

**PREPARATION OF ENRICHED FRACTIONS OF  
 $\alpha$ -LACTALBUMIN AND  $\beta$ -LACTOGLOBULIN FROM  
CHEESE WHEY USING CARBON DIOXIDE**

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**ABSTRACT**

*A new process for preparing enriched fractions of  $\alpha$ -lactalbumin ( $\alpha$ -La) and  $\beta$ -lactoglobulin ( $\beta$ -Lg) from whey protein concentrate was developed. To prepare the fractions, solutions containing from 7% (w/w) to 25% (w/w) whey protein concentrate (WPC75) were sparged with carbon dioxide ( $\text{CO}_2$ ) in a batch reactor. The effects of pressure, temperature, concentration, and residence time on the distribution of the individual whey proteins in each fraction were investigated. Best results were obtained for 7% (w/w) WPC75 solutions, at reactor fill pressures of 4140 kPa and 5520 kPa, temperature of 64C and reactor residence time of 10 min. Gel electrophoresis of unpurified enriched samples showed that recovery of  $\alpha$ -La was approximately 55% and that of  $\beta$ -Lg was 78%. The resulting fractions have a pH of 6.0 and contain no added salts.*

**INTRODUCTION**

Sweet whey, a watery by-product of the cheese manufacturing process, consists of almost 7% solids. Proteins account for about 10-12% of the solids content, the rest being mainly lactose (74%), minerals (8%), milkfat (3%) and lactic acid (3%) (Morr 1989). With the advent of ultrafiltration, whey protein concentrates (WPC), with protein contents ranging from 35 to 85%, have been obtained. WPC have high lipid and lactose contents. Their functional properties may be variable because of different manufacturing processes or because of varying amounts of lactose, minerals, and lipids (Schmidt *et al.* 1984). Whey

protein isolates (WPI), with protein contents greater than 90%, are obtained by ultrafiltration followed by ion-exchange. WPI typically contain less than 1% fat or lactose. There is less variability in their functional properties (Morr 1989).

The proteins,  $\alpha$ -La,  $\beta$ -Lg, bovine serum albumin (BSA), heavy and light chain immunoglobulins (Igs), and proteose-peptones, comprise the principal whey proteins (DeWit 1989).  $\beta$ -Lg accounts for approximately 50% of the whey protein, the rest being  $\alpha$ -Lg (12%), Igs (10%), BSA (5%), and proteose-peptones (23%) (Morr 1989). Commercial WPI contain approximately 60%  $\beta$ -Lg and 20%  $\alpha$ -La, the remainder being Igs and BSA. WPI provide high gel strength and are used to replace larger quantities of egg white, caseinates, WPC, or soy protein in formulations.

Enriched fractions of  $\alpha$ -La and  $\beta$ -Lg could provide the food industry with proteins that have new functional properties. The  $\alpha$ -La enriched fraction may find use in humanized infant formulas (Heine *et al.* 1992). The  $\beta$ -Lg fraction may provide enhanced gel strength in formulas compared to commercial WPI.

Various methods for fractionation of the whey proteins, resulting in enriched fractions of  $\alpha$ -La and  $\beta$ -Lg, have been reported. Methods that rely on addition of salts with or without pH adjustment (Aschaffenburg and Drewry 1957; Kaneko *et al.* 1985; Kuwata *et al.* 1985; Al-Mashikh and Nakai 1987; Mailliar and Ribadeau-Dumas 1988; Mate and Krochta 1994) result in precipitation of  $\beta$ -Lg. Chen (1992) used the polyethylene glycol (PEG)/potassium phosphate aqueous two-phase system to partition and separate the fractions. Heine *et al.* (1992) used extraction with organic solvents in heat-treated acidified whey to isolate  $\alpha$ -La. Various chromatographic methods (Biscans *et al.* 1985; Ohtomo *et al.* 1988; Thibault 1991; Chiancone and Gattoni 1993; Carrere *et al.* 1994) have been used to separate the whey proteins. Methods have also been proposed based on differences in the solubilities of  $\alpha$ -La and  $\beta$ -Lg. Amundson *et al.* (1982) and Slack *et al.* (1986) used the processes of ultrafiltration and electrodialysis along with gentle heat treatment and pH adjustment, to obtain a precipitate of  $\beta$ -Lg. Pearce (1983) and Pearce *et al.* (1991) used gentle heat treatment and pH adjustment to aggregate  $\alpha$ -La.

The importance of Ca binding for stabilization of the structure of  $\alpha$ -La has been discussed by several workers (Hiraoka *et al.* 1980; Kronman *et al.* 1981; Bernal and Jelen 1984).  $\alpha$ -La, containing one mole of Ca per mole of protein, unfolds and precipitates at temperatures in the range of 50 to 65°C at low Ca, that results from lowered pH in the range of 3.8 to 4.2.  $\alpha$ -La aggregates renature when pH is readjusted. Bramaud *et al.* (1995) showed that addition of  $\text{CaCl}_2$  stabilizes  $\alpha$ -La at its isoelectric pH and under heat treatment, resulting in reduced  $\alpha$ -La in the precipitated fraction. Removal of Ca destabilized  $\alpha$ -La resulting in a higher precipitated fraction.

The use of  $\text{CO}_2$  for isolation of casein from milk (Tomasula *et al.* 1995; Tomasula *et al.* 1997) has been demonstrated in batch and continuous systems.

The associated whey had a residual pH of 6.0. The precipitant was eliminated from the whey upon depressurization. The precipitant remains in the whey in other casein precipitation processes that use acid, salt, ethanol (Fox 1989). As discussed above, current methods for fractionation of the whey proteins generally rely on the addition of agents which contaminate the products and would, if included as part of a process, require steps for their removal.

The purpose of this study was to investigate the use of CO<sub>2</sub> for the fractionation of whey proteins resulting in enriched fractions of  $\alpha$ -La and  $\beta$ -Lg from WPC and to identify the optimal processing conditions. The degree of separation and yield of the component whey proteins in the  $\alpha$ -La and  $\beta$ -Lg enriched fractions were also determined.

## MATERIALS AND METHODS

Solutions were prepared from whey protein concentrate containing approximately 75% protein (WPC75) (Calpro Ingredients, Inc., Corona, CA). The nominal analysis was the following: protein, 77.4%; moisture, 4.7%; fat, 6.5%; ash, 2.7%; lactose by difference, 8.7% and Ca, 4510 ppm.

A 1000 mL Model 4521 316SS Parr batch reactor (Parr Instrument Co., Moline, IL) was used to obtain  $\alpha$ -La and  $\beta$ -Lg enriched fractions from the WPC75 solutions. Details on the construction and use of the reactor are found in Tomasula *et al.* (1995).

Preliminary studies were conducted using 500 g samples of 7% (w/w) and 25% (w/w) WPC75 solutions prepared with triple distilled water to establish the minimum temperature, pressure, and reactor residence time required for separation of the fractions. In a typical trial, WPC75 solution refrigerated at 6C was poured into the batch reactor and the lid was secured. CO<sub>2</sub> (AirCo., BOC Group, Murray Hill, NJ) was allowed to fill the reactor until a pressure of 2760 kPa, 4140 kPa, or 5520 kPa was indicated on the pressure gauge. This pressure will be referred to as the fill pressure. The contents of the reactor were then rapidly heated to the desired temperature, thereby increasing pressure, and held for residence times up to 30 min. Pressure was then released and the reactor contents were held for 15 min at 45C. This was to ensure that all samples were handled similarly before further treatment. The resulting slurries were centrifuged using a Model RC-5B Sorvall Refrigerated Superspeed Centrifuge (Newtown, CT) at approximately 5000 g for 1 h. The precipitate or the  $\alpha$ -La enriched fraction was freeze-dried. The extent of precipitation was estimated by dividing the dry weight of precipitate by the weight of protein in the initial WPC sample on a dry basis. Precipitate was observed only at temperatures greater than 50C and at fill pressures greater than 2760 kPa. Half as much precipitate was produced at a residence time of 5 min than at 30 min. The same amount of precipitate was obtained at 10 min and 20 min. Experiments were not conducted

above 64C because the pressure limits of the vessel were exceeded upon heating the CO<sub>2</sub>-WPC75 solutions to higher temperatures.

Additional trials were conducted using solutions with total solids content greater than 25% (w/w) but the  $\alpha$ -La fraction was difficult to remove because of the solution viscosity. 25% (w/w) was selected as the upper limit for total solids content.

For the main experimental trials, the reactor was charged with 500 g of 7% (w/w), 16% (w/w), or 25% (w/w) WPC75 solution. The reactor was then filled with CO<sub>2</sub> at 6C until a fill pressure of 2760 kPa, 4140 kPa or 5520 kPa was reached. The reactor contents were then heated to 54, 60, or 64C and held for a residence time of 10 min. Pressure was noted. The reactor was then depressurized and the reactor contents were cooled to 45C and held for 15 min. The resulting slurries were centrifuged at 5000 g for 1 h. Experiments were performed in triplicate and the data were analyzed by ANOVA.

The  $\alpha$ -La enriched fraction was washed with triple distilled water and then freeze-dried. The  $\beta$ -Lg fraction was not treated further.

#### **A Control Study**

Runs were carried out using N<sub>2</sub> instead of CO<sub>2</sub> to pressurize the vessel. After filling the reactor with a 7% (w/w) WPC75 solution at 6C, N<sub>2</sub> was added to the reactor until a fill pressure of 4140 kPa was reached. The reactor contents were then heated to 64C and held for 10 min. The reactor was then depressurized and the reactor contents were cooled to 45C and held for 15 min. The product was centrifuged at 5000 g for 1 h. This experiment was performed in triplicate.

#### **Analytical Methods**

Compositions of the fractions were determined according to AOAC (1984) methods. To determine solids content, 2 g of the freeze-dried  $\alpha$ -La enriched fraction or 10 mL of the  $\beta$ -Lg enriched fraction were placed in a crucible, evaporated to near dryness on a steam bath (16.032), placed in a vacuum oven overnight for 17 h at 70C, cooled in a desiccator and weighed (16.212). Fat content was obtained using the Roese-Gottlieb Method (16.064). Protein content was obtained using the Kjeldahl method given in (16.213) using a factor of 6.38. To determine ash content, the crucible was fired overnight in a muffle furnace at 550C, cooled, and weighed. Ca was determined using an atomic absorption spectrometer (AAS) (Perkin-Elmer 1100B, Norwalk, CT). Ash samples were dissolved in an AAS blank solution (2% HNO<sub>3</sub> and 0.1% LaCl<sub>3</sub>) to suppress ionization. AAS blank solution was also used to zero the instrument, to prepare samples, and to dilute samples. The spectrometer was calibrated using certified Ca standards in the range of the ash samples according to the procedure detailed

in the Perkin-Elmer Handbook (Perkin-Elmer 1994). Lactose was obtained by difference. Analyses were carried out in triplicate.

#### **pH Measurement**

pH values were measured with a high pressure probe (Innovative Sensors, Inc., Anaheim, CA) designed to withstand pressures up to 69 MPa. pH of the untreated whey solutions was 6.35.

#### **Determination of the Whey Proteins**

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of protein samples was carried out on a Phast System (Pharmacia, Piscataway, NJ) with a phast gel gradient of 8-25% acrylamide. Samples were prepared and proteins separated according to the method of Parris *et al.* (1990). The protein bands were stained with Coomassie R350 dye. Molecular weight standards were run with WPC75 (Calpro Ingredients, Inc., Corona, CA). The stained gels were dried and the intensity of the bands was scanned with ImageQuant™ (Molecular Dynamics, Inc., Sunnyvale, CA). The coefficient of variation for  $\alpha$ -La and  $\beta$ -Lg in 5 replicate whey samples using this method were 11.9% and 9.1%, respectively.

### **RESULTS AND DISCUSSION**

Preliminary runs showed that very small amounts of  $\alpha$ -La enriched precipitate — containing ash, protein, lactose and fat — were obtained at temperatures less than 54C. Aggregation of  $\alpha$ -La occurred under the conditions of temperature ( $54C \leq T \leq 64C$ ), fill pressure ( $2760 \text{ kPa} \leq P \leq 5520 \text{ kPa}$ ), concentration ( $7\% \text{ (w/w)} \leq C \leq 25\% \text{ (w/w)}$ ), and residence time ( $10 \text{ min} \leq t \leq 30 \text{ min}$ ). Precipitates derived from the 25% (w/w) WPC75 solutions were difficult to recover because of the solution viscosity. Maximal precipitation of  $\alpha$ -La was obtained from the 7% (w/w) WPC75 solution at 64C and at any of the fill pressures. pH at conditions of maximal precipitation ranged from 4.3 to 4.6. The corresponding  $\beta$ -Lg remained in solution.

Some slurry was held up in the exit lines of the reactor after depressurization. Material balance calculations around the reactor showed that reactor losses averages 3.1% of the initial WPC75.

#### **Effect of pH or Pressure**

The effects of CO<sub>2</sub> pressure on the corresponding precipitation pH obtained after heating the vessel contents to 54C are shown in Fig. 1. The results at 64C are not significantly different and are not reported here. The fill pressures that

correspond to the pressure reached upon heating the contents of the batch reactor are shown in parentheses along the x-axis. At both temperatures, pH decreased dramatically with increasing pressure up to a fill pressure of 2760 kPa. The pH then leveled off with further increases in pressure, because of the buffering effects of whey. Buffering effects result in a higher pH for a whey solution compared to water under the same pressure (Tomasula *et al.* 1995).

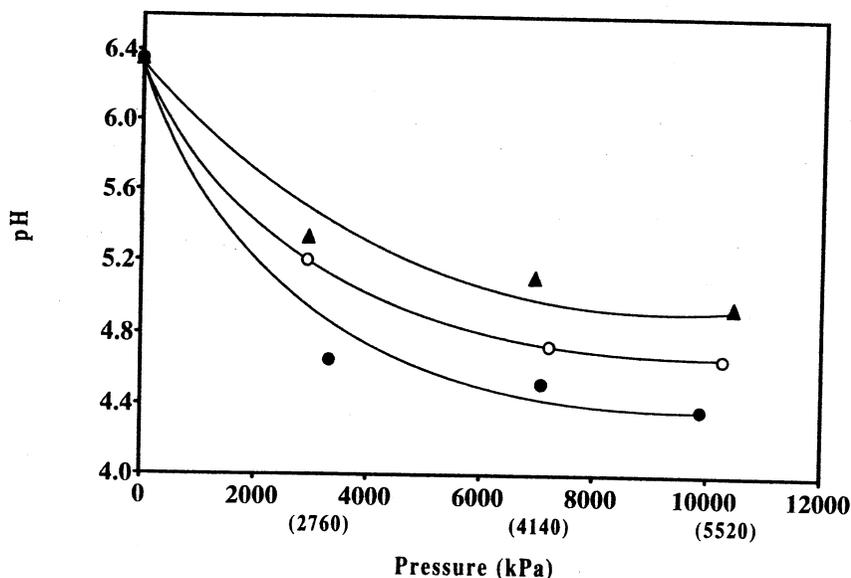


FIG. 1. pH AS A FUNCTION OF PRESSURE FOR 7% (W/W) ●, 16% (W/W) ○, AND 25% (W/W) ▲, WPC SOLUTIONS AT 54C  
Fill pressures are shown in parentheses.

A pH of 6.0 was obtained after depressurization of the reaction vessel. This is a significant advantage over processes that use acids for fractionation because neutralization of the enriched fractions with bases such as KOH or NaOH is not required.

Pearce (1983) reported that maximal precipitation of  $\alpha$ -La using HCL occurred at pH 4.2 and temperature of 65C. In this study, maximal precipitation of  $\alpha$ -La occurred in the pH range from 4.3 to 4.6 at 64C.

Precipitate was not obtained when the vessel was pressurized with N<sub>2</sub> instead of CO<sub>2</sub>. There was no pH effect. This result suggests that precipitate formation is a result of pH adjustment of the WPC75 solution through carbonic

acid formation and application of heat, not pressure induced aggregate formation. Pressure effects on proteins are generally seen at pressures far above those used in this study (Dumay *et al.* 1994; Nakamura *et al.* 1993).

### Composition of the Precipitates

The effects of temperature and pressure on composition of the  $\alpha$ -La and  $\beta$ -Lg enriched fractions are shown in Table 1. Results are shown for the 7% WPC75 solutions only.

TABLE 1.  
EFFECT OF TEMPERATURE AND PRESSURE ON PROXIMATE COMPOSITION OF THE  
 $\alpha$ -LA AND  $\beta$ -LG WHEY PROTEIN FRACTIONS FROM 7% (W/W) WPC75.  
MOISTURE-FREE BASIS<sup>1</sup>

T C	Fill Pressure (kPa)	Fraction	%Protein	%Fat	%Ash	%Lactose	%Calcium <sup>2</sup>
54	2760	$\alpha$ -La	83.3 $\pm$ 0.8a	10.6 $\pm$ 0.3a	0.93 $\pm$ 0.15a	5.2 $\pm$ 3.3a	14.7 $\pm$ 7.0a
		$\beta$ -Lg	87.9 $\pm$ 0.5f	2.8 $\pm$ 0.4d	3.60 $\pm$ 0.52e	5.7 $\pm$ 1.4d	85.3 $\pm$ 1.5d
54	4140	$\alpha$ -La	75.0 $\pm$ 2.5b	11.1 $\pm$ 0.4a	1.93 $\pm$ 0.15b	12.0 $\pm$ 3.1b	41.0 $\pm$ 10.0b
		$\beta$ -Lg	90.3 $\pm$ 2.5f	.8 $\pm$ 0.1e	2.71 $\pm$ 0.50e	6.2 $\pm$ 3.1d	59.0 $\pm$ 2.0e
54	5520	$\alpha$ -La	78.8 $\pm$ 2.5b	11.8 $\pm$ 0.1b	1.82 $\pm$ .07b	7.6 $\pm$ 2.7bc	59.9 $\pm$ 7.0c
		$\beta$ -Lg	88.5 $\pm$ 1.8f	.1 $\pm$ 0.1f	2.97 $\pm$ 0.49e	8.5 $\pm$ 2.3d	40.9 $\pm$ 2.5f
64	2760	$\alpha$ -La	85.9 $\pm$ 0.2c	9.0 $\pm$ 0.4c	1.04 $\pm$ 0.15a	4.1 $\pm$ 0.8a	28.7 $\pm$ 7.9a
		$\beta$ -Lg	78.7 $\pm$ 1.3g	3.1 $\pm$ 0.4d	3.60 $\pm$ 0.08e	14.6 $\pm$ 1.8e	71.3 $\pm$ 1.8h
64	4140	$\alpha$ -La	88.2 $\pm$ 1.0d	8.6 $\pm$ 0.8c	1.09 $\pm$ 0.15a	2.2 $\pm$ 2.0a	24.4 $\pm$ 7.1a
		$\beta$ -Lg	77.4 $\pm$ 2.4g	3.1 $\pm$ 0.5d	3.59 $\pm$ 0.08e	15.9 $\pm$ 3.0e	73.7 $\pm$ 1.8h
64	5520	$\alpha$ -La	85.2 $\pm$ 1.3c	8.4 $\pm$ 0.5c	1.21 $\pm$ 0.04a	5.2 $\pm$ 2.1a	58.0 $\pm$ 8.0bc
		$\beta$ -Lg	87.4 $\pm$ 1.8f	3.3 $\pm$ 0.4d	3.13 $\pm$ 0.07f	6.2 $\pm$ 2.3d	41.8 $\pm$ 1.7f

<sup>1</sup>Values are the mean of 3 experiments, each with 3 replicates.

<sup>2</sup>%Ca calculated relative to amount of Ca in initial 7% WPC75 solution.

<sup>a-h</sup>Means with different superscripts in the same column for either  $\alpha$ -La or  $\beta$ -Lg are significantly different (P < 0.05).

Increasing temperature, at constant pressure, resulted in slightly greater increases in protein content of the  $\alpha$ -La fraction because of increased precipitation (Table 1). Increasing pressure resulted in less precipitate at 54C and almost no change in amount of precipitate at 64C. After centrifugation of the  $\alpha$ -La -  $\beta$ -Lg mixture, a distinct fat layer was observed throughout the  $\beta$ -Lg supernatant which could not be removed by centrifugation. Amundson *et al.* 1982 also observed a "lipid -  $\beta$ -Lg complex" that either settled to the bottom or floated to the surface of the container after centrifugation. It was explained that in cases where the density of the complex is less than that of water, it floats to the surface of the container, but settles to the bottom when the reverse is true. Fauquant *et al.* (1985) have shown that the residual fat layer in WPC consists mostly of phospholipids, which can be removed prior to processing through treatment with  $\text{CaCl}_2$ , pH adjustment, and heating, followed by microfiltration. As shown in Table 1, the  $\alpha$ -La enriched fraction has a lower fat content at 64C than at 54C. This suggests either an expulsion of phospholipid, which is larger in size relative to the whey proteins (Jelen 1991), with increased aggregate formation and possibly interaction with  $\beta$ -Lg, or a thermoaggregation of the lipids and Ca.

The % ash contents of  $\alpha$ -La and  $\beta$ -Lg at 54C and 2760 kPa are not significantly different than those at 64C and 2760 kPa or 4140 kPa. The increase in % ash content of  $\alpha$ -La with increase in pressure follows the increase in Ca discussed below.

Values of % lactose were obtained by difference. The values for % ash and also % lactose are highly variable because the enriched fractions were not purified. The enriched  $\alpha$ -La fraction can be washed further by resuspension of the pellet in water. Diafiltration can be used to remove ash and lactose from the enriched  $\beta$ -Lg fraction.

At 54C, Ca content of the enriched  $\alpha$ -La fraction, reported as the percentage of Ca relative to the amount in the starting material, was lowest at 2760 kPa and increased with increasing pressure. At 64C, Ca content of the same fraction increased when pressure was increased to 5520 kPa. The variability in Ca content cannot be attributed to changes in pH, pressure, or temperature. The pH is not significantly different at either temperature and drops only slightly with increasing pressure. It was expected under these conditions that a constant value of Ca would be obtained. The lack of repeatability in values of reported Ca content is likely the result of not washing the precipitates before analysis. Overall, the results support the observations of Hiraoka *et al.* (1980), Kronman *et al.* (1981), and Bernal and Jelen (1984), because precipitation of the  $\alpha$ -La fraction occurred at lowered pH and in the temperature range of 50C to 65C.

## Effect of Temperature

The influence of temperature and pressure on recovery of  $\alpha$ -La and of  $\beta$ -Lg was determined by applying densitometry to the stained gels to quantify the results. The results for temperature effects at 4140 kPa are reported in Table 2 as percentage yield or recovery. Recovery is defined as the amount of the whey protein in the enriched fraction divided by the amount in the initial protein. The distribution of the individual whey proteins in the feed WPC75 was:  $\alpha$ -La (22.4%),  $\beta$ -Lg (50.8%), BSA (9.9%) Igs (9.5%), and Lf (7.5%).

TABLE 2.  
EFFECT OF TEMPERATURE ON THE DEGREE OF FRACTIONATION<sup>1</sup> OF WHEY  
PROTEINS IN THE  $\alpha$ -LA AND  $\beta$ -LG ENRICHED FRACTIONS PRECIPITATED  
FROM 7% WPC75 SOLUTIONS AT 4140 KPA

Enriched Fraction	54C		64C	
	$\alpha$ -La	$\beta$ -Lg	$\alpha$ -La	$\beta$ -Lg
$\alpha$ -La	27.5%	72.5%	55.4%	44.6%
$\beta$ -Lg	30.1%	69.9%	21.8%	78.2%
BSA	63.5%	36.5%	55.6%	44.4%
Igs	69.4%	30.6%	49.4%	50.6%
Lf		100.0%	47.9%	52.1%

<sup>1</sup>Degree of Fractionation is defined as the amount of the whey protein component in the enriched fraction divided by the amount of the same component in the feed WPC.

BSA - Bovine serum albumin

Igs - Immunoglobulins

Lf - Lactoferrin

As shown in the table, the percentage of  $\alpha$ -La in the  $\alpha$ -La enriched fraction increased with increase in temperature, while the percentages of  $\beta$ -Lg, BSA and Igs decreased. At 54C, Lf was not detected in the  $\alpha$ -La enriched fraction.

### **Effect of Concentration of Initial WPC**

At a fixed temperature, and concentration of either 7% (w/w), 16% (w/w), or 25% (w/w), the effect of pressure (or pH) on the degree of fractionation (results not shown) was negligible at the three pressures studied. This is because pH, as shown in the figures, changes slowly or the change is insignificant, with pressure increase from 2760 kPa to 5520 kPa. For the 7% (w/w) solution at 64C, the percentage of  $\beta$ -Lg in the  $\beta$ -Lg enriched fraction with respect to total protein, was approximately 78% at the three pressures. The percentage of  $\alpha$ -La or  $\beta$ -Lg recovered in either fraction at 16% (w/w) and 25% (w/w) did not improve with the increase in WPC75 feed concentration. These results, even though much higher concentrations were used in this study, are consistent with Bramaud *et al.* (1995). Further increases in  $\alpha$ -La concentration did not change the amount of the precipitated fraction by much. This was attributed to the variation in ionic content or to the variation in the other protein concentrations.

### **Effect of Residence Time**

Effects of residence time on the extent of  $\alpha$ -La precipitation were noted for reactor residence times up to 30 min. Every sample was cooled for 10 min after reactor depressurization and before centrifugation. For residence times  $> 10$  min, changes in the amount of  $\alpha$ -La precipitated were negligible. These results are unlike those of Bramaud *et al.* (1995) for  $\alpha$ -La precipitated at pH 4.2 with mineral acid at 64C. They noted an almost linear increase in the amount of  $\alpha$ -La precipitated for residence times to 30 min followed by a leveling off in precipitation rate up to 180 min. The discrepancies between the results of Bramaud *et al.* (1995) and the results of this study are perhaps due to the differences in feed WPC concentration, much higher concentrations were used in this study. Different analytical methods were also used to determine the whey proteins in the enriched fractions. In addition, the experiments in this study were carried out on a much larger scale. Differences in the amount of precipitate obtained appear to be only on the order of 10%. Bramaud *et al.* (1995) carried out their experiments on the laboratory scale, with no reported loss of material.

### **Comparison with Other Methods**

Recovery of yield of  $\beta$ -Lg is compared in Table 3 to that obtained in other studies. Yield of  $\alpha$ -La is not reported in the table because in most instances, the

objective of the salting-out methods was to eliminate  $\beta$ -Lg from whey in order to prepare a simulated human milk containing  $\alpha$ -La, immunoglobulins (Igs), and lactoferrin (Lf). In most cases, values are given for the crude products as reported by the respective workers. The salting-out methods generally resulted in high yields of  $\beta$ -Lg and almost pure fractions of  $\beta$ -Lg, but purification of the protein must be accompanied by salt removal steps for edible product. Amundson *et al.* (1982), using ultrafiltration and electro dialysis along with heat treatment and pH adjustment, prepared a  $\beta$ -Lg enriched precipitate fraction and an  $\alpha$ -La enriched supernatant fraction. The  $\beta$ -Lg fraction contained mostly  $\beta$ -Lg and some other unspecified whey proteins. The  $\alpha$ -La fraction contained some  $\beta$ -Lg, BSA, and Igs. The weight fractions of the components were not reported. Pearce *et al.* (1991) reported that the  $\alpha$ -La enriched fraction also contained enzymes, Igs, and BSA. The  $\beta$ -Lg enriched fraction also contained casein derived peptides.

TABLE 3.  
AMOUNT OF  $\beta$ -LG RECOVERED BY DIFFERENT METHODS

Method	Recovery of $\beta$ -Lg, (w/w)%	Reference
Salting-out		
Method 1	>99	Kuwata <i>et al.</i> (1985)
Method 2	62	Kuwata <i>et al.</i> (1985)
Salting-out	90	Kaneko <i>et al.</i> (1985)
Salting-out	84	Maillart and Ribadeau-Dumas (1988)
Salting-out	>65	Mate and Krochta (1994)
Salting-out	80	Al-Masheik and Nakai (1987)
Ultrafiltration and Electrodialysis	90	Amundson <i>et al.</i> (1982)
pH	>75	Pearce <i>et al.</i> (1983)
CO <sub>2</sub>	78	Tomasula (This study)

The recovery of  $\beta$ -Lg in this study is comparable to that reported by Pearce *et al.* (1991).  $\beta$ -La, Igs, Lf and BSA were distributed almost evenly in both fractions as shown in Table 2 for the data at 64C. It is believed that the mechanism for aggregation of  $\alpha$ -La is similar in the two cases even though in this study, CO<sub>2</sub>, was used to lower pH.

## CONCLUSIONS

Processes that use high pressure or supercritical CO<sub>2</sub> as alternatives to mineral acids, organic solvents, or salts, are of growing interest. Fractionation of the whey proteins in a WPC using heat and CO<sub>2</sub>, in a pilot-scale batch process, resulted in uncontaminated fractions with a residual pH of 6.0. Recovery of  $\alpha$ -La and  $\beta$ -Lg was comparable to other methods.

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