S1}- and α_{S2}-caseins suggests that high pressure destabilised the internal structure of casein micelles.

After pressurisation of RSM at 9 °C for 15 min (Fig. 1b), no increase in casein solubilisation was observed from 0·1 to 150 MPa, but the contents of soluble α_{S2}-, (α_{S1}+β)- and κ-caseins clearly increased in the range 200–300 MPa. Same tendancy was observed from urea-MSH-PAGE (Fig. 1b). Comparatively, casein solubilisation increased as early as 100 MPa after RSM pressurisation at 20 °C (Fig. 1a). Besides, in agreement with the protein concentration determined by the BCA method (Table 2), there was much less pressure-induced solubilisation of caseins at 9 than at 20 °C, as determined after pressure release. These results are difficult to explain, since the formation of small-sized micelles was enhanced at 9 °C compared with 20 °C (Regnault et al. 2004). However, recent data published by Anema et al. (2005) confirm our results. They observed lower levels of non-sedimentable casein after pressurisation (200–300 MPa for 15 min) of reconstituted skim milk at 10 °C than at 20 °C.

After pressurisation of the PC dispersion at 20 °C for 15 min (Fig. 1a′), the contents of soluble (α_{S1}+β)- and κ-caseins increased from 0·1 to 200 MPa then remained constant at the highest pressure levels, as already observed for RSM (HP treatment at 20 °C) but to a lesser extent. These results are in accordance with determination of protein concentration by BCA method (Table 2). The level of soluble α_{S2}-casein increased from 150 to 250–300 MPa. For PC dispersion pressurised at 9 °C for 15 min, Fig. 1b′ indicated casein solubilisation at ⩾200 MPa. In contrast to RSM, the amount of released casein in aqueous phase of PC dispersion was of the same order of magnitude or slightly higher at 9 than at 20 °C.

SDS-MSH-PAGE also permitted investigation of the effects of pressurisation on the behaviour of whey proteins. SDS-MSH-PAGE of the UCF supernatant of RSM pressurised at 100–300 MPa and 20 °C showed that the proportion of soluble β-lg decreased at ⩾200 MPa to reach ∼83% of the total initial β-lg after treatment at 300 MPa (Fig. 2a). This is in agreement with the findings of Needs et al. (2000a) who observed that ∼86% of the total initial whey proteins were present in the pH 4·6 soluble fraction of RSM pressurised at 300 MPa and 20 °C for 15 min. This decrease in soluble β-lg probably corresponds to loss of its native structure followed by protein aggregation, as previously published (Funtenberger et al. 1995, 1997; Kolakowski et al. 2001), and subsequent entrapment of β-lg aggregates in the UCF pellet. However, some soluble β-lg molecules could be denatured into

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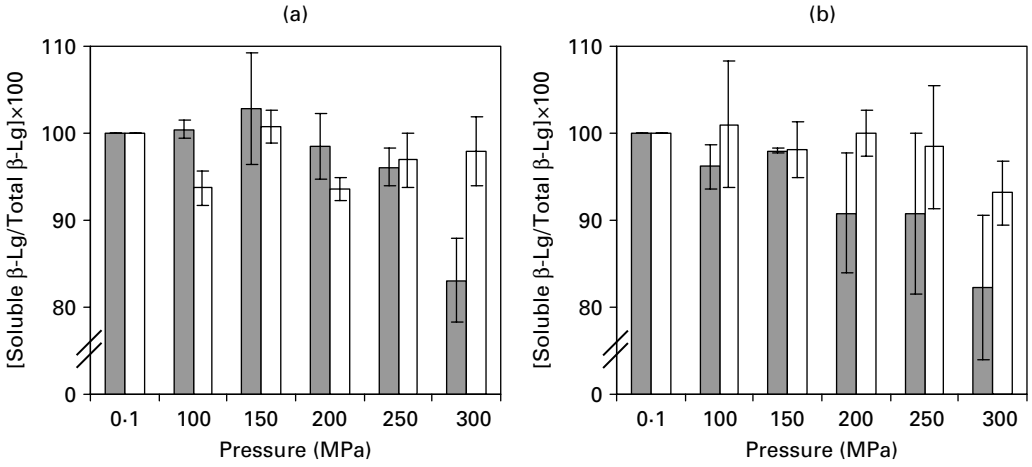

Fig. 2. Effects of treatment at 100–300 MPa for 15 min at 20 °C (■) or 9 °C (□) on the relative proportion (×100) of soluble β-lactoglobulin (β-Lg) in the ultracentrifugation supernatants of raw skim milk (a) or phosphocaseinate dispersion (b). Determination from SDS-MSH-PAGE.

Values are the means ± deviation to the mean from 2 independent experiments

non sedimentable aggregates associated or not to small sub-units of casein micelles (Huppertz et al. 2004a).

SDS-MSH-PAGE of the UCF supernatant of RSM pressurised at 9 °C showed that the proportion of soluble β-lg was little modified after pressurisation at 100–300 MPa (Fig. 2a). Thus, β-lg is more pressure-resistant at low temperature than at 20 °C, as already observed by Kolakowski et al. (2001) for β-lg solutions (2·0–2·5%, w/w, in Tris buffer, pH 7) and by Gaucheron et al. (1997) for β-lg in reconstituted skim milk. These results are in accordance with the slight decrease in total soluble proteins observed by BCA method after pressurisation of RSM at 300 MPa (compared with 200 MPa) and 20 °C but not 9 °C (Table 2).

SDS-MSH-PAGE of the UCF supernatant of the PC dispersion pressurised at 100–300 MPa and 20 or 9 °C (Fig. 2b) indicates that β-lg was more resistant to pressure at 9 than at 20 °C, as already seen for RSM. After pressurisation at 300 MPa, soluble β-lg accounted for ∼82% of total β-lg at 20 °C and ∼93% at 9 °C.

α-la and bovine serum albumin of RSM or of the PC dispersion were not sensitive to pressure from 100 MPa to 300 MPa at either temperature (results not shown), as previously published (López-Fandiño & Olano, 1998; Gaucheron et al. 1997; López-Fandiño et al. 1996; Needs et al. 2000; Huppertz et al. 2004a).

Conclusion

Pressurisation of raw skim milk (RSM) or of a dispersion of phosphocaseinate (PC) at 20 or 9 °C for 15 min were previously shown to induce irreversible partial micelle dissociation at or above 200 MPa (Regnault et al. 2004), while the present study reveals partial solubilisation of Ca, P and casein constituents already at 100–150 MPa. This treatment (at 100–150 MPa) induced a slight increase in the hydrodynamic volume of casein micelles which probably resulted from water penetration in the core of the micelles. This micellar hydration is not incompatible with starting instability of micellar structure under moderate pressure (⩽150 MPa), allowing a partial release of minerals (at 20 and 9 °C) and of caseins (at 20 but not at 9 °C) into the soluble phase. At higher pressure levels (⩾200 MPa), the progressive dissociation of casein micelles (Regnault et al. 2004) is accompanied by an additional solubilisation of minerals and caseins.

It is generally accepted that electrostatic and hydrophobic interactions are weakened under pressure, due to electrostriction phenomena and rearrangement of water molecules around charged groups and hydrophobic areas. This could result in the dissociation of casein micelles into smaller units, including individual caseins, and the dissociation of CCP. Moreover, the increase in the ionic product of water and the resulting pH decrease under pressure, together with low temperatures, enhance the solubilisation of calcium phosphate. However, reversible electrostatic and hydrophobic interactions may occur during and after pressure release, allowing a partial re-association of dissociated caseins into new micellar sub-units. This latter hypothesis is supported by spectroscopic measurements carried out under pressure (unpublished data; Needs, 2003). It is not excluded that reassociation phenomena through hydrophobic interactions may even take place during pressurisation at high pressure levels (250–300 MPa) (Payens & Heremans, 1969; Ohmiya et al. 1989).

After treatment at 300 MPa and 9 or 20 °C, relatively small proportions of minerals (7–9% of micellar Ca and 6–13% of micellar P) and caseins (8–14% of micellar

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caseins) are released into the soluble phase, and are not integrated into the newly formed micellar subunits (~30 nm in diameter; Regnault et al. 2004). These pressure-solubilised minerals are not free but bound to the soluble proteins. Thus, Ca released from casein micelles could be bound to soluble proteins (caseins and whey proteins) as calcium ions and/or indirectly bound to soluble caseins as Ca phosphate salts. In parallel, P released from the micelles could be directly bound to seryl residues of caseins or indirectly as Ca phosphate salts. Several investigators (Schrader et al. 1997; Gaucheron et al. 1997; López-Fandiño et al. 1998) have suggested that pressure-released Ca and P could result from the solubilisation of colloidal calcium phosphate. The present study suggests that CCP would be bound to caseins during its solubilisation, or would bind to soluble proteins once in soluble phase of RSM or PC dispersions.

β-Lg was more affected by pressurisation at 20 than at 9 °C. Thus, the formation of small amounts of large micellar aggregates observed after processing at 250–300 MPa and 20 °C (Regnault et al. 2004) could result from the self-association of pressure-denatured β-lg molecules, inducing their sedimentation in the UCF pellet. It is not excluded, however, that pressure-denatured β-lg associates to caseins after pressure release to form large micellar aggregates. The notable marked release of κ-casein (15–25% of micellar κ-casein) into the soluble phases of RSM or PC dispersions after pressurisation at 250–300 MPa and 20 °C could also contribute to the formation of these large aggregates by decreasing electrostatic repulsions and increasing hydrophobic attractions between micelles.

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References

Alais C 1984 La constitution du lait. In *Science du lait: principes des techniques laitières*, pp. 29–35 Paris: SEPAIC

Anema SG, Lowe EK & Stockmann R 2005 Particle size changes and casein solubilisation in high-pressure-treated skim milk. *Food Hydrocolloids* **19** 257–267

Bastier P, Dumay E & Cheftel JC 1993 Physico-chemical and functional properties of commercial caseinates. *International Journal for Food Science* (*LWT*) **26** 529–537

Byler DM & Farrell HM 1989 Infrared spectroscopic evidence for calcium ion interaction with carboxylate groups of casein. *Journal of Dairy Science* **72** 1719–1723

Cheftel JC & Dumay E 1998 Effect of high presssure on food biopolymers with special reference to β-lactoglobulin. In *The properties of water in foods – ISOPOW 6* pp. 369–397 (Ed. DS Reid). London: Blackie Academic & Professionnal

Dalgleish DG & Law AJR 1989 pH-induced dissociation of bovine casein micelles. II. Mineral solubilization and its relation to casein release. *Journal of Dairy Research* **56** 727–735

Desobry-Banon S, Richard F & Hardy J 1994 Study of acid and rennet coagulation of high pressurized milk. *Journal of Dairy Science* **77** 3267–3274

Fédération Internationale de Laiterie 1990 *Lait. Determination de la teneur en phosphore total* (*méthode spectrométrique*). Brussels: IDF (*Norme Internationale* 42B).

Funtenberger S, Dumay E & Cheftel JC 1995 Pressure-induced aggregation of β-lactoglobulin in pH 7·0 buffers. *International Journal for Food Science* (*LWT*) **28** 410–418

Funtenberger S, Dumay E & Cheftel JC 1997 High pressure promotes β-lactoglobulin aggregation through SH/S-S interchange reactions. *Journal of Agricultural and Food Chemistry* **45** 912–921

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** 439–447

Holt C, Van Kemenade MJJM, Nelson JLS, Sawyer L, Harries JE, Bailey RT & Hukins DWL 1989 Composition and structure of micellar calcium phosphate. *Journal of Dairy Research* **56** 411–416

Huppertz T, Kelly AL & Fox PF 2002 Effects of high pressure on constituents and properties of milk. *International Dairy Journal* **12** 561–572

Huppertz T, Fox PF & Kelly AL 2004a High pressure treatment of bovine milk: effects on casein micelles and whey proteins. *Journal of Dairy Research* **71** 97–106

Huppertz T, Fox PF & Kelly AL 2004b Dissociation of caseins in high pressure-treated bovine milk. *International Dairy Journal* **14** 675–680

Huppertz T, Fox PF & Kelly AL 2004c Properties of casein micelles in high pressure-treated bovine milk. *Food Chemistry* **87** 103–110

Jenness R & Koops J 1962 Preparation and properties of a salt solution which simulates milk ultrafiltrate. *Netherlands Milk and Dairy Journal* **16** 153–164

Kolakowski P, Dumay E & Cheftel JC 2001 Effects of high pressure and low temperature on β-lactoglobulin unfolding and aggregation. *Food Hydrocolloids* **15** 215–232

Law AJR 1996 Effects of heat treatment and acidification on the dissociation of bovine casein micelles. *Journal of Dairy Research* **63** 35–48

Le Graet Y & Brulé G 1993 Les équilibres minéraux du lait: influence du pH et de la force ionique. *Le Lait* **73** 51–60

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** 929–936

López-Fandiño R, De la Fuente MA, Ramos M & Olano A 1998 Distribution of minerals and proteins between the soluble and colloidal phases of pressurised milks from different species. *Journal of Dairy Research* **65** 69–78

López-Fandiño R & Olano A 1998 Effects of high pressures combined with moderate temperatures on the rennet coagulation properties of milk. *International Dairy Journal* **8** 623–627

Needs EC 2003 High pressure processing of dairy products. In *Ultra High Pressure Treatment of Foods* pp. 269–296 (Eds MEG Hendrickx & D Knorr). New-York: Klumer Academic/Plenum Publishers

Needs EC, Stenning RA, Gill AL, Ferragut V & Rich GT 2000 High-pressure treatment of milk: effects on casein micelle structure and on enzymic coagulation. *Journal of Dairy Research* **67** 31–42

Ohmiya K, Kajino T, Shimizu S & Gekko K 1989 Effect of pressure on the association states of enzyme-treated caseins. *Agricultural and Biological Chemistry* **53** 1–7

Payens TAJ & Heremans K 1969 Effect of pressure on the temperature dependent association of β-casein. *Biopolymers* **8** 335–345

Pierre A & Brulé G 1981 Mineral and protein equilibria between the colloidal and soluble phases of milk at low temperature. *Journal of Dairy Research* **48** 417–428

Regnault S, Thiebaud M, Dumay E & Cheftel JC 2004 Pressurisation of raw skim milk and 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 1982 Association of caseins and casein micelle structure. In *Developments in Dairy Chemistry* pp. 61–86 (Ed. PF Fox). London: Applied Science Publishers

Schrader K, Buchheim W & Morr CV 1997 High pressure effects on the colloidal calcium phosphate and the structural integrity of micellar casein in milk. Part 1. High pressure dissolution of colloidal calcium phosphate in heated milk systems. *Nahrung* **41** 133–138

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 Forschungberichte* **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

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ & Klenk DC 1985 Measurement of protein using bicinchonic acid. *Analytical Biochemistry* **150** 76–85

Tarodo de la Fuente B, Lablee J & Cuq JL 1999 Le lait – Coagulation et synérèse. *Industries Alimentaires et Agricoles* **116** 19–26

Van Dijk HJM 1990 The properties of casein micelles. 1. The nature of the micellar calcium phosphate. *Netherlands Milk and Dairy Journal* **44** 65–81

Walstra P 1990 On the stability of casein micelles. *Journal of Dairy Science* **73** 1965–1979

Walstra P & Jenness R 1984 Proteins. In *Dairy Chemistry and Physics,* pp. 98–122 (Eds P Walstra & R Jenness). New-York: John Wiley & Sons

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