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Preparation of Casein Using Carbon Dioxide[1]

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ABSTRACT

The effects of pressure, temperature, residence time, and mass of skim milk on some characteristics of casein, prepared by precipitation with high pressure CO_2, were examined in a batch reactor. For a 500-g milk sample, precipitation occurred at pressures >2760 kPa and temperatures >32°C. Residence time was not significant and was held at 5 min.

Yields were maximum at 2750 to 5520 kPa and at 38 to 49°C for a 500-g milk sample. The resulting whey had a pH of 6.0. The casein product had an acceptable appearance and had greater solids, ash, and Ca contents than commercial acid caseins. Particle size distribution studies showed that the mean particle size was sensitive to precipitation pressure and temperature and was similar to that of acid caseins produced under laboratory conditions.

The HPLC studies of the casein and whey fractions showed that precipitation by CO_2 did not result in fractionation of casein or whey proteins to their component proteins.

(**Key words**: supercritical carbon dioxide, casein, whey, high pressure)

Abbreviation key: $\mathbf{d_{50}}$ = mean curd particle size, **PSD** = particle size distribution.

INTRODUCTION

Casein is isolated from milk through the action of lactic acid bacteria or the acidification of skim milk by an acid such as HCl or H_2SO_4. In either case, casein precipitates at the isoelectric point of approximately pH 4.6. Many theories have been proposed to explain the mechanism for acid coagulation of casein micelles (2, 4).

The acid precipitation of casein by dissolution of CO_2 in milk has been demonstrated on a laboratory scale (6). Experiments were conducted in a batch reactor using up to 300 g of milk. Yields >99% were achieved at 50°C and 3500 kPa. Contents of solids, ash, and Ca for casein depended on precipitation pressure and temperature, and values fell between those of the acid and rennet caseins. The whey had a pH of 6.0 compared with the pH of 4.6 obtained in acid precipitation processes. Acid precipitation may require less processing of whey before its further use or disposal.

The use of CO_2 as a precipitant is attractive because it eliminates the presence of the precipitant from the products. However, CO_2 use is currently limited to small batch operation. Batch operation is associated with high labor costs of charging and discharging the reactor as well as the possibility of variations among batches. For CO_2 precipitation processes to be competitive, continuous operation on a large scale is required.

A continuous process requires a vessel for reaction and precipitation as well as a method for simultaneous removal of casein, whey, and CO_2 from the high pressure vessel as milk is fed to the vessel. A continuous flow tubular reactor and precipitator is the best choice for large-scale operation because, in addition to its high operation capacity, it can be built using thick-walled tubing and jacketed for efficient temperature control using a double-pipe arrangement. Equipment costs for a continuous mixed flow reactor would be prohibitive for both high pressure service and high output. Methods have not been reported for simultaneous removal of casein, whey, and CO_2 from

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[1]Mention of brand or firm names does not constitute an endorsement by the USDA over others of a similar nature not mentioned.

the high pressure vessel as milk is charged to the vessel.

The laboratory data of Jordan et al. (6) have established the boundaries of temperature and pressure for optimal yield. Mixing effects and poor contact between milk and CO_2 were minimized because of the small volume of milk used in these studies. For the design of the reactor and precipitator, additional data are necessary to test the feasibility of working with larger volumes of milk and to identify the factors, if any, that would lead to decreased yield.

In this study, data were obtained in a large batch reactor to determine the effects of pressure, temperature, residence time, and weight of milk on casein precipitated by dissolving CO_2 into milk. In addition, conditions leading to optimal precipitation were identified, and the effects of CO_2 addition to milk on the distribution of the individual casein and whey proteins were examined.

MATERIALS AND METHODS

Source of Milk

Pooled raw milk, skimmed before use, from a local dairy was used in all trials. The mean analyses were 3.5% total protein, 2.8% casein, 9.0% total solids, .6% ash, 4.9% lactose, and .12% (1200 ppm) Ca. The mean pH at 20°C was 6.6.

Design and Operation of Reactor

The reactor shown in Figure 1 is a 1000-ml, model 4521 316SS Parr reactor (Parr Instrument Co., Moline, IL). The reactor was jacketed for temperature control. The CO_2 sparger was fabricated from a 2-μm porous metal filter (Supelco, Inc. Belfonte, PA), which was then attached to a .6-cm tube. For removal from the pressurized vessel, whey was filtered through a series of screens packed into a disc. A schematic diagram of the entire process is shown in Figure 2.

In a typical trial, a weighed amount of milk was poured into the vessel, the lid was secured, and the milk was heated. A timer was started, and CO_2 (Union Carbide, Linde Division, Danbury, CT) was allowed to fill the reactor until the desired pressure was indicated. Filling and pressurizing required 30 to 60 s. After a residence time of 5 min, the vessel was depressurized and opened. The casein was removed, rinsed several times with distilled water, and weighed. The pH of the casein and whey samples was measured at 25°C.

Initial tests showed that casein precipitated only when CO_2 was sparged or bubbled through the milk into the reactor; very little precipitated if CO_2 was added to the headspace of the reactor. Stirring rate did not affect casein yield. The vessel contents were not stirred because stirring broke up the curd.

Experimental Design and Statistical Analysis

Removal of casein from the batch reactor after each run was labor intensive because the 16-kg reactor had to be tilted forward and held while the casein was removed from the bottom of the reactor. A spring system was added to the reactor to make this job easier. Cleaning and sanitizing the reactor after each run and sample preparation for analysis took at least 3 h per run in many cases because casein would cling to the sparger and the walls of the reactor at higher temperatures. Minimizing the number of runs while looking at as many factors as possible was desirable in this study.

A 2^4 factorial experimental design was used to screen for the effects of two levels each of temperature, pressure, mass of milk, and residence time on casein yield and curd appearance. In this screening study, runs were not replicated. The data used in the design are shown in Table 1. The levels for each variable were selected based on preliminary tests that were performed to identify the region where casein yield was optimal, i.e., where yield was approximately 2.8%. The laboratory data and results found by Jordan et al. (6) were also used as a guide in selecting the levels for each variable. The results were analyzed using a normal probability plot (1, 10).

Based on the results obtained from the screening study, trials were then conducted in triplicate at 32, 38, 43, 49, and 60°C at constant milk mass from 200 to 900 g. Pressure was varied from 2760 to 5520 kPa. A residence time of 5 min was used for all trials. The effects of these variables were evaluated on yield, solids content, ash content, and Ca con-

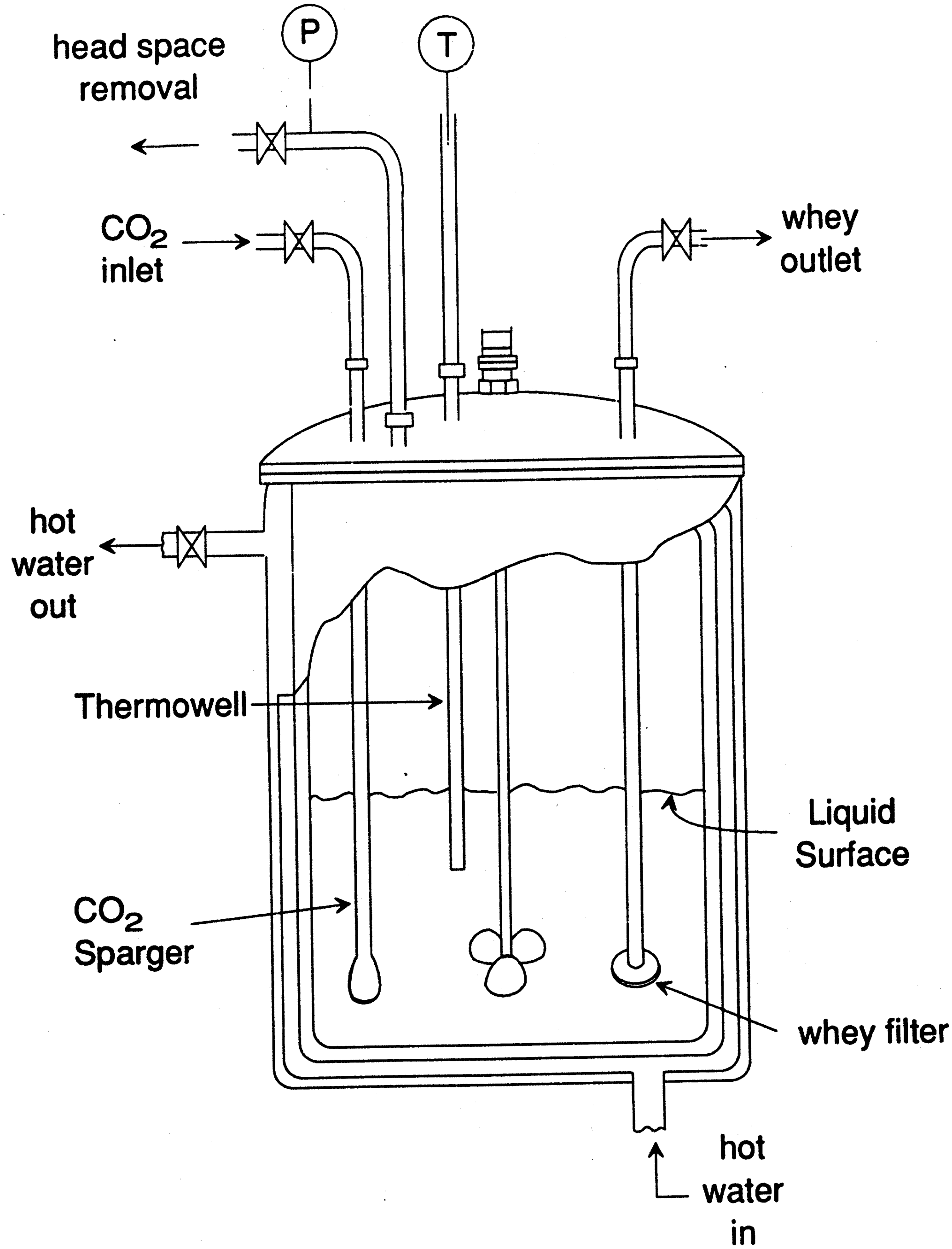

Figure 1. Batch reactor.

tent as well as on appearance and particle size distribution. Two trials were also conducted at 25°C and 2760 and 5520 kPa, respectively.

The data were analyzed with ANOVA, and means were compared using the *t* test (1). Differences were considered to be significant at $P < .05$.

TABLE 1. Data used in a screening study (2^4 factorial design).

	Level design variable	
	Low	High
Pressure, kPa	2760	5520
Temperature, °C	38	60
Residence time, min	5	10
Milk sample size, g	500	750

Determination of Solids, Ash, and Ca Contents

To determine solids contents, 2 g of casein or 10 ml of whey were placed in a crucible, evaporated to near dryness on a steam bath, placed in a vacuum oven overnight for 17 h at 70°C, cooled in a desiccator, and weighed. To determine ash content, the crucible was fired overnight in a muffle furnace at 550°C, cooled, and weighed.

The Ca was determined using an atomic absorption spectrometer (Perkin-Elmer 1100B; Norwalk, CT). Ash samples were dissolved in 10 ml of 50% (vol/vol) HNO_3 and then rinsed in a 100-ml volumetric flask with water. The spectrometer was calibrated using certified Ca standards prepared with acid in the range of the ash samples.

Semiquantitative Determinations of the Major Milk Proteins by HPLC

The HPLC method was used to determine the relative weight percentages (wt/wt) of the major milk proteins in some of the casein and

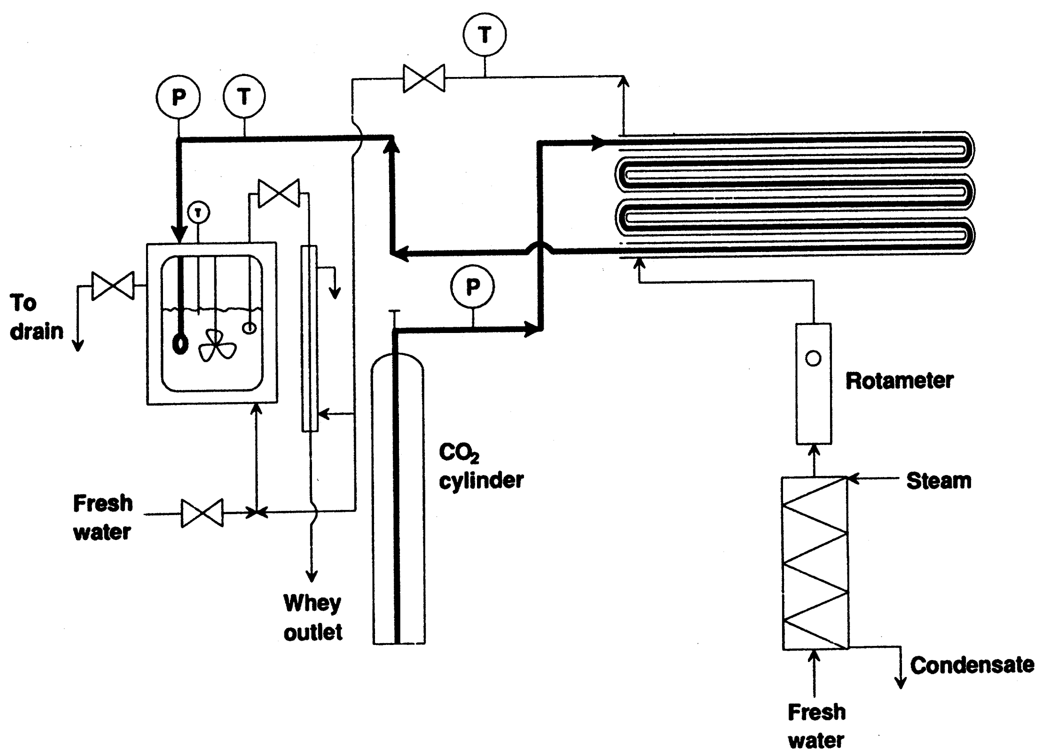

Figure 2. Schematic diagram of experimental apparatus.

whey samples (8). The system consisted of a Hewlett-Packard 1050 quaternary pump module (Wilmington, DE) interfaced with an HP 1050 autosampler and injector module, a Spectra Physics SP 8440 variable UV/VIS detector (San Jose, CA), and an HP 3396A integrator. Aliquots (50 μl) were injected onto the column. The HPLC was calibrated using purified proteins standards with .35 mg/ml of ribonuclease A as an internal standard. Responses were calculated from peak area measurements at an absorbance of 280 nm.

HPLC Determination of Lactose

Lactose was determined using the method given by Kwak and Jeon (7). An Aminex HPX-87-H 300 × 7.8-mm ion-exclusion column was used preceded by a Cation-H, 40 × 4.6-mm guard column (Bio-Rad Laboratories, Melville, NY); .013*N* HNO_3 was the mobile phase. Refractive index of lactose was measured using a Spectra Physics differential refractometer and integrator and used to determine lactose concentration from a standard refractive index curve.

Particle Size Distribution

Particle size distribution (PSD) data were determined using a wet sieving method (5).

pH Measurement at High Pressures

The pH conditions were measured as a function of pressure with a high pressure probe (Innovative Sensors, Inc., Anaheim, CA) designed to withstand pressures of up to 6.9 MPa. The probe, which could not be inserted directly into a vessel port, was inserted outside the vessel in the whey removal line where pressures in the line were the same as those in the vessel.

pH was recorded for a 750-g milk sample at 38°C as the vessel was pressurized with CO_2 and then depressurized.

For purposes of comparison, casein was precipitated from a 750-g milk sample at 38°C by addition of 1*N* HCl. A glass rod was used to stir the beaker contents slowly, and care was taken not to disturb the precipitated casein. pH was recorded as a function of volume of HCl added.

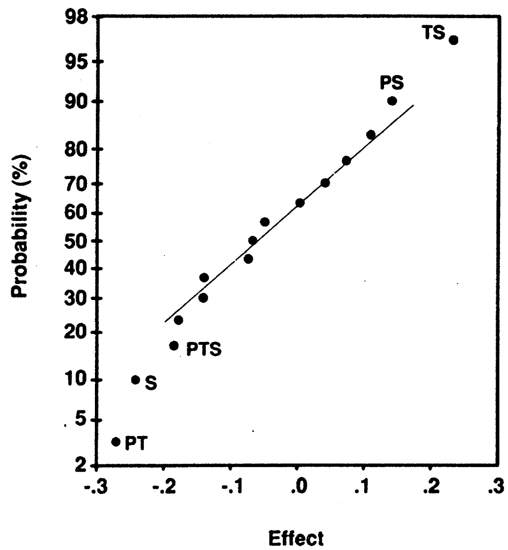

Figure 3. Normal probability plot of effects for 2^4 factorial experimental design. TS = T, × S two-factor interaction; PS = P × S two-factor interaction; and PTS = P × T × S, three factor interaction, where T = temperature, P = pressure, and S = mass of milk.

RESULTS AND DISCUSSION

The results of the screening study were analyzed using a normal probability plot (1, 10) and are shown in Figure 3. The main effect, mass of milk, and the interactions of pressure × temperature, temperature × mass of milk, and pressure × mass of milk all affected yield. Effects of residence time were not significant, as observed previously (6).

The screening study also showed that curd appearance was strongly influenced by temperature. At 38°C and either 2760 or 5520 kPa, the curd was moist and had an appearance similar to that of cottage cheese. At 60°C and either pressure, the curd was dry, had a rubbery feel, and was stringy. Individual particles were not apparent.

Casein Yield

For all runs, casein yields were normalized to 100% and were considered to indicate complete precipitation if yield was >2.8% of the weight of the milk, which is the mean casein content of milk. Yield increased to 100% for milk temperatures >38°C and pressure of 4140 kPa as milk sample size was increased from 200 to 750 g and then decreased sharply with further increases in mass (Figure 4). On average, yields were maximal when milk mass ranged from about 400 to 750 g and for temperatures >32°C. Trends were the same at 5520 kPa, except for data at 60°C in which yields were maximal from 400 to 850 g. At both pressures, high yields were expected for smaller milk mass, but more scatter in the data was apparent because the reactor was large for this sample size, making contact of milk and CO_2 difficult.

The trial conducted at 25°C and 2760 kPa, which was well below the coagulation temperature of 38°C generally used in casein manufacture, resulted in a highly wet, aerated solid that dissolved soon after removal from the reactor. The trial at 25°C and 5520 kPa resulted in a wet solid with a few suspended curds.

Increases in reactor pressure increased casein yield depending on temperature (Figure 5). Differences in the data at 32°C and pressures <4140 kPa are not significant but are for the other temperatures. Consistent data were difficult to obtain at 60°C and 2760 kPa because the casein dried onto the walls of the reactor and was difficult to remove from the reactor. For a milk mass of 750 g and over the entire temperature range of this study, yields were 100% only at 5520 kPa; at the other pressures, yields were the highest at the highest temperatures.

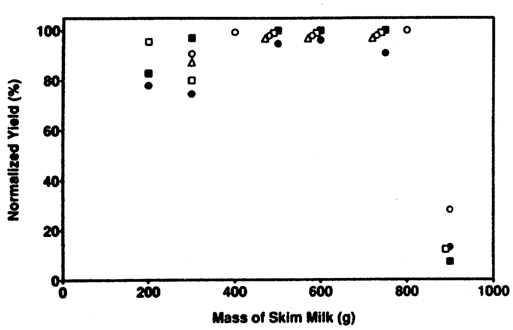

Figure 4. Normalized yield from a skim milk mass of 500 g as a function of temperature for precipitation pressure of 4140 kPa at 32 (●), 38 (■), 43 (□), 49 (O), and 60 (Δ)°C.

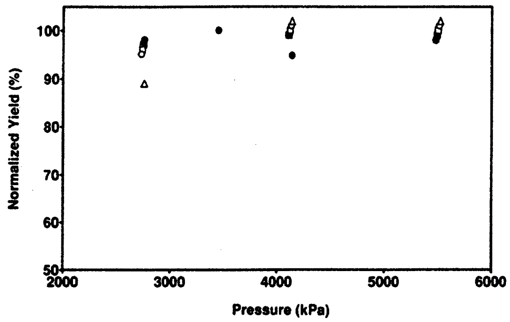

Figure 5. Normalized yield from a skim milk mass of 500 g as a function of precipitation pressure at 32 (●), 38 (■), 43 (□), 49 (O), and 60 (Δ)°C.

Total Solids, Ash, and Ca Contents

At constant system pressure and mass of skim milk, total solids, ash, and Ca contents of casein generally increased as temperature increased (Table 2). At temperatures >43°C, total solids content of casein was slightly sensitive to increasing pressure. Values for total solids observed in this study were greater than those previously reported (6) at 50°C: 22.4% total solids at 3000 kPa and 27.9% total solids at 4000 kPa. Ash content decreased slightly as pressure increased at 38, 43, and 49°C. Values reported (11) for ash of acid and rennet caseins were 1.8 and 7.8%, respectively.

The Ca contents show no consistent trend as temperature increased at a fixed pressure or pressure increased at a fixed temperature. However, variation among batches was apparent as the Ca content of replicates varied. At 50°C, in the pressure range from 2000 to 4000 kPa, Ca ranged from 1.1 to 1.4% (6).

Variation in solids, ash, or Ca contents as a function of temperature or pressure was not significant for the whey samples. For a 500-g milk sample at 38°C and 5520 kPa, the mean analyses of the whey were 6.7% solids, .6% ash, and .1% Ca.

Curd Appearance and Texture

Curd appearance was watery and slushy only when precipitation was <90% complete. Samples precipitated at 32 to 49°C with yields >90% (Figures 4 and 5) had discernible particles or granules. At temperatures ≥38°C, the

TABLE 2. Effects of pressure and temperature on the solids, ash, and Ca contents of casein.

	Solids			Ash[1]			Ca[2]		
Temperature	2760 kPa	4140 kPa	5520 kPa	2760 kPa	4140 kPa	5520 kPa	2760 kPa	4140 kPa	5520 kPa
(°C)					(%)				
32	20.0^{f}	15.3^{g}	19.4^{f}	1.24^{h}	.98^{j}	1.12^{i}	1.62^{a}	1.03^{b}	1.60^{a}
38	21.3^{f}	22.7^{f}	23.6^{f}	1.33^{g}	1.27^{h}	1.24^{h}	1.28^{b}	.84^{c}	.84^{c}
43	27.7^{e}	29.3^{e}	28.6^{e}	1.77^{d}	1.66^{e}	1.44^{f}	1.13^{b}	1.42^{a}	1.46^{a}
49	47.0^{bc}	35.9^{c}	32.8^{d}	2.48^{b}	2.05^{c}	1.78^{d}	1.77^{a}	1.25^{b}	1.85^{a}
60	41.3^{a}	43.3^{a}	38.3^{bc}	2.88^{a}	2.53^{b}	2.58^{b}	. . .	1.88^{a}	2.20^{a}

[a,b,c,d,e,f,g,h,i,j]Within each category, means with no superscript in common are significantly different ($P < .05$).

[1]Wet basis.

[2]Dry basis.

casein could be described as friable. Casein precipitated at 60°C was always dry and stringy and had no discernible particles. At constant pressure, samples produced at 32°C had a creamy appearance, but samples produced at the higher temperatures became progressively drier in appearance as temperature increased. At constant temperature and with increases in pressure from 2760 to 5520 kPa, visual inspection showed a slight decrease in curd particle size.

pH Measurements

The following equation represents the equilibrium that governs the dissolution of CO_2 in H_2O:

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^- . \quad [1]$$

Dissolved CO_2 hydrolyzes water to form carbonic acid as shown in Equation [1]. Solubility of CO_2 increases with pressure at a given system temperature. The amount of CO_2 required for complete precipitation of casein from a given quantity of milk may be approximated from solubility data for the CO_2-H_2O system and thermodynamic property data to calculate the amount of CO_2 required to fill the headspace of the batch reactor. At 38°C, the solubility of CO_2 (grams of CO_2/100 g of H_2O) is approximately .1 at 0 kPa, approximately 2.2 at 2760 kPa, and 4.2 at 5500 kPa. At 60°C, the solubility of CO_2 is approximately 1.6 at 2760 kPa and 3.1 at 5500 kPa (3). The amount of CO_2 required to fill the headspace is easily calculated using the generalized compressibility factor (9). The batch reactor system used in this study could not be used for precise determination of CO_2 solubility in milk. The solubilities of CO_2 in H_2O and skim milk were similar (6).

When the vessel was pressurized, the solubility of CO_2 increased, resulting in increased H^+ concentration (Figure 6). Further increases in pressure to 6900 kPa dropped pH to 5.4. A buffering effect occurred over the entire pressure range, a result of interactions of Ca, PO_4^-, and other buffers in milk (4) and H^+ and HCO_3^-. As expected, depressurization from 7000 to 1000 kPa was accompanied by a constant pH of 5.4. Depressurization from 1000 to 0 kPa resulted in a final pH of 6.0. The mean pH of the casein was 5.8 upon removal from the vessel directly after depressurization,

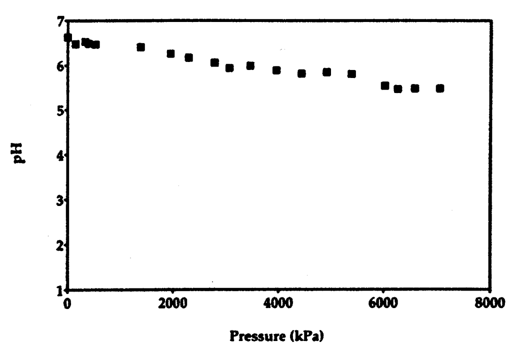

Figure 6. Effect of CO_2 pressure on pH for skim milk mass of 750 g at 38°C.

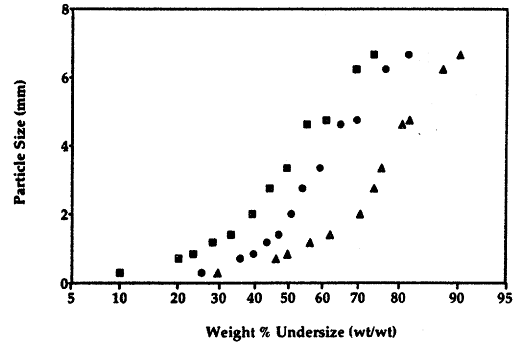

Figure 7. Effect of pressure on casein particle size distribution at 38°C and 2760 kPa (■), at 38°C and 5520 kPa (▲), and at 43°C and 5520 kPa (●).

which is about .4 pH units higher than the pH at 7000 kPa. The final pH corresponds to that of casein with high Ca content. However, the curd was not as fibrous or sticky as that produced by HCl precipitation at pH 5.4. In addition, the whey of casein precipitated by CO_2 had the slightly yellow, opalescent appearance of acid whey even though pH was 5.8 and not 4.6. At pH of 5.8, the whey associated with the casein precipitated by HCl still had a milky appearance. The impact of pressure on the curd was not investigated in this study. No denaturation of proteins was expected because proteins are not denatured by pressures <600 MPa (12).

Particle Size Distribution Studies

The mean curd particle size (d_{50}) of curd made at 38°C decreased from 3.35 mm to 841 μm when the pressure was increased from 2760 to 5520 kPa, respectively. These data were consistent with plots of particle size versus weight percentage (g/100 g) undersize (Figure 7). At a constant pressure of 5520 kPa, particles precipitated at 43°C were slightly larger (d_{50} = 2.00 mm) than those precipitated at 38°C. The d_{50} in this study at 43°C and 5520 kPa (2.0 mm) is consistent with the values at low pH and at 45°C (5). The shift in the curves away from linearity was most likely due to the use of the sparger with a 2-μm pore size to introduce CO_2 to the milk. The lack of an acidulation period, the time required for casein to agglomerate after removal from the reactor, may also have been a factor.

Protein Fractionation

The relative weight percentages (g/100 g) of the major milk proteins in the casein and whey samples were determined. The casein samples were relatively free of whey proteins, and the whey samples were relatively free of casein proteins. A single peak was noted for α_{s1}-casein and BSA of the casein samples only, indicating that these components were coeluted. However, because the fractions of the other whey proteins in the casein samples were very small or zero, this fraction probably was predominantly α_{s1}-casein.

Lactose was determined for the initial skim milk and whey samples only. Material balance calculations indicate that the casein product was free of lactose.

Predicted Operating Conditions for Batch or Continuous Precipitation

High casein yields were obtained in a batch reactor only when CO_2 was sparged directly through the milk. The placement of the sparger was critical for optimal contact between milk and CO_2. Although the volume of milk used in this study was over two times the volume of milk used by Jordan et al. (6), larger volumes were impractical. Sparging a large volume of milk was difficult because of the almost instantaneous formation of precipitate, which promoted poor contact between unreacted milk and CO_2. Stirring improved contact between milk and CO_2 but broke up the precipitate. Continuous operation would ensure that precipitate formation did not interfere with mixing of the reactants.

The batch data showed that batch or continuous operation should take place at temperatures >38°C and pressures >4140 kPa to obtain the highest yields. The amount of CO_2 needed to effect precipitation was determined from the solubility data (3) and the mass of milk. However, the curd appearance data showed that casein precipitated at 60°C and possibly 49°C would be difficult to process because of its dryness. In initial design considerations, the size of continuous flow tubular reactor and precipitator may be estimated by assuming that the residence time required for batch operation, 5 min in this study, is equal to the residence time in the continuous flow reactor (9).

CONCLUSIONS

At constant milk mass and temperature, increasing pressure resulted in higher casein yields and a curd that became progressively firmer in appearance. Results were best at 38 and 43°C. At 32°C, the curd appeared watery, and, at 60°C, the curd was stringy or rubbery and dry and had no discernible particles. Solids, ash, and Ca contents of casein were higher than those for caseins precipitated using mineral acids. Solids and ash contents increased as temperature increased. Solids, ash, and Ca contents of the whey were constant with pressure but decreased as temperature increased.

At constant milk mass and temperature, mean particle size decreased as pressure increased, which is the same as decreased particle size as pH decreased. Mean particle size increased with temperature.

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