

Modified water solubility of milk protein concentrate powders through the application of static high pressure treatment

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The effects of high pressure (HP) treatment (100–400 MPa at 10–60 °C) on the solubility of milk protein concentrate (MPC) powders were tested. The solubility, measured at 20 °C, of fresh MPC powders made with no HP treatment was 66%. It decreased by 10% when stored for 6 weeks at ambient temperature (~20 °C) and continued to decrease to less than 50% of its initial solubility after 12 months of storage. Of the combinations of pressure and heat used, a pressure of 200 MPa at 40 °C applied to the concentrate before spray drying was found to be the most beneficial for improved solubility of MPC powders. This combination of pressure/heat improved the initial cold water solubility to 85%. The solubility was maintained at this level after 6 weeks storage at ambient temperature and 85% of the initial solubility was preserved after 12 months. The improved solubility of MPC powders on manufacture and on storage are attributed to an altered surface composition arising from an increased concentration of non-micellar casein in the milk due to HP treatment prior to drying. The improved solubility of high protein powders (95% protein) made from blends of sodium caseinate and whey protein isolate compared with MPC powders (~85% protein) made from ultrafiltered/diafiltered milk confirmed the detrimental role of micellar casein on solubility. The results suggest that increasing the non-micellar casein content by HP treatment of milk or use of blends of sodium caseinate and whey proteins are strategies that may be used to obtain high protein milk powders with enhanced solubility.

Keywords: High pressure, MPC, milk protein concentrate, solubility.

Milk protein concentrate (MPC) powders are high protein powders produced by ultrafiltration (UF) and diafiltration (DF) of skim milk whilst maintaining the same ratio of casein to whey protein as in the original milk. The powders produced have increased content of protein and decreased amounts of lactose and milk serum salts. Changes in filtration conditions will impact on the composition and the functionality of the MPC powders produced. One of the factors that limit the full exploitation of MPC powders in particular powders containing over 80% protein, produced by the conventional filtration processes of UF/DF of skim milk and spray-drying is the loss of solubility (measured at 20 °C), on storage. This detracts from the functionality of the powder in a range of applications. The loss of solubility on storage has been attributed to cross-linking of casein (predominantly α_s - and β -casein) at the surface of powder particles (Havea, 2006) and migration of residual fat to the surface of the powder particle (Gaiani et al. 2007, 2009). Increasing the

time and temperature of reconstitution improves the solubility of MPC powders (McKenna, 2000; Mistry, 2002; Gaiani et al. 2006, 2007; Mimouni et al. 2009, 2010a; Jeantet et al. 2010). However, low solubilities even at high temperatures of reconstitution after extended storage of MPC powders have been reported (McKenna, 2000). The loss of solubility has been attributed to the slow release of casein micelles from the dispersed powder particle due to the increased crosslinking of micelles rather than to the formation of insoluble material (Mimouni et al. 2010a, b).

Technological approaches such as the addition of citrate (Bhaskar et al. 2001) or sodium chloride (Schuck et al. 1999, 2002; Carr, 2002) have been suggested for improving the cold solubility of MPC powders. The addition of citrate or sodium chloride dissociate the casein micelle and increase the amount of serum protein (Udabage et al. 2000). This increases the available surface-active protein that can migrate to the interface of a droplet during atomization and spray drying. Competition of various surface active species for the droplet interface during drying has been reviewed and this is a strategy that can be used to alter the surface composition of a powder with consequent effects on

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powder properties (Jayasundera et al. 2009). It may be expected that the increased free protein in the serum due to addition of salts or citrate will compete with the casein micelles for the air–water interface during spray drying and that this competition leads to a reduced level of casein micelles at the interface of MPC powders and therefore a reduced number of micellar–micellar crosslinks. Furthermore, the increased amount of casein in the serum phase could act as ‘inert’ fillers thereby spatially separating the casein micelles.

We hypothesise that an increased concentration of serum protein at the droplet interface is desirable for improved solubility of MPC powders on storage and that this may be achieved by dissociation of the casein micelle in the milk stream by high pressure (HP) treatment prior to drying of the concentrate. Depending on the magnitude of static pressure applied, generally an increase in pH and calcium activity (López-Fandiño et al. 1996) and whey protein denaturation (López-Fandiño et al. 1996; Huppertz et al. 2004a, b; Hinrichs & Rademacher, 2005; López-Fandiño, 2006), a decrease in casein aggregation (Needs et al. 2000; Huppertz et al. 2004c) and a disruption of colloidal calcium phosphate (Anema et al. 1997; López-Fandiño et al. 1998) are expected.

This study was undertaken to evaluate the potential of HP treatment as a strategy to improve the water solubility of MPC powders at ambient temperatures. Unlike the use of a strategy based on added salts, the application of HP as a processing intervention is a physical process and does not increase the amounts of salts in the final powder. The effects of HP treatment on the properties of milk and milk protein concentrates were examined. HP treatment of commercial liquid concentrates were undertaken to assess the effects of HP on the processability of the milk streams and the properties of the MPC powders after manufacture and during extended storage. Assessments of solubility on reconstitution of MPC powders in water at ambient and at elevated temperatures were examined. In addition the solubility of reconstituted MPC in milk, in place of water, was also investigated. In order to further elucidate the role of micellar and non-micellar casein on solubility, the solubility of high protein milk powders made from blends of sodium caseinate and whey protein isolate were compared with that of MPC powders.

Materials and Methods

Materials

Fresh whole milk was collected, skimmed, thermized (65 °C for 15 s) at Tatura Milk Industries (236 Hogan Street, Tatura, VIC 3616, Australia) and transported cold to CSIRO Division of Food and Nutritional Sciences (CFNS), Werribee, Australia. This milk was used to characterise the pressure induced changes on milk and concentrate.

Fresh commercial liquid milk protein concentrate (~18% total solids, TS) prepared by pasteurisation and UF with DF

for conversion into MPC powders (~85% protein, dry basis) was collected from Murray Goulburn Co-operative Ltd, (Victoria) and transported refrigerated to CFNS, Werribee, pressure treated and converted to MPC powders.

For the production of high protein powders with altered casein to whey protein ratios, sodium caseinate was purchased from Fonterra Co-operative Ltd., (Auckland, New Zealand) and whey protein isolate (WPI; Alacen 895) was purchased from New Zealand Milk Products.

High pressure treatment of fresh liquid milk streams and characterisation of pressure induced changes

Treatment of milk streams. Upon receipt, the skim milk was pasteurized at 72 °C for 15 s. The pasteurized milk was either high pressure treated followed by UF/DF or UF/DF followed by high pressure treatment. Milk or concentrate were subjected to 10 min of static HP treatment at 100 MPa/20, 40, 90 °C or 200 MPa/40 °C. All pressure treatments were done using a 35L HP unit (Quintus Press, Avure Technologies, USA). Adiabatic heating (~3 °C per 100 MPa) was taken into account when reporting temperature at pressure holding (10 min). The rate of compression was 5 MPa/s and the rate of decompression was 40 MPa/s. The HP treated streams were stored at ambient temperature (~20 °C) and characterised within 2 h of release of pressure.

Characterisation of milk streams. The properties measured were pH, Ca activity (Ca²⁺), total protein and pH 4·6 precipitable proteins (casein (C)+denatured whey (DW); C+DW). A Radiometer combined electrode and a calcium selective electrode was used to measure pH and calcium activity respectively at ambient temperature (20±2 °C). For calcium activity measurements, calibrations were carried out with CaCl₂ solutions, with an ionic strength of 0·08 M, adjusted with KCl. The standard curve of potential vs. In Ca²⁺ was used to evaluate the calcium activity of the milks, 0·425 was used as the activity co-efficient of Ca²⁺, with c⁰ = 1 mmol/l.

Crude nitrogen content was determined in a Leco FP-2000 nitrogen analyser where a factor of 6·38 was used in the conversion to protein contents. The proteins precipitable at pH 4·6 were isolated by precipitation at pH 4·6 following a modification of the method of Lynch & Barbano (1998).

% pH 4·6 precipitable proteins

$$= \frac{(\text{casein} + \text{denatured whey proteins})}{\text{total crude protein}} * 100$$

Preparation and characterisation of protein powders

High pressure treatment of commercial milk concentrates for production of MPC powders. Upon receipt, the concentrates were subjected to concurrent high pressure and temperature treatment in the pilot plant of CFNS, Werribee, Australia. Concentrates were subjected to 10 min of static HP

Table 1. Experimental design used to test effects of pressure and temperature on nitrogen solubility of MPC powders

Day	Run	Pressure (MPa)	Temperature (°C)	
1	1	0.1	10	<i>Control</i>
	2	100	10	
	3	0.1	25	
	4	200	25	
	5	400	40	
	6	0.1	60	
2	1	0.1	25	<i>Control</i>
	2	400	25	
	3	100	25	
	4	0.1	40	
	5	400	40	
	6	0.1	60	
3	1	100	10	<i>Control</i>
	2	100	25	
	3	0.1	25	
	4	100	40	
	5	400	60	
	6	200	60	
4	1	200	10	<i>Control</i>
	2	0.1	25	
	3	400	25	
	4	0.1	40	
	5	200	40	
	6	100	60	
5	1	0.1	10	<i>Control</i>
	2	200	10	
	3	0.1	25	
	4	200	40	
	5	100	40	
	6	400	60	

treatment using a fully randomised statistical design within the practical restrictions placed on the design. There were four pressures [0.1 MPa (atmospheric pressure), 100, 200 and 400 MPa] and four temperatures (10, 25, 40 and 60 °C) used. The combination of no pressure treatment (0.1 MPa) and temperature (25 °C) was designated as the 'control' condition. A new control powder, made from milk concentrate given no pressure treatment, was made every time a fresh batch of concentrate was received.

Within the practical constraints it was possible to produce 30 powders in a 5-day working week. As a control was included each day, there were 25 runs available for the other 15 treatment combinations, and so 10 combinations could be replicated, and 5 combinations were conducted once. The temperature of 40 °C was of particular interest as preliminary experiments showed that this temperature of processing provided substantial improvements in powder solubility. This meant that all four treatment combinations involving this temperature were replicated.

The fully randomized experimental design used is given in Table 1. Since the plant was not capable of maintaining a temperature of 10 °C with the pressure 400 MPa, it was

decided to lower the pressure to 100 MPa. In addition, the experimental design had the following features: (a) excluding the control, the four pressures were replicated as evenly as possible on each experimental day, (b) excluding the control and the 40 °C treatments which were included on each day, the four temperatures were replicated as evenly as possible across the experiment, and (c) the number of times treatments occurred with other particular treatments within a day was as even as possible.

All liquid concentrates were spray dried using a Drytec Compact Spray Dryer (Drytec, Kent, UK) with an inlet temperature of 180 °C and an outlet temperature of 85 °C. The MPC powders were packed and sealed in aluminium foil bags and stored at ambient temperature 20 ± 2 °C until analysed. Once opened for testing, the unused powder was discarded and a new bag was opened for subsequent testing during storage.

High protein powders with differing casein to whey protein ratios. High protein powders with differing casein to whey ratios (10:1 and 1:10) were also made by blending appropriate amounts of a 15% total solids sodium caseinate solution and a 15% total solids whey protein isolate solution. The blends were subjected to pressure treatments (no pressure, 100, 200 and 400 MPa at 25 or 40 °C for 10 min) and spray dried using a Drytec Compact Spray Dryer (Drytec, Kent, UK) with an inlet temperature of 180 °C and an outlet temperature of 85 °C. The TS content of all concentrates were between 14 and 17%, the average protein content of the powder was 95%.

Solubility of MPC and high protein powders. The IDF standard (173:1995) was used to determine the nitrogen solubility of MPC and high protein powders at 20 °C. To test the nitrogen solubility of MPC powders in water at 45 °C, the IDF method was followed except that the reconstitution temperature was 45 °C. When testing the nitrogen solubility of MPC powders in skim milk, skim milk was fortified with 1% MPC powder and the same IDF method was followed. The moisture content of the powders was determined using a Sartorius moisture balance (Sartorius AG Gottingen, Germany).

Treatment of data on nitrogen solubility of MPC powders.

The model fitting was performed in GenStat using the REML algorithm. All the models are 'linear mixed models' which account for the three levels of variation (between days, between runs within days, and between measurements within runs) and missing values.

Results and Discussion

High pressure treatment of fresh liquid milk streams and characterisation of pressure induced changes

The pH, calcium activity (Ca²⁺) and pH 4.6 precipitable protein (both casein (C) and denatured whey proteins (DW)

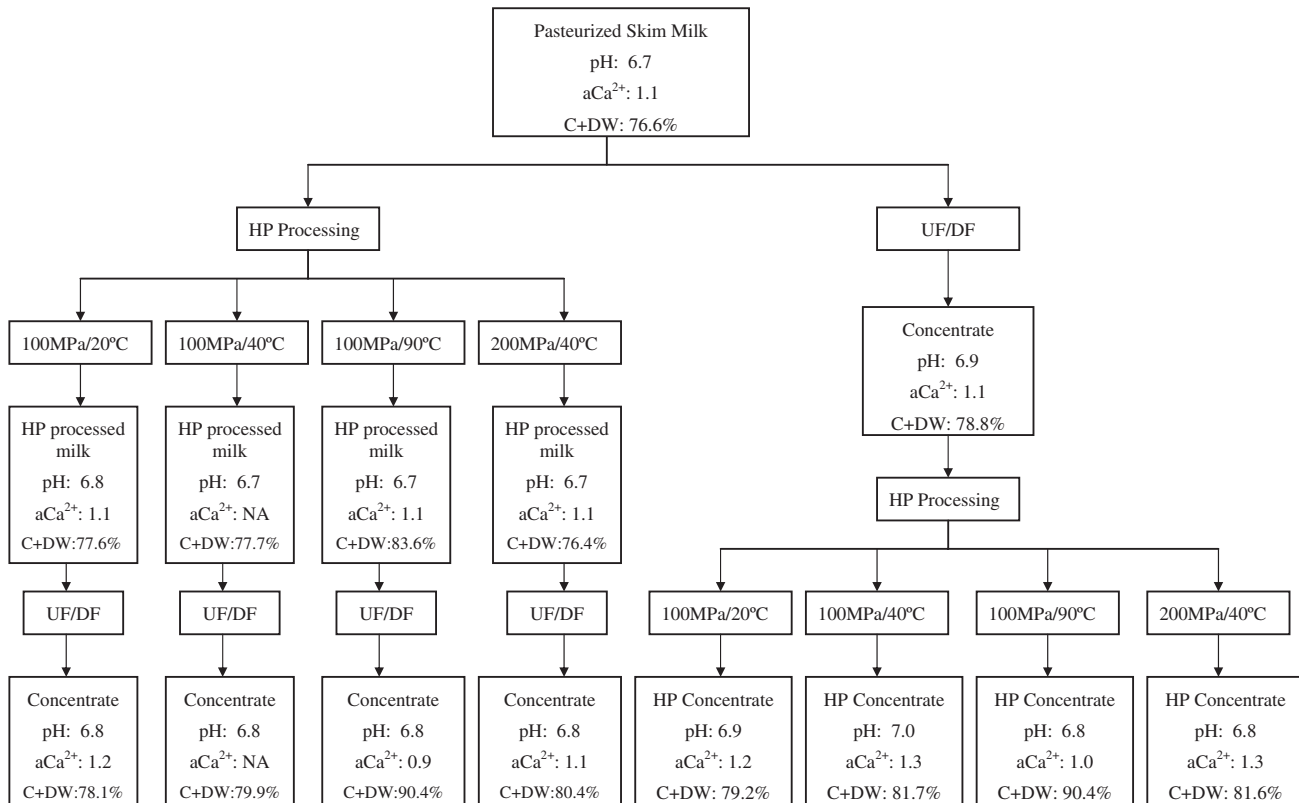


Fig. 1. Changes in properties of milk due to ultra filtration (UF) with diafiltration (DF) and high pressure treatment. aCa^{2+} : calcium activity; C+DW: casein and denatured whey proteins (pH 4-6 insoluble protein); HP: high pressure. N/A data not available.

precipitate at pH 4-6) of HP treated milk, HP treated UF/DF concentrate and UF/DF concentrate made from HP treated milks are given in Fig. 1. The characterisation of the HP treated streams show that there are slight changes to the pH, mineral and protein components of the milk when HP is applied (Fig. 1).

pH. There was a slight increase in the pH (0.1–0.3 pH units) of the streams after UF/DF concentration (Fig. 1). The slight increase in pH in milks produced by UF/DF observed is due to the changes induced in the mineral equilibria of milk. With the application of UF/DF, most of the minerals including Ca and inorganic phosphate (Pi) in the serum phase are removed during the DF stage so that Ca and Pi are concentrated in the micellar phase. This leads to the solubilisation of the colloidal calcium phosphate and an increase in the pH. This is in contrast to concentration of milk by evaporation, where the serum Ca and Pi concentrations are increased with the removal of water, resulting in increased ionic strength and a transfer of serum Ca and Pi into colloidal state and a decrease in pH (Bienvenue et al. 2003).

Calcium activity. Both the original skim milk and UF/DF concentrate without HP treatment had similar calcium

activities. If milk or concentrate was subject to HP, the calcium activity remained unchanged or increased when the temperature at pressure holding was $\leq 40^\circ\text{C}$. A decrease in calcium activity in the liquid concentrates was observed when the temperature at pressure holding was 90°C (Fig. 1). Our results showed that calcium activity was dependent on the temperature and pressure used (Fig. 1). It is known that calcium activity increases with a decrease in pH and with a decrease in temperature (Augustin & Clarke, 1991a, b; Chandrapala et al. 2010). Pressure-induced increases in calcium activity are expected as pressure causes dissolution of colloidal calcium phosphate. The overall effect on calcium activity is the result of the interplay of all these factors. Our results suggest that the effects of pressure dominate at lower temperatures under the conditions used in this study.

pH 4-6 precipitable protein. Concentration of milk by UF/DF increased the amount of protein insoluble at pH 4-6 under all conditions, but particularly if the HP treatment was conducted at 90°C (Fig. 1). The increase in the amount of protein insoluble at pH 4-6 with UF/DF may be due to complexation of denatured whey protein with casein or aggregation of the denatured whey proteins. Both heat and pressure increase the amount of denatured whey proteins. At all the pressure – temperature combinations tested, HP

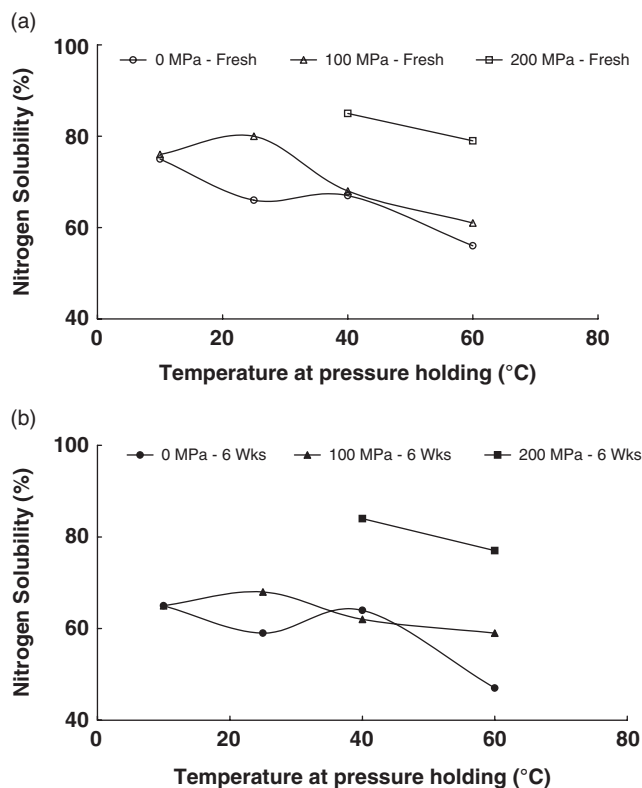


Fig. 2. Effect of treatment temperature on the nitrogen solubility of MPC powders reconstituted at 20 °C in water (a) solubility of fresh powders and (b) powders stored at 20 °C for 6 weeks. Concentrates HP treated at 200 MPa and 10 or 25 °C could not be dried into powders due to gelling.

treatment of milk concentrate caused greater insolubility of the proteins than HP treatment of milk. This accounts for a noticeable increase in the amount of protein insoluble at pH 4.6 when concentrate was pressurized in comparison with the concentration of pressurized single strength milks.

High protein powders made from HP treated concentrates

Processability of MPC powders. The ability to handle and spray dry the concentrates to MPC powders was dependent on the magnitude of the applied pressure and the temperature at pressure holding. MPC powders were successfully manufactured from concentrates (a) processed at various temperatures (10, 25, 40 and 60 °C) without pressure, (b) processed at those same temperatures at 100 MPa and (c) processed at 40 and 60 °C at 200 MPa. All these concentrates did not thicken excessively after treatment. However, MPC powders could not be produced from concentrates that were processed at 10 or 25 °C at 200 MPa and neither could they be manufactured from concentrates that were treated at 25, 40 or 60 °C and 400 MPa, as all these concentrates gelled after treatment. Excessive thickening is due to increased whey protein denaturation and dissociation of caseins at increased

pressure. The improved processability of concentrates processed at 200 MPa at 40–60 °C compared with that treated at lower temperatures (10 or 25 °C) could simply be due to the overriding effects of reduced viscosity with increasing temperature.

Solubility of fresh MPC powders. Nitrogen solubility of fresh MPC powders, measured after overnight storage, is given in Fig. 2(a). The response surface model, fitted with linear terms in pressure and temperature, showed that:

Nitrogen solubility(Initial/fresh)

$$= 70.8 + 0.0907 \times \text{pressure} - 0.293 \times \text{temperature}$$

The overall *P*-value ($P=0.084$) is consistent with no difference between the means, but comparisons between pairs of means produce a few significant differences. Although the lack of balance caused by the absence of $T=10$ °C and $T=25$ °C for a pressure treatment of 200 MPa, due to gelling of the concentrates, limits the interpretive value of this exercise.

The response surface model suggests that solubility increases on average by about 9% for an increase of 100 MPa units in pressure, and decreases by about 3% for an increase of 10 °C in temperature. The response surface model was also fitted with a pressure \times temperature interaction term and quadratic terms in both pressure and temperature. None of these additional terms were significant, which confirmed that the most appropriate model was the one with only linear terms.

Solubility of MPC powders after storage. The solubility after 6 week storage is shown in Fig. 2(b). The statistical analysis of the results, with pressure and temperature fitted as separate factors, resulted in similar conclusions to those for solubility of freshly prepared powders. The response surface model fitted with linear terms in pressure and temperature showed that pressure is significant but temperature is not and hence this model was not considered appropriate for the 6 week nitrogen solubility data. The response surface model with a pressure \times temperature interaction term and quadratic terms in both pressure and temperature resulted in similar findings to those for overnight solubility in that none of these additional terms are significant. Hence the most appropriate model for the nitrogen solubility at 6 week is the one with a linear term in pressure only and the fitted equation was:

Nitrogen solubility(after 6 week storage)

$$= 63.8 + 0.101 \times \text{pressure}$$

This equation implies that the solubility at 6 weeks increases on average by about 10% for an increase of 100 MPa units in pressure.

Of the treatments tested (0–400 MPa, 10–60 °C), a pressure treatment of 200 MPa at 40 °C was processable and the most beneficial for improved initial solubility and after

Table 2. Nitrogen solubility of MPC powders stored for 6 weeks at 20 °C – Effect of temperature and medium of reconstitution

Treatment		Nitrogen solubility (%)			
		Dispersed in water		Dispersed in milk	
Pressure (MPa)	Temperature (°C)	At 20 °C	At 45 °C	At 20 °C	At 45 °C
Not applied	10	65±1	94±1	90±2	99±2
	25	59±8	90±3	88±2	99±2
	40	64±6	86±6	89±2	98±3
	60	47±2	86±3	87±1	100±1
100	10	65±20	88±3	90±4	100±0
	25	68±9	91±2	91±2	100±2
	40	62±17	85±13	89±4	97±2
	60	59±1	90±1	86±1	96±1
200	10	No powder due to gelling of concentrate during pressurization			
	25	No powder due to gelling of concentrate during pressurization			
	40	84±2	91±2	88±1	96±1
	60	77±1	80±1	86±1	98±1
400	25	No powder due to gelling of concentrate during pressurization			
	40	No powder due to gelling of concentrate during pressurization			
	60	No powder due to gelling of concentrate during pressurization			

Data average of $n \geq 2$ with SD

6 weeks of storage. Hence the solubility of this powder was compared with the powder given no pressure treatment after 12 months of storage at 20 °C. MPC powders produced from concentrates processed at 200 MPa/40 °C maintained a higher nitrogen solubility (72%) compared with that of powders produced from non-pressure treated concentrates held at 10 or 25 °C where solubility on reconstitution in water was 41 and 34% respectively. The solubilities after 12 months of storage were lower than those obtained for corresponding powders after 6 weeks of storage (Fig. 2(b)), confirming the expected trend that increased storage was detrimental for MPC solubility.

However high protein powders made from recombined concentrates of sodium caseinate and whey protein isolates with differing casein to whey ratios (10:1 and 1:10) were completely soluble (~100%) after manufacture. This was irrespective of the pressure treatment (no pressure, 100, 200 and 400 MPa at 25 or 40 °C) or ratio of casein to whey protein. Assessment of powders stored at ambient temperature or 40 °C showed that high nitrogen solubilities (~100%) were maintained even after 1 month of storage at elevated temperatures (40 °C) in contrast to the MPC powders which lost solubility on storage. Taken together our results suggest that the decrease in solubility of high protein powders on storage is an issue only when caseins are predominantly present in micellar form (as in milk) but not when a critical amount of caseins are in the non-micellar state (as in caseinates). Our results are consistent with findings in the literature where an improvement in solubility was observed when citrate or monovalent salts was added prior to drying (Schuck et al. 1999, 2002; Bhaskar et al. 2001; Carr, 2002). The added salts and citrate (Udabage et al. 2000) solubilise the colloidal calcium phosphate and cause the dissociation

of micellar casein. A similar effect of solubilisation of colloidal calcium phosphate and dissociation of the micellar casein is observed when milk streams are subjected to HP (Schrader et al. 1997), thereby decreasing the ratio of micellar to non-micellar casein. Thus suggesting that an improved solubility can be achieved by decreasing the ratio of micellar to non-micellar casein. The difference between the addition of salts and a HP treatment is that the salt remains in the MPC powder if added to the concentrate prior to drying to achieve the critical decrease in the ratio of micellar to non-micellar casein which may be undesirable. However, applying HP treatment does not result in an increased salt level in the MPC. It is suggested that an increased concentration of serum protein in the milk stream due to HP alters the surface composition of the MPC powder and leads to improved solubility of MPC powders on storage. This would be in line with the hypothesis that an increased concentration of non-micellar protein at the droplet interface which delays the micellar–micellar crosslinking and skin formation (which delays the release of the caseins from the dispersed powder particle) is desirable for improved solubility of MPC powders on storage.

Effects of conditions of reconstitution on MPC powder solubility

Effects of temperature of reconstitution. After 6 weeks of storage, the nitrogen solubility of MPC powders was also tested at elevated reconstitution temperature (45 °C). As expected increasing the temperature of the water used for reconstitution of MPC powders increased solubility (Table 2). This is in line with previous research (McKenna, 2000). However, it was evident that powders with low

solubilities at 20 °C also had somewhat lower solubilities at 45 °C. The practice in industrial application of MPC powders is to increase the temperature of reconstitution when a powder has low solubility at 20 °C. However the decrease in solubility on extended storage may not be reversed sufficiently by increasing the temperature of reconstitution. Our results also agree with previous findings where low solubilities were obtained even at high temperatures of reconstitution on extended storage (McKenna, 2000).

Effects of medium of reconstitution. When the medium of reconstitution for MPC powders was changed to skim milk, solubility was markedly improved compared with those obtained for powders reconstituted in water at the same temperatures (Table 2). The alternative approach of reconstituting MPC powders in milk in situations where fortification of milk with milk solids is more effective than reconstitution in water at elevated temperature. The solubility in skim milk was superior to the solubility in water, one possible reason for this could be the higher mineral salt content of milk compared with water, which could promote the re-equilibration of mineral equilibria, providing a driving force for the release of the casein from the dispersed powder particle. This interpretation is consistent with the proposition that the loss of solubility is mainly due to the slower release of the casein micelles from aggregated protein particles, rather than the formation of insoluble material (Mimouni et al. 2010a, b). Famelart et al. (1999) and Hussain et al. (2011) have attributed changes in rehydration behaviour to changes in the hydration of casein micelles with the addition of NaCl and CaCl₂, the changes depending on the type and concentration of salt added.

Although in most industrial applications the use of elevated temperatures or liquid milk as the dissolving medium are feasible options, in many potential consumer and food service applications of MPC, solubility in water at ambient temperature is desirable. HP treatment of the liquid MPC prior to drying may be an attractive additive-free option to improve the solubility and storage stability of MPC powders especially powders with higher protein content (milk protein isolate >90% protein) with poor initial solubilities.

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