Impact of Milk Preacidification with CO₂ on Cheddar Cheese Composition and Yield*

B. K. Nelson, J. M. Lynch, and D. M. Barbano

Northeast Dairy Foods Research Center

Department of Food Science, Cornell University, Ithaca, NY 14853

ABSTRACT

Preacidification of milk for cheese making may have a beneficial impact on increasing proteolysis during cheese aging. Unlike other acids, CO₂ can easily be removed from whey. The objectives of this work were to determine the effect of milk preacidification on Cheddar cheese composition, the recovery of individual milk components, and yield. Carbon dioxide was injected inline after the cooling section of the pasteurizer. Cheeses with and without added CO₂ were made simultaneously from the same batch of milk. This procedure was replicated 3 times. Carbon dioxide in the cheese milk was about 1600 ppm, which resulted in a milk pH of about 5.9 at 31°C. The starter culture and coagulant addition rates were the same for both the CO₂ treatment and the control. The whey pH at draining of the CO₂ treatment was lower than the control. Total make time was shorter for the CO₂ treatment compared with the control. Cheese manufactured from milk acidified with CO₂ retained less of the total calcium and fat than the control cheese. The higher fat loss was primarily in the whey at draining. Preacidification with CO₂ did not alter the crude protein recovery in the cheese. The CO₂ treatment resulted in a higher added salt recovery in the cheese and produced a cheese that contained too much salt. Considering the higher added salt retention, the salt application rate could be lowered to achieve a typical cheese salt content. Cheese yield efficiency of the CO₂ treated milk was 4.4% lower than the control due to fat loss. Future work will focus on modifying the make procedure to achieve a normal fat loss into the whey when CO₂ is added to milk. (**Key words:** carbon dioxide, Cheddar, cheese, yield)

INTRODUCTION

Mozzarella cheeses made from milk preacidified with acetic and citric acids had higher pH 4.6 and 12% TCA

soluble nitrogen levels, indicating a greater extent and depth of proteolysis than the control cheese (Metzger et al., 2001). Proteolysis is important to Cheddar cheese flavor development (Aston and Creamer, 1986). Any treatment that would enhance the rate of normal proteolysis and increase the rate of flavor development may be attractive to cheese manufacturers. An acidulant added to milk that remains in the cheese and whey may be a detriment if not allowed by standards of identity or if not desired in the finished product. Carbon dioxide appears to be suited for milk preacidification because it behaves as an acidulant when dissolved in milk, could be removed from the whey by applying a vacuum, and may not remain in the cheese.

Carbon dioxide has been used to increase refrigerated shelf life of cottage cheese (Hotchkiss and Lee, 1996) and raw milk (Rashed et al., 1986; King and Mabbitt, 1987; Ma et al., 2003). Carbon dioxide addition resulting in a milk pH above 6.0 has also been used to inhibit the growth of spoilage organisms in raw milk prior to cheese manufacture (Calvo et al., 1993; McCarney et al., 1995). Other investigators (Montilla et al., 1995; St-Gelais et al., 1997) have reported that milks preacidified with CO₂ (to a milk pH of 6.0 and 6.56, respectively) coagulate in a manner similar to the controls, but with 75 and 30% less coagulant, respectively. Van Slyke et al. (1903) reported that treating milk with CO₂ after heating the milk to 98°C restores the coagulation properties. Increased milk shelf life, bacterial inhibition, and lower coagulant usage rates are positive effects of CO₂ preacidification. A business decision regarding CO₂ addition to milk prior to Cheddar cheese making requires knowledge of the value of benefits, summarized previously, and costs related to CO₂ usage. Anything that decreases the yield of cheese made from milk acidified with CO₂ should be considered as a cost relating to CO₂ usage. Therefore, studies focused on the impact of milk preacidification with CO₂ on Cheddar cheese yield will enable decision makers to better quantify costs and balance the disadvantages vs. the advantages of CO₂ usage.

McCarney et al. (1995) and St-Gelais et al. (1997) have reported Cheddar cheese yield data on cheese made with CO₂ added to milk. McCarney et al. (1995) did not report total fat and protein accountability, but did report that

Received May 30, 2004.

Accepted August 9, 2004.

Corresponding author: D. M. Barbano; e-mail: dmb37@cornell.edu.

*Use of names, names of ingredients, and identification of specific models of equipment is for scientific clarity and does not constitute any endorsement of product by authors, Cornell University, or the Northeast Dairy Foods Research Center.

only 85.9% of the fat was recovered in the control cheese and 88.5% in the CO₂ treatment cheese. Compared with the yield theoretical value of 93% for Cheddar cheese, both the treatment and control fat recovery of McCarney et al. (1995) were very low. There must have been systematic fat loss caused by the make procedure that resulted in the lower fat recovery in the control cheese. Likewise, the casein recovery was low in the control and CO₂ cheeses (87.5 and 89.9%) compared with the Van Slyke theoretical yield value of about 96%. Without more information, comparisons and interpretation of the data of McCarney (1995) would not provide adequate decision-making information. The study by St-Gelais et al. (1997) did not report total fat and protein accountability, only fat and protein recovery in the cheese. The fat recovery in the control cheese was 91.93%, which was slightly lower than the theoretical yield value (93%) and much lower than the reported value for their CO₂ treatment (98.54%). Neither of these studies reported CO₂ levels in the whey or calcium recoveries. The pH values of the preacidified milks in the study of Metzger et al. (2001) were 5.8 and 6.0. Metzger et al. (2000) reported a lower calcium and fat recovery in the Mozzarella cheeses manufactured from preacidified milk. A need exists for complete data on component accountability and recovery as well as CO₂ levels in the whey and Cheddar cheese made from milk with added CO₂. Therefore, the objective of our study was to determine the effect of added CO₂ (resulting in a milk pH of 5.9) on calcium, fat, crude protein, and total milk solids and on added salt recoveries, Cheddar cheese composition, and yield. The impact of preacidification of milk with CO₂ on proteolysis and cheese CO₂ content during aging will be presented in another paper.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

One 18-kg block of full-fat Cheddar cheese was made from milk with added CO₂ and one control without added CO₂. Cheese was made on 3 different days (using a different batch of milk each day) over a 1-wk period. A one-way ANOVA was used to determine if there was an impact of CO₂ on the composition and yield of the cheese. The least significant difference ($P \leq 0.05$) test was used to compare treatment means if the *F*-test for the statistical model was significant ($P \leq 0.05$). The PROC GLM procedure of SAS was used for all data analysis (SAS version 8.02, 1999 to 2001, SAS Institute Inc., Cary, NC).

Milk Processing and Cheese Manufacture

Milk processing and cheese manufacture were completed on the same day. Each day raw whole bovine milk was received from the Cornell University dairy farm.

The raw whole milk was pasteurized with a plate heat exchanger at 72°C and a holding time of 15 s. The pasteurized milk was then cooled to 4°C utilizing the regeneration and cooling sections of the system. Carbonated milk was collected after about 250 kg of pasteurized milk was collected for the control treatment. A stainless steel sparger (7 μ m) was inserted inline after the cooling section of the pasteurization system. An additional 8 m of 2.54-cm diameter stainless steel pipe, with a sanitary conical-seat flow controlling valve at the end, were added after the point of CO₂ injection to allow 15 s of holding time for CO₂ incorporation into the cold pasteurized milk. A pressure gauge was located inline at the point of injection and another just before the flow control valve. Carbonation conditions (CO₂ flow at 1.13 m³/h with 172 kPa back pressure at the flow control valve) were equilibrated with water (16.6 L/min) before milk was started through the pasteurization system. Milk was carbonated to approximately 1650 ppm to achieve a CO₂ level of about 1600 ppm in the cheese vat, which produced a milk pH of 5.9 at 31°C. The pH of 5.9 is between the 2 pH levels reported by Metzger et al. (2001) where increased soluble nitrogen levels were observed.

All components for cheese making including milk, whey, salt whey, cheese, and samples were weighed to the nearest gram (model PE24, Mettler Instrument Co., Highstone, NJ). After pasteurization, approximately 240 kg of milk was weighed and placed into the control vat. The second vat was filled with about 240 kg of pasteurized and carbonated milk, and cheese making was conducted simultaneously. The milk in each vat was heated to 31°C while agitating (model 4MX; Kusel Equipment Co., Watertown, WI). The milk was ripened for 45 min at 31°C after the starter culture (911 DVS pellets, Chr. Hansen Inc., Milwaukee, WI) was added, at 0.27 g/kg of milk. When ripening was complete, annatto color (AFC WOS 550, Rhodia Inc., Madison WI) was added (0.0033 mL/kg of milk) to each vat. The ripened milk, 31°C, was coagulated with double strength chymosin (0.1 mL/kg of milk; Chymax Extra, Chr. Hansen Inc., Milwaukee, WI). The chymosin was diluted in 200 mL of water processed by reverse osmosis, immediately before addition to the milk. After 30 min, the coagulum was cut (1.2-cm wire knives), and the curds and whey were not stirred for 5 min. After 5 min, the curds and whey were stirred gently without added heat for 10 min. The temperature was increased from 31 to 33°C over 15 min and then from 33 to 38°C over an additional 15 min. The curds and whey were continuously stirred and a temperature of 38°C maintained until the target whey draining pH of 6.35 was attained. When the whey was drained, the curds were piled and allowed to knit together for 15 min. The large slab of curd was cut into 2 smaller slabs, then turned. The 2 curd slabs were stacked after 15 min. Curd

slabs were maintained at 38°C, piled 2 high, and turned over every 15 min throughout the Cheddaring process. Curd slabs were milled when the curd pH reached 5.30. Salt was added at 2.7% of the curd weight. The salt was divided equally into 3 portions. The milled curds were dusted with a small amount of the first portion of salt, then stirred for 2 min and allowed to sit for 10 min. The remainder of the first portion of salt was then added, after which the curds were stirred and allowed to sit for 10 min. The curds were salted with the 2 other portions of salt at 10-min intervals. The salted milled curds were placed in a 18-kg capacity stainless steel Wilson hoop and pressed in an A-frame press (model AFVS, Kusel Equipment Co., Watertown, WI) for 30 min at 70 kPa and then overnight, about 17 h, at 420 kPa. The cheese blocks were vacuum packaged and placed in a 4°C cooler for 24 h before being placed in a cooler set at 6°C for aging.

Sampling and Sample Preparation

Sampling. Raw whole milk at 4°C was mixed and sampled immediately before pasteurization. Pasteurized control and CO₂ treated milks were collected after heating in the cheese vat to 31°C prior to starter addition. The whey collected from the start of curd draining to the end of draining was placed in a separate vat for each treatment and sampled for CO₂ analysis. Additional whey collected throughout Cheddaring was added to the vats containing the whey. When all the whey from each vat was collected, the whey was heated to 38°C and mixed to assure uniform composition before a sample was taken for compositional analysis and used in mass-balance calculations. Salt whey was collected and weighed separately after milling at the vat and mixed with the press whey, which was weighed. Press whey was collected during pressing by placing the hooped curds in large 8-mil plastic bags (model number S-5851, Uline, Waukegan, IL). Hot water was run on the outside of the bags to liquefy fat that may have solidified on the inside surface of the bag during pressing, and all of the whey was removed from the bags. A 1-cm thick × 28 cm × 19 cm cross-sectional slice from the center of the rectangular 18-kg block of cheese was removed immediately after the block was removed from the press. The slice of cheese for compositional analysis was vacuum packaged and cooled to 4°C prior to analysis.

Sample preparation. Liquid samples were placed in 59-mL snap lid vials and analyzed fresh or stored frozen at -40°C. Frozen liquid samples were thawed in a microwave oven in a manner that kept the sample temperature below 10°C. Cheese slices were cut into 2-cm pieces and then ground (model 31BL92, Waring, New Hartford, CT) into 2- to 3-mm pieces and packed into 59-mL snap

lid vials (Capital Vial, Inc., Fultonville, NY) with no head space and either analyzed fresh or held frozen at -40°C before analysis. Frozen cheese samples were thawed overnight at 4°C prior to analysis.

Standard Plate, Coliform, and Somatic Cell Counts

Standard plate and total coliform counts of pasteurized whole milks were determined by standard methods (Marshall, 1992; 6.2 and 7.8). Somatic cell counts of raw whole milk (AOAC 2000; 17.13.01, 978.26) were determined using a fluorimetric method (MilkoScan Combi 4000, Integrated Milk Testing; A/S N, Foss Electric, Hillerød, Denmark) by a New York State licensed commercial laboratory (Dairy One, Ithaca, NY).

Chemical Analyses

Milk, whey, and salt whey composition. Fat, TS, total nitrogen, NPN, and noncasein nitrogen content of the milk, whey, and salt whey were determined using ether extraction (AOAC, 2000; 33.2.26, 989.05), forced air oven drying (AOAC, 2000; 33.2.44, 990.20), Kjeldahl (AOAC, 2000; 33.2.11, 991.20), Kjeldahl (AOAC, 2000; 33.2.12, 991.21), and Kjeldahl (AOAC, 2000; 33.2.64, 998.05), respectively. Crude protein was calculated by multiplying total nitrogen by 6.38. The calcium content was determined using atomic absorption (Metzger et al., 2000). The CO₂ content of the milk and whey was determined (Ma et al., 2001) using a CO₂ analyzer (MOCON Pac Check 650, MOCON, Minneapolis, MN). The Volhard method (Marshall, 1992; 15.5.B) was used to determine the salt content in the salt whey, using a 0.5-g test portion. Milk, whey, and salt whey compositions were determined in triplicate, with the exception of calcium and CO₂, which were determined in duplicate.

Cheese composition and pH. Fat content was determined using the Babcock method (Marshall, 1992; 15.8.A). Cheese moisture was determined gravimetrically by drying 2 g of cheese in a forced-air oven at 100°C for 24 h (AOAC, 2000; 33.2.44, 990.20). Salt content was determined using the Volhard method (Marshall, 1992; 15.5.B). The Kjeldahl method (1 g of cheese) was used to determine total nitrogen (Lynch et al., 2002). The cheese calcium content was determined by atomic absorption (Metzger et al., 2000).

Cheese pH was measured using a Xerolyt combination electrode (model HA405; Mettler Toledo, Columbus, OH) and an Accumet pH meter (model AR 25, Fisher Scientific, Pittsburgh, PA) after tempering to 23°C. All analyses were carried out in duplicate except total nitrogen, moisture, and fat, which were performed in quadruplicate.

Component Recoveries

Fat, CP, calcium, total milk solids, and added salt recoveries were determined by multiplying the weights (determined to the nearest gram) of milk, whey, salt whey, and cheese by the compositions determined by chemical analysis, then dividing by the total weight of either fat, CP, calcium, total milk solids, or added salt, and multiplying by 100. Total milk solids recovery calculations did not include salt in the salt whey or in the cheese. If the mean actual total unadjusted recoveries between treatments for the component were not significantly different ($P > 0.05$), then the recoveries were adjusted by dividing the actual recoveries by the mean total recovery for each day of cheese making and multiplying by 100.

Yield and Yield Efficiency

Actual cheese yields were calculated by ((cheese weight + curd sample weight)/(milk weight – milk sample weight) $\times$ 100. Moisture and salt adjusted yield was calculated accordingly (actual yield $\times$ (100 – (cheese moisture content + cheese salt content)))/(100 – (37 + 1.5)). The moisture and salt adjustment allows for comparison between treatments. Cheese yield efficiency was calculated by dividing the adjusted yield by the theoretical yield and multiplying by 100. Both Van Slyke and Barbano theoretical cheese yield formulas (Neocleous et al., 2002) were used for a cheese yield efficiency calculation.

The Van Slyke cheese yield formula for Cheddar cheese was calculated according to the following formula: yield = (((0.93 $\times$ percentage fat in milk) + (percentage CN in the milk – 0.1) $\times$ 1.09)/(1 – (target cheese moisture/100))). The Barbano formula for Cheddar cheese differs from the Van Slyke formula in that the nonfat solids of the whey were used to determine the nonfat whey solids retained in the water phase of the cheese (Barbano, 1996). The Barbano formula is useful when manufacturing a preacidified cheese because it can compensate for the loss of calcium into the whey. Theoretical Cheddar cheese yield using the Barbano formula was calculated using the following formula: yield = (A + B + C)/(1 – ((target cheese moisture + target cheese salt)/100)), where A = (0.93 $\times$ percentage fat in milk), B = (percentage CN in milk – 0.1) $\times$ (calcium phosphate retention factor), C = (((A + B)/(1 – (actual cheese moisture percentage/100))) – (A + B)) $\times$ (percentage nonfat whey solids/100)) $\times$ (solute exclusion factor). The calcium phosphate retention factors used in this study for the control and CO₂ treatments were 1.092 and 1.082, respectively. The same calcium phosphate retention factor for the control theoretical yield calculation was used by Neocleous et al. (2002). The lower calcium phosphate retention factor

Table 1. Pasteurized control milk composition and pH for each day of cheese making.

Component	Day of cheese making		
	1	2	3
Fat, %	3.44	3.42	3.35
CP, %	2.90	2.92	2.93
NCN, ¹ %	0.66	0.67	0.66
NPN, ² %	0.20	0.18	0.19
TS, %	11.88	11.75	11.74
CN/TP, ³ %	82.96	82.26	82.99
CN/fat, %	0.65	0.66	0.68
Calcium, %	0.105	0.106	0.106
pH ⁴	6.63	6.68	6.64
SCC, per mL	268,000	260,000	226,000

¹NCN = Noncasein nitrogen $\times$ 6.38.

²NPN = NPN $\times$ 6.38.

³CN/TP = [(CP – NCN)/(CP – NPN) $\times$ 100], where TP = true protein (CP – NPN), CP = total N $\times$ 6.38.

⁴Measured at 31°C.

used for CO₂ treatment theoretical yield calculation was obtained by plotting calcium retention factor data (acetic acid treatments) of Metzger et al. (2000) and using the second order polynomial equation (solute exclusion factor = $-0.0333(x^2) + 0.4333(x) - 0.316$) to compute a calcium retention factor to use for the milks (mean milk pH of 5.93) with added CO₂, where x = milk pH. The solute exclusion factor of 0.6941 used by Neocleous et al. (2002) was also used in this study for both the control and added CO₂ theoretical yield formulas.

RESULTS AND DISCUSSION

Milk Composition and Quality

Milk composition data are presented in Table 1. The mean CN as a percentage of true protein was about 83%, which in turn gave a mean CN-to-fat ratio of 0.66. The pH of the milk at 31°C before CO₂ addition (Table 1) was normal for good quality milk. The standard plate counts of the control and preacidified milks of 440 and 1150 cfu/mL were low and not significantly different. The coliform count was <10 cfu/mL for both treatments. The mean SCC of the milks used each day for cheese making are shown in Table 1. The CO₂ levels of the control and CO₂ treated milks were significantly different after CO₂ addition (Table 2). The target milk pH of

Table 2. Mean (n = 3) carbon dioxide content (ppm) of the control and treatment milk and whey.

	Control	Treatment	LSD	SEM
	ppm			
Milk	88 ^b	1615 ^a	77	19.7
Whey at draining	85 ^b	1000 ^a	115	29.4

^{a,b}Means within a row that do not share a common superscript differ ($P \leq 0.05$).

Table 3. Mean (n = 3) milk, whey, and curd pH during cheese making.

	Control	CO ₂	LSD	SEM
Milk	6.65 ^a	5.93 ^b	0.054	0.014
Coagulant addition ¹	6.57 ^a	5.93 ^b	0.052	0.013
Drain ²	6.35 ^a	5.96 ^b	0.074	0.019
Mill ³	5.30	5.30	NS	0.000

^{a,b}Means within a row that do not share a common superscript differ ($P \leq 0.05$).

¹Ripened (45 min) milk sample.

²Whey sample.

³Curd sample.

about 5.9 was achieved (Table 3) by the addition of about 1600 ppm of CO₂ (Table 2).

Effect of CO₂ on Cheese Making

The mean (n = 3) temporal patterns of pH for the control and CO₂ treatment during cheese making are shown in Figure 1. The milk pH before starter culture addition, at coagulant addition, and of the whey at draining was higher ($P \leq 0.05$) for the control than for the CO₂ treatment (Table 3). The slope of the control pH curve from 0 to 120 min (Figure 1) was negative, whereas the CO₂ treatment pH was constant over the same period. The downward slope of the control pH curve was expected because the starter culture was producing lactic acid during the 45 min of ripening. Although the starter culture growing in the milk of the CO₂ treatment was producing lactic acid, the pH of the whey did not change much from the initial pH of the milk until after about 130 min into cheese making because the milk was also

Figure 1. Mean (n = 3) temporal pattern of pH of the control (squares) and CO₂ (circles) treatment during cheese making. Samples at time 0 and 45 min were milk, samples from 100 to 140 min were whey, and samples after 140 min were curd.

Table 4. Mean (n = 3) Cheddar cheese make times for control and treatment cheeses.

	Control	CO ₂	LSD	SEM
	min			
Coagulant addition to drain	88 ^a	78 ^b	2.6	0.67
Drain to mill	88	83	NS	1.72
Total	176 ^a	161 ^b	8.6	2.20
Stir out	10 ^a	0 ^b	0.0	0.00

^{a,b}Means within a row that do not share a common superscript differ ($P \leq 0.05$).

losing CO₂. The time from coagulant addition to whey draining (Table 4) was shorter for the CO₂ treatment ($P \leq 0.05$). Moreover, if CO₂ produced the milk pH decrease usually caused by lactic acid during ripening, then the lactic acid content of the sweet whey collected at draining would be reduced. The CO₂ remaining in the whey could be removed with a vacuum chamber. This might improve the quality of whey products in certain applications. The separate impacts of lactic acid and CO₂ on the pH observed for the CO₂ treatment (Figure 1) cannot be determined from the data collected.

The total make time was shorter with the milk preacidified with CO₂ (Table 4) mostly due to the decrease in cooking time before whey draining. The target whey draining pH of 6.35 at the end of cooking was surpassed by the CO₂ treatment when the curd reached the final cooking temperature (38°C), while the control required an additional 10 min of stir-out time at 38°C before the target pH was achieved (Table 4). The curds from the CO₂ treatment and control were both milled at pH 5.3. There was no significant difference in time from drain to mill between the control and CO₂ treatment (Table 4). A shorter ($P < 0.05$) total make time of the CO₂ treatment was also reported by St-Gelais et al. (1997) using CO₂ to preacidify milk to pH 6.56 prior to cheese making; however, they reported a similar time from cut to whey draining and a 30 min shorter Cheddaring time.

The effect of CO₂ was visually apparent during cheese making in our study. Preacidification with CO₂ produced a firmer coagulum that could be detected by touch and by the resistance of the coagulum when cut with wire knives. Other investigators have observed more rapid coagulation (Montilla et al., 1995; St-Gelais et al., 1997), and Van Slyke et al. (1903) observed that the rennetability of milk that had been heat treated above 85°C was restored with the addition of CO₂ to milk prior to cheese making. After cutting, the curds of the CO₂ treatment floated to the surface with increasing temperature during cooking, whereas the curds of the control settled to the bottom if stirring was not constant. Floating curds may require a change in procedure if a portion of the whey is normally drained from the cheese vat through

Table 5. Mean (n = 3) whey, salt whey, and cheese composition.

Component	Control	CO ₂	LSD	SEM
	%			
Whey				
Fat	0.27 ^b	0.37 ^a	0.058	0.015
CP ¹	0.85	0.86	NS	0.020
NPN ²	0.25	0.25	NS	0.005
TP ³	0.60	0.60	NS	0.019
TS	6.71 ^b	6.87 ^a	0.098	0.025
Calcium	0.05 ^b	0.07 ^a	0.002	0.0004
Salt whey				
Fat	1.28 ^b	4.07 ^a	1.174	0.299
CP	1.08 ^b	1.10 ^a	0.023	0.006
NPN	0.40	0.40	NS	0.008
TP	0.68 ^b	0.71 ^a	0.024	0.006
TS	16.15	15.73	NS	0.210
Salt	8.73 ^a	6.73 ^b	0.856	0.218
Calcium	0.21 ^a	0.16 ^b	0.023	0.006
Cheese				
Fat	34.19 ^a	32.83 ^b	1.024	0.261
FDB ⁴	54.12 ^a	52.67 ^b	0.859	0.219
CP	23.72	24.02	NS	0.213
PDB ⁵	37.49	38.09	NS	0.423
Moisture	36.84	37.67	NS	0.285
MNFS ⁶	55.97	56.08	NS	0.246
Salt	1.44 ^b	2.24 ^a	0.119	0.030
Salt-in-the-moisture	3.92 ^b	5.96 ^a	0.385	0.098
Calcium	0.69 ^a	0.52 ^b	0.035	0.009
Calcium/CP (× 100)	2.91 ^a	2.15 ^b	0.185	0.047

^{a,b}Means within a row that do not share a common superscript differ ($P \leq 0.05$).

¹CP = Crude protein (CP = total nitrogen × 6.38).

²NPN = Nonprotein nitrogen × 6.38.

³TP = True protein (TP = CP – NPN).

⁴FDB = Fat on a dry basis.

⁵PDB = Protein on a dry basis.

⁶MNFS = Moisture in the nonfat substance.

an outlet located about half-way between the surface of the whey and the bottom of the vat (i.e., predraining). In the case of floating curds, predraining could be accomplished by draining a portion of the whey through the outlet located at the bottom of the cheese vat. If horizontally stirred cheese vats were used to manufacture cheese from milk containing CO₂, the thickness of the floating curd mass may be an issue that needs to be investigated with regard to curd integrity and fines. The fact that curds can be made to float in this process provides an opportunity to think about a different design of cheese vat and curd handling system that could reduce curd shattering.

Whey, Salt Whey, and Cheese Composition

Whey and salt whey composition. A major portion of the CO₂ added to the milk was removed with the whey at draining (Table 2). Means and composition differences of whey and salt whey due to CO₂ treatment are reported in Table 5. Carbon dioxide treatment resulted in a higher

($P \leq 0.05$) fat content in the whey and salt whey. The calcium content was higher ($P \leq 0.05$) in the whey from the CO₂ treatment, and calcium content in the salt whey was lower ($P \leq 0.05$) than in the control. There was no significant difference in whey CP content, but there was a slight increase in the CP content of the salt whey. There was substantially less salt in the salt whey of the CO₂ treatment than in the control (Table 5). St-Gelais et al. (1997) reported higher ($P < 0.05$) fat content in the control whey than in the CO₂ treatment (0.54 vs. 0.35%, respectively). The level of fat in whey reported by St-Gelais et al. (1997) would normally be associated with much lower fat recovery in cheese (Barbano and Sherbon, 1984) and does not seem consistent with the high fat recovery in the cheese (91.93 and 98.54%, respectively) reported in the same paper. The same authors did not detect a significant difference in the ash content of the whey, and salt whey composition was not reported.

Ultrafiltration is often used to fractionate whey before spray drying. Whey proteins, α -lactalbumin in particular, have been implicated in membrane fouling (Tong et al., 1989). In addition to protein, soluble calcium has been found to reduce flux (Ramachandra Rao et al., 1994). With respect to fouling due to protein, there was no significant difference in CP content of the whey (Table 5) between the control and the CO₂ treatment. The calcium content of whey from the CO₂ treatment (Table 5) was higher ($P \leq 0.05$). Experiments are needed to determine if the whey from CO₂ preacidification differs in ultrafiltration flux from that of typical Cheddar cheese whey. From the current literature it was unclear whether the higher calcium content of the whey from the CO₂ treatment would impact the process of dehydration during spray drying, the rehydration of whey powder, or the functionality of the whey protein concentrate compared with the control.

Cheese composition and pH. No difference ($P \leq 0.05$) in cheese CP, protein on a dry basis, moisture, or moisture in the nonfat substance was detected between the control and CO₂ treatment. The fat content of the control cheese was higher ($P \leq 0.05$) than that of the CO₂ treatment (Table 5). The CO₂ treatment cheese contained less calcium (Table 5) due to the reduced milk pH prior to rennet addition (Table 3). The calcium content of the control was similar to the value of 0.721%, standard error 10.770, listed in the USDA National Nutrient Database (USDA, 2003), but the CO₂ treatment calcium content was lower. Additional experiments need to be conducted to determine if the lower calcium content of the CO₂ treatment cheese could reduce calcium lactate crystal formation during aging. The control cheese pH, 5.00, was lower ($P \leq 0.05$) than the CO₂ treatment cheese pH, 5.09. The largest difference ($P \leq 0.05$) between the control and treatment cheeses was salt content. The control

Table 6. Adjusted mean (n = 3) calcium, fat, CP, milk solids, and added salt recovery in whey, salt whey, and cheese.

	Control	CO ₂	LSD	SEM
	%			
Calcium recovery				
Whey	37.27 ^b	54.28 ^a	1.502	0.382
Salt whey	3.55 ^a	2.44 ^b	0.362	0.092
Cheese	59.07 ^a	43.38 ^b	1.669	0.425
Fat recovery				
Whey	6.98 ^b	9.79 ^a	1.708	0.435
Salt whey	0.68 ^b	1.89 ^a	0.391	0.100
Cheese	93.08 ^a	87.57 ^b	1.921	0.489
CP recovery				
Whey	25.48	25.65	NS	0.448
Salt whey	0.66	0.60	NS	0.030
Cheese	74.11	73.51	NS	0.462
Milk solids recovery				
Whey	50.55 ^b	52.00 ^a	1.132	0.288
Salt whey	1.15	1.24	NS	0.069
Cheese	48.65 ^a	46.41 ^b	1.281	0.326
Salt recovery				
Salt whey	55.28 ^a	37.62 ^b	4.981	1.269
Cheese	43.04 ^b	64.05 ^a	5.748	1.464

^{a,b}Means within a row that do not share a common superscript differ ($P \leq 0.05$).

cheese had a salt content of 1.44% compared with 2.24% for the CO₂ treatment. Thus, the salt-in-the-moisture content for the CO₂ treatment (5.96%) was higher than the typical value (about 4.6%) for aged Cheddar. This could impact enzymatic changes during aging that will be addressed in a separate paper.

Component Recoveries

The actual total recoveries (i.e., accountability) for all components were not influenced by the CO₂ treatment. Actual total calcium, CP, fat, milk solids, and added salt recoveries for cheeses made from milk without and with added CO₂ were 101.91 and 102.13%, 101.89 and 101.39%, 100.60 and 99.11%, 99.94 and 99.24%, and 96.54 and 99.81%, respectively. Therefore, the actual recoveries were adjusted as described in the materials and methods section of this paper.

Calcium recovery. More ($P \leq 0.05$) calcium was recovered in the whey of the milk treated with CO₂ (Table 6) than in the control (54.28 vs. 37.27%, respectively). Mean calcium recoveries were higher ($P \leq 0.05$) in the control salt whey and cheese compared with the CO₂ treatment (Table 6). Adding CO₂ to milk lowers the pH (Table 3) and causes an increase in calcium and phosphate concentrations in the serum phase of milk (Law and Leaver, 1998). The higher milk serum calcium content at coagulant addition was the likely cause for the firmer coagulum of the CO₂ treatment and the higher calcium content of the CO₂ treatment whey. The lower calcium recovery in the cheese reduced cheese calcium content

from 0.69 to 0.52% (Table 5), which was also due to the increased soluble calcium at coagulant addition.

Fat recovery. Carbon dioxide treatment cheeses had a lower ($P \leq 0.05$) fat recovery in the cheese than the control cheeses (Table 6). Almost 10% of the total milk fat was recovered in the whey from the CO₂ treated milk compared with about 7% in the control. The fat recovery for the control was consistent with the assumption of 93% fat recovery in the Cheddar cheese theoretical yield formulas described in the Materials and Methods section. The mean fat recovery in the salt whey of the CO₂ treatment was more than twice that of the control (Table 6). Although the difference in fat content between the control and the CO₂ treatment was greater for the salt whey than for the whey (Table 5), more fat was lost in the whey than in the salt whey, as shown in Table 6.

The lower pH of the milk with CO₂ added prior to rennet addition changes the rate and firmness of milk coagulation. The coagulation for the milk with added CO₂ was faster and firmer in our study. Johnson et al. (2001) varied coagulation firmness in a controlled study of composition and yield of 50% reduced fat Cheddar cheese and found an increase in fat loss with increased coagulation time and firmness at cut. Johnson et al. (2001) indicted that a coagulation that is cut soft will lose less fat and serum than a coagulation that is cut firm, if sufficient time is allowed for formation of the skin on the surface of curd particles after cutting and before stirring. In the present study, the curd for the CO₂ treatment was firmer than the control, but also was much lower in bound calcium content.

Why was less fat recovered in the CO₂ treatment cheese than in the control cheese? The milk pH of the CO₂ treatment (5.9) was closer to the acid pH optimum for chymosin. Higher enzyme activity may lead to excessive casein hydrolysis at coagulation rather than specific action on κ -CN. Nonspecific proteolysis of CN by chymosin would reduce the casein structure's ability to hold fat, and higher fat losses would be observed. However, no significant difference in whey CP content or CP recovery in the cheese was detected between the control and CO₂ treatment.

It is more likely that the lower calcium (and phosphate, although phosphate was not measured in this study) recovery, not casein hydrolysis, in the cheese played a role in the lower fat recovery in the cheese of the CO₂ treatment. Increasing the milk serum calcium level by CO₂ treatment is similar to adding CaCl₂, in that both produce a more firm milk coagulum. The result of the 2 methods may be similar, but their impact on cheese composition differs. Adding CaCl₂ to milk (0.01 to 0.02% wt/wt) would not be expected to decrease the bound calcium. On the other hand, acidifying milk (i.e., adding CO₂ to a milk pH of 5.9) would decrease bound

calcium and colloidal phosphorus (Law and Leaver, 1998) and increase soluble calcium. Thus the bound calcium and probably the colloidal phosphorus content of the curd in the present study was lower than if the coagulum had been formed with added calcium. Also, the bound calcium and phosphorus were probably lower in the CO₂ treatment than in the control cheese, indicated by the higher calcium content in the whey of the CO₂ treatment. The lower calcium content may have altered the ability of the curd to retain fat during cooking, Cheddaring, salting, and pressing. More work is needed to understand the exact point in time and the cause for the higher fat loss in the whey when CO₂ is used to decrease the milk pH to 5.9 for the manufacture of full-fat Cheddar cheese, and this will aid in the development of strategies to reduce fat loss during manufacture of full-fat Cheddar when CO₂ is used in cheese making.

CP and milk solids recovery. No differences ($P \geq 0.05$) were detected in mean CP recoveries between the treatments for the whey, salt whey, or cheese (Table 6). Total milk solids recovery did not include added salt, only milk solids. Total milk solids recovery of the CO₂ treatment was higher in the whey and lower in the cheese. The differences in milk solids recovery were generally consistent with the differences in fat and calcium recovery due to CO₂ treatment.

Salt recovery. An unexpected result of the current study was the difference ($P \leq 0.05$) in salt recovery in the cheese between the control and CO₂ treatments (Table 6). About 64% of the added salt was recovered in the cheese of the CO₂ treatment, compared with only 43% in the control cheese. The large difference (Table 5) in salt content between control and CO₂ cheeses occurred even though the curd-salting rate was the same for both treatments. No difference ($P > 0.05$) was detected in cheese moisture (Table 5), but the CO₂ treatment caused the salt-in-the-moisture to be one and a half times that of the control. St-Gelais et al. (1997) did not detect a difference in cheese salt content between the control and CO₂ treatment. The CO₂ treatment of St-Gelais et al. (1997) not only had a higher milk pH at coagulant addition (6.47), but also had a higher curd pH at salting (5.46). In our study, the coagulant and salt were added at lower pH values for the CO₂ treatment than in the work of St-Gelais et al. (1997). It is unclear whether the lower calcium content of the curd at salting was responsible for the high salt uptake of CO₂ treatment cheese. A marked improvement of added salt retention in Cheddar cheese, like the results shown in Table 6, would reduce salt wastes from a cheese manufacturing facility.

Cheese Yield and Yield Efficiency

Actual and adjusted cheese yields were significantly lower for the CO₂ treatment (Table 7). Other investiga-

Table 7. Mean ($n = 3$) actual moisture, and salt adjusted, Van Slyke, and Barbano theoretical cheese yields and cheese yield efficiencies.

	Control	CO ₂	LSD	SEM
	— kg/100 kg —			
Yield				
Actual	9.26	9.07	NS	0.052
Adjusted ¹	9.29 ^a	8.86 ^b	0.302	0.077
Van Slyke	9.21	9.21	NA ²	NA
Barbano	9.21	9.18	NA	NA
Yield efficiency				
Van Slyke	100.9 ^a	96.2 ^b	2.599	0.662
Barbano	100.9 ^a	96.5 ^b	2.760	0.703

^{a,b}Means within a row that do not share a common superscript differ ($P \leq 0.05$).

¹Moisture adjusted to 37% and salt to 1.5%.

²NA = Not applicable.

tors reporting cheese yield did not detect differences in cheese yield between the control and CO₂ treatment (Calvo et al., 1993; McCarney et al., 1995; St-Gelais et al., 1997). The Van Slyke theoretical yield formula predicted the same yield for both the control and the CO₂ treatment because the milk compositions were the same (Table 7). Cheese yields predicted by the Barbano theoretical yield formula for the CO₂ treatment cheeses were lower because of the different calcium phosphate retention factor used for the CO₂ treatment that allowed for the expected lower retention of calcium phosphate in the cheese. The cheese yield efficiency of the control was 100.9%. The mean fat recovery attained with the control cheese (Table 6) was consistent with the theoretical fat recovery (93%) of the theoretical yield formulas, which indicates that the cheese making methods alone did not create the fat loss observed in the CO₂ treatment cheese. Preacidification with CO₂ resulted in a 4.7% lower Van Slyke yield efficiency and a 4.4% lower Barbano yield efficiency than the control. The difference in yield efficiency within the CO₂ treatment (0.3%) represents the reduction in yield due to mineral loss. The fat loss in the whey caused a greater difference in yield efficiency between the control and treatment than did the calcium loss.

CONCLUSIONS

Cheese manufactured from milk acidified to a pH of 5.9 with approximately 1600 ppm of CO₂ retained less calcium and fat than the control cheese. The higher loss of fat was primarily in the whey at draining. Preacidification with CO₂ did not alter the CP recovery in the cheese. The CO₂ treatment resulted in a higher added salt recovery in the cheese and produced a cheese that contained too much salt. Considering the higher added salt retention of the CO₂ treatment, the salt application rate could be lowered to achieve a typical cheese salt

content. Cheese yield efficiency of the CO₂ treated milk was 4.4% lower than the control due to fat loss. The benefits of better quality raw milk, better milk coagulation, reduced rennet use, and potentially reduced problems with calcium crystal formation are enough to warrant more research to modify the cheese making procedure to decrease fat loss and salt content in the cheese. Successful modification of the make procedure to reduce fat loss will be necessary for manufacturers to implement this level of CO₂ addition to milk prior to full-fat Cheddar cheese making.

ACKNOWLEDGMENTS

The authors thank Tom Burke, Maureen Chapman, Laura Landolf, Bob Kaltaler, Amar Olabi, and Pat Wood for technical assistance and Dairy Management Inc. (Rosemont, IL), and the Northeast Dairy Foods Research Center for partial financial support.

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