Effect of Temperature of CO₂ Injection on the pH and Freezing Point of Milks and Creams¹

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ABSTRACT

The objectives of this study were to measure the impact of CO₂ injection temperature (0°C and 40°C) on the pH and freezing point (FP) of (a) milks with different fat contents (i.e., 0, 15, 30%) and (b) creams with 15% fat but different fat characteristics. Skim milk and unhomogenized creams containing 15 and 30% fat were prepared from the same batch of whole milk and were carbonated at 0 and 40°C in a continuous flow CO₂ injection unit (230 ml/min). At 0°C, milk fat was mostly solid; at 40°C, milk fat was liquid. At the same total CO₂ concentration with CO₂ injection at 0°C, milk with a higher fat content had a lower pH and FP, while with CO₂ injection at 40°C, milks with 0%, 15%, and 30% fat had the same pH. This indicated that less CO₂ was dissolved in the fat portion of the milk when the CO₂ was injected at 0°C than when it was injected at 40°C. Three creams, 15% unhomogenized cream, 15% butter oil emulsion in skim milk, and 15% vegetable oil emulsion in skim milk were also carbonated and analyzed as described above. Vegetable oil was liquid at both 0 and 40°C. At a CO₂ injection temperature of 0°C, the 15% vegetable oil emulsion had a slightly higher pH than the 15% butter oil emulsion and the 15% unhomogenized cream, indicating that the liquid vegetable oil dissolved more CO₂ than the mostly solid milk fat and butter oil. No difference in the pH or FP of the 15% unhomogenized cream and 15% butter oil emulsion was observed when CO₂ was injected at 0°C, suggesting that homogenization or physical dispersion of milk fat globules did not influence the amount of CO₂ dissolved in milk fat at a CO₂ injection temperature of 0°C. At a CO₂ injection temperature of 40°C and at the same total CO₂ concentration, the 15% unhomogenized

cream, 15% vegetable oil emulsion, and 15% butter oil emulsion had similar pH. At the same total concentration of CO₂ in cream, injection of CO₂ at low temperature (i.e., <4°C) may produce a better antimicrobial effect during refrigerated shelf life due to the higher concentration of CO₂ in the skim portion of the cream.

(**Key words:** carbon dioxide, pH, freezing point, milk and cream)

Abbreviation key: FP = freezing point.

INTRODUCTION

The addition of CO₂ to dairy foods, such as cottage cheese, has been used to extend product shelf life (Hotchkiss and Lee, 1996). The commercial success of CO₂ technology in the dairy industry has initiated interest in better understanding the impact of CO₂ addition on the chemistry of milk components. The antimicrobial effect of CO₂ is dependent on the concentration of CO₂ in the aqueous phase of the food (Fernandez et al., 1997; Devlieghere et al., 1998). Once CO₂ is dissolved in the aqueous phase, milk pH decreases (Ma et al., 2001). Although the antimicrobial effect of CO₂ is not pH dependent (King and Mabbitt, 1982 1987; Ma et al., 2003), many dairy processing steps are pH dependent (Corredig and Dalgleish, 1996; Singh et al., 1996). The extent of pH reduction is related to the amount of CO₂ dissolved, hydrated, and protonated in the aqueous phase and thus depends on the intrinsic properties of the aqueous phase, such as buffering capacity and initial pH (Gill, 1988; Löwenadler and Rönner, 1994; Devlieghere et al., 1998). To achieve any pH reduction, CO₂ needs to be dissolved in the aqueous phase of a dairy food.

Dairy foods consist of an aqueous (skim portion) and a lipid (milk fat) phase. It is generally accepted that CO₂ has a higher solubility in a nonpolar solvent, such as lipid, than in a polar solvent, such as water (Fogg and Gerrard, 1991). This is because the molecular structure of CO₂ (O=C=O) is nonpolar and CO₂ has a dipole moment of zero. However, most dairy products are stored at refrigeration temperatures and at these temperatures, a high percentage (e.g., 60 to 80%) of milk

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Table 1. The ANOVA models used for analysis of pH and freezing point (FP) data for experiments 1 and 2.

	df for pH	df for FP	Analyzed as	Error term
Replicate	1	1	Block	
Treatment ¹	2	2	Fixed effect	Replicate × treatment
Replicate × treatment	2	2	Interaction	
CO ₂ linear	1	1	Continuous variable	E
CO ₂ quadratic	1	...	Continuous variable	E
CO ₂ linear × treatment	2	2	Interaction	E
CO ₂ quadratic × treatment	2	...	Interaction	E
Error (E)	24	27		

¹For experiment 1, the three treatments were skim milk, 15% fat unhomogenized cream, and 30% fat unhomogenized cream. For experiment 2, the three treatments were 15% fat unhomogenized cream, 15% butter oil emulsion homogenized in skim milk, and 15% vegetable oil emulsion homogenized in skim milk.

fat is solid (Brunner, 1978). When the fat is solid, very little CO₂ should be dissolved in the fat portion and most of the dissolved CO₂ in the product should be in the skim portion. Although some of the milk fat remains liquid at refrigeration temperature, within spherical fat globules the solid fat forms a layer on the inside surface of milk fat globule membrane (Buchheim, 1970) and could act as a barrier to prevent migration of CO₂ in and out of the fat globules. Above 38°C when milk fat melts, it may be expected that CO₂ can dissolve easily in both the skim and the fat portions of milk. Therefore, when the effect of CO₂ addition on the pH of milk is measured, milk temperature needs to be clearly specified and controlled.

Excellent temperature control during milk pH measurement is important because even in milk without added CO₂, milk pH decreases linearly with increasing temperature and the decrease is related to shifts in degree of association of calcium phosphate (Dixon, 1963; Chaplin and Lyster, 1988; Hege and Kessler, 1986; Fox and McSweeney, 1998). Using the linear coef-

ficient of -0.0073 pH unit/°C reported by Chaplin and Lyster (1988), the pH of noncarbonated milk is expected to decrease about 0.3 pH unit when its temperature is increased from 0 to 40°C. To obtain accurate pH measurement, the pH probe and calibration buffers need to be tempered to the temperature at which the pH of the milk will be measured. In addition, the appropriate reference pH for each calibration buffer at that temperature needs to be used. The change in the pH of calibration buffers as a function of temperature can be obtained from the manufacturer of the calibration buffer. Tempering the pH electrode to the same temperature as the buffers and the milk samples will provide more rapid response and more stable readings.

The concentration of CO₂ in a dairy food is often expressed in units of ppm (Ma et al., 2001) and mM (King and Mabbitt, 1987; Hotchkiss et al., 1999). The common method of CO₂ determination measures the amount of CO₂ in the entire dairy food (King and Mabbitt, 1987; Ma et al., 2001) and thus the CO₂ concentration is an average concentration of CO₂ in both the skim

Table 2. Sum of squares (SS) and probabilities (*P*) from the ANOVA of pH (with CO₂ injection at 0 and 40°C) and freezing point (FP, measured using milk and creams with CO₂ injection at 0°C) data in experiment 1. The treatments were skim milk, 15% fat unhomogenized cream, and 30% fat unhomogenized cream.

	pH at 0°C SSM ¹ = 2.2821 Model R ² = 0.997		FP (CO ₂ injection at 0°C) SSM = 0.03566 Model R ² = 0.996		pH 40°C SSM = 1.8616 Model R ² = 0.998	
	SS ²	<i>P</i>	SS	<i>P</i>	SS	<i>P</i>
Replicate	0.0058		0.0000		0.0042	
Treatment	0.0855	<0.05	0.0037	NS ³ (0.06)	0.0000	NS
Replicate × treatment	0.0040		0.0002		0.0001	
CO ₂ linear	2.0457	<0.01	0.0306	<0.01	1.5642	<0.01
CO ₂ quadratic	0.0788	<0.01	...	...	0.0456	<0.01
CO ₂ linear × treatment	0.0016	NS (0.07)	0.0003	<0.01	0.0000	NS
CO ₂ quadratic × treatment	0.0024	<0.05	...	...	0.0000	NS
Error (E)	0.0066		0.0001		0.0034	

¹SSM = Sum squares of model.

²SS = Sum squares of individual factors.

³NS = Not significant, *P* > 0.05.

Figure 1. pH (0°C) of skim milk (□, ■), 15% fat unhomogenized cream (●, ○), and 30% fat unhomogenized cream (▲, △) after CO₂ injection at 0°C as a function of total CO₂ content (ppm) of the milk or cream. The solid and open symbols represent data from replication 1 and 2, respectively. The dotted lines are the least square second order polynomial regression lines.

and fat portions. The CO₂ dissolved in the fat portion should not contribute to pH reduction in the skim portion of a milk. Depending proportionally how much of the total dissolved CO₂ is in the skim portion versus the fat portion, two dairy systems with the same skim portion properties, such as buffering capacity and initial pH and the same amount of total dissolved CO₂ (in units of ppm or mM) but with different fat concentration and properties may exhibit different pH. A simple example would be two milks with the same skim portion composition but different fat concentration. If these two milks have the same total CO₂ concentration and if more CO₂ is proportionally dissolved in the fat portion, then the milk with a higher fat content would be expected to have less CO₂ dissolved in the skim portion and thus exhibit a higher pH and vice versa.

Apart from pH, freezing point (FP) is another parameter that reflects the amount of CO₂ dissolved in the skim portion of milk. Upon CO₂ addition, milk FP decreases (Ma et al., 2001). No detailed research has been conducted to determine the extent of pH reduction and FP depression in milks and creams when CO₂ injection is conducted at different temperatures. Because pH and FP are indicators of the amount of CO₂ dissolved in the skim portion and thus also the effectiveness of the antimicrobial effect of CO₂, it is important to understand the effect of temperature of CO₂ injection on the pH and FP in dairy systems. The objectives of this study were to measure the impact of CO₂ injection temperature (0°C and 40°C) on the pH and freezing point (FP) of (a) milks with different fat contents (i.e., 0%, 15%, and 30%) and (b) creams with the same fat content (15%) but containing fat with different melting characteristics (i.e., solid versus liquid) and physical dispersion (i.e., homogenized versus unhomogenized).

MATERIALS AND METHODS

Milk and Cream Preparation and Analysis

The process of preparation and analysis of milk and cream occurred over a four day period and was replicated two times. On the first day, fresh raw milk (Cornell Dairy Plant, Ithaca, NY) was HTST pasteurized (72°C/15 s, Processing Machinery and Supply Co., Philadelphia, PA) and separated at 60°C into a skim milk and a cream portion using a lab-scale cream separator (Model 100, Delaval, Poughkeepsie, NY). Immediately after separation, both the skim milk and cream were cooled on ice to 4°C and stored at 4°C. Fat content of the separated cream (AOAC, method number 995.18; 33.3.18) and the skim milk (Marshall, 1993, method number 15.8B) was determined by the Babcock method.

Five batches, about 25 kg each, were prepared: skim milk, 15% fat unhomogenized cream, 30% fat unhomogenized cream, 15% butter oil emulsion in skim milk, and 15% vegetable oil emulsion in skim milk. All fat percentages were based on weight. Vegetable oil was used, for the purpose of this experiment to provide a source of low melting fat and it was liquid at 0°C. The 15% and 30% unhomogenized creams were prepared by blending the appropriate amounts of skim milk and cream containing about 40% fat. For the preparation of 15% vegetable oil emulsion, skim milk was heated to 71°C in a steam kettle and vegetable oil (Mazola 100% pure corn oil, Best Foods, Eaglewood Cliffs, NJ) was added slowly to the heated skim milk with continuous stirring for 5 min using a high shear mixer (Model AXR, Silverson Machines Ltd., Waterside, England). The mixture was homogenized at 71°C (1st stage: 13.78

Table 3. Regression coefficients for predicting the pH (0 and 40°C) and freezing point (FP) of milk and creams upon CO₂ (ppm) addition in experiments 1 and 2.

Experiment 1							
	pH at 0°C			FP, °H ¹			pH at 40°C
	Skim milk	15% cream	30% cream	Skim milk	15% cream	30% cream	Skim milk 15% and 30% creams
Intercept	6.99	6.99	6.99	-0.534	-0.539	-0.540	6.64
X (CO ₂)	-5.78×10^{-4}	-6.60×10^{-4}	-7.41×10^{-4}	-4.20×10^{-5}	-4.77×10^{-5}	-5.40×10^{-5}	-7.24×10^{-4}
X ²	1.05×10^{-7}	1.30×10^{-7}	1.60×10^{-7}	...	...	...	1.80×10^{-7}
R ²	0.99	0.99	0.99	0.98	0.99	0.99	0.99

Experiment 2				
	pH at 0°C		15% unhomogenized cream, 15% butter oil emulsion and 15% vegetable oil emulsion	
	15% unhomogenized cream, 15% butter oil emulsion	15% vegetable oil emulsion	FP, °H	pH at 40°C
Intercept	6.97	6.96	-0.541	6.62
X (CO ₂)	-6.31×10^{-4}	-5.83×10^{-4}	-4.77×10^{-5}	-7.04×10^{-4}
X ²	1.19×10^{-7}	1.13×10^{-7}	...	1.70×10^{-7}
R ²	0.99	0.99	0.99	0.99

¹°H = degrees Hortvet.

MPa (2000 psi), 2nd stage: 3.445 MPa (500 psi); Gaulin Model 75E, Everett, MA) and cooled to 4°C. The preparation procedure for the 15% butter oil emulsion in skim milk was similar to that of the vegetable oil emulsion, except that the butter oil was melted before addition to skim milk. Efficiency of homogenization was verified by measuring fat globule size distribution (Smith et al., 1995) using a laser light scattering particle size analyzer (Malvern MasterSizer E, Worcestershire, U.K.). The actual fat content of the five batches was confirmed using the Mojonnier method (AOAC, method number 989.05; 33.2.26). Each of the five batches was split into two 12.5 kg batches: one carbonated on d 2 at 0°C (actual temperature varied between 0 and 1°C) and the other one carbonated on d 4 at 40°C.

On the second day, the first group of the five batches were carbonated at 0°C in a continuous flow inline CO₂ injection system. The injection system was modified from a countercurrent stainless steel tubular heat exchanger (internal diameter = 0.5 cm) cooled by circulating ice-water. Milk was pumped, from a constantly stirred feed tank, through the system with a peristaltic pump and CO₂ (beverage grade) was injected through a stainless steel tube (internal diameter = 0.08 cm) inserted through a Tee-fitting perpendicular to the milk flow immediately after the feed pump. The residence time of milk and creams in the heat exchanger was approximately 60 s at a backpressure of about 0.034 MPa (5 psi). Six different carbonation levels between control (none) and 2000 ppm were achieved by adjusting the flow rate of CO₂ while keeping the flow rate of milk

and creams constant (230 ml/min). After carbonation, milk and creams were stored in 40-ml plastic vials (Capitol Vial Corp., Fultonville, NY) at 0 to 1°C overnight to allow equilibration and were analyzed on the third day for pH (0°C), CO₂ concentration (Ma et al., 2001), and FP (AOAC, method number 990.22; 33.2.04). Freezing point was measured using a cryoscope (Model 4D3, Advanced Instruments, Inc., Norwood, MA) and reported in degrees Hortvet (°H). The conversion of °H to °C = $(0.1915 \times (-|°H|) - 0.0004785)/0.199$ (AOAC, method number 980.15, 33.2.33). Before pH measurement, milk samples were left in ice-water for at least 2 h. The pH probe (model HA 405 DXK-58/120 combination pH probe; Mettler Toledo, Columbus, OH) was calibrated with pH 7.13 and 4.01 buffers (Fisher Scientific, Fair Lawn, NJ) at 0°C.

On the fourth day, the second group of the five batches were warmed to 40°C in a steam kettle and then carbonated. CO₂ injection was conducted in a continuous flow inline system as described above, except that the product temperature in the tubular heat exchanger was maintained at 40°C. Carbonated skim milk and creams were collected at 40°C into 40-ml plastic vials (Capitol Vial Corp., Fultonville, NY) at the heat exchanger exit, immediately capped, and put into a 40°C water bath. Sample pH was determined using a pH probe that was calibrated with pH 6.97 and 4.03 buffers at 40°C. Carbonated milk and cream samples at 40°C were immediately (without cooling) weighed into Erlenmeyer flasks and sealed for CO₂ analysis (Ma et al., 2001).

Figure 2. Change of freezing point (FP) in degrees Hortvet of skim ($\square$, $\blacksquare$), 15% fat unhomogenized cream ($\circ$, $\bullet$), and 30% fat unhomogenized cream ($\triangle$, $\blacktriangle$) after CO_2 injection at 0°C as a function of total CO_2 content (ppm) of the milk or cream. The solid and open symbols represent data from replication 1 and 2, respectively. The dotted lines are the least square linear regression lines.

Experimental Design and Statistical Analysis

Experiment 1: effect of temperature of CO_2 injection on pH and FP of skim milk and creams. Skim milk ($< 0.1\%$ fat) and two unhomogenized creams (ca. 15% and 30% fat) were prepared, as described above. CO_2 injection was conducted at 0°C and 40°C to achieve six levels of added CO_2 ranging from control (none) to approximately 2000 ppm. At 40°C , milk fat was completely melted. The pH and FP of the skim milk and the two creams with CO_2 injection at 0°C were determined using milk samples that were equilibrated overnight at 0°C to 1°C after processing. The pH of the milk and creams with CO_2 injection at 40°C was determined immediately after CO_2 injection. The experiment was rep-

licated two times using different batches of pasteurized, unhomogenized milk and cream.

Experiment 2: effect of temperature of CO_2 injection on pH and FP of creams with different fat characteristics. A 15% fat concentration was chosen because homogenization of a cream with a fat level $> 15\%$ tends to cause the fat globules to cluster (Metzger and Barbano, 1996). Creams were carbonated at 0°C and 40°C to contain six different levels of added CO_2 from control (none) to 2000 ppm. Cream pH (with CO_2 injection at 0 and 40°C) and FP (with CO_2 injection at 0°C) were determined as described in experiment 1. The experiment was replicated two times using different batches of ingredients.

Statistical analysis. The ANOVA models used for the analysis of pH and FP data are shown in Table 1. The concentration of CO_2 was treated as a continuous variable. A quadratic term of CO_2 concentration was included in the model for the analysis of pH data but not for the FP data, based on experience from previous research (Ma et al., 2001).

Because the concentration of CO_2 was treated as a continuous variable in the ANOVA model (Table 1), any model terms involving CO_2 concentration would be correlated. For example, the CO_2 linear and the CO_2 quadratic or the CO_2 quadratic and CO_2 quadratic $\times$ treatment would be correlated. Distortion of the ANOVA by multicollinearity of these terms in the model was resolved by centering the CO_2 data using a mathematical transformation of the data for CO_2 concentration (Glantz and Slinker, 2001). The transformation was done by subtracting the mean CO_2 concentration from each of the individual CO_2 concentrations and using these "mean-centered" data in the statistical analysis. This type of linear transformation minimized correlation between the model terms involving CO_2 concentration but did not alter the mathematical nature of the statistical testing (Glantz and Slinker, 2001). Based on the results of ANOVA, regression analysis was used to produce equations to predict pH and FP at different CO_2 concentrations in milk or cream. A quadratic term of CO_2 concentration was used in the regression for pH but not for FP. For estimation of the regression parameters (i.e., intercepts and slopes), the original CO_2 concentrations were used. All statistical analyses were done using SAS (version 8.02, 1999–2001).

RESULTS AND DISCUSSION

Experiment 1: Effect of Temperature of CO_2 Injection on pH and FP of Skim Milk and Creams

Noncarbonated milk and creams. Mean ($n = 2$) fat content of the skim milk and the two creams were

Table 4. Sum of squares (SS) and probabilities (*P*) from the ANOVA of pH (0°C) and freezing point (FP) data using CO₂ concentration in the skim portion of the milk and creams, assuming that no CO₂ was dissolved in the fat portion and all of the CO₂ was in the skim portion when CO₂ injection was conducted 0°C. The treatments were skim milk, 15% fat unhomogenized cream, and 30% fat unhomogenized cream.

	pH at 0°C		FP	
	SSM ¹ = 1.6782, Model R ² = 0.999		SSM = 0.0206, Model R ² = 0.994	
	SS ²	<i>P</i>	SS	<i>P</i>
Replicate	0.0001		0.0004	
Treatment	0.0012	NS ³	0.0000	NS
Replicate × treatment	0.0002		0.0000	
CO ₂ linear	1.0523	<0.01	0.0196	<0.01
CO ₂ quadratic	0.0478	<0.01	...	
CO ₂ linear × treatment	0.0002	NS	0.0000	NS
CO ₂ quadratic × treatment	0.0002	NS	...	
Error (E)	0.0021		0.0001	

¹SSM = Sum squares of model.

²SS = Sum squares of individual factors.

³NS = Not significant, *P* > 0.05.

0.07, 15.2, and 30.2%. The concentration of CO₂ in the noncarbonated skim milk, 15% cream, and 30% cream were 84, 66, and 53 ppm, respectively. At 0°C, the pH of the noncarbonated skim milk (6.94), 15% cream (6.95), and 30% cream (6.95) were similar and they each showed about 0.35 unit decrease in pH when the temperature was increased from 0°C to 40°C. This decrease in pH was expected and the extent of decrease was comparable to literature values (Chaplin and Lyster, 1988). Before carbonation, the FP of skim milk (−0.539°C), 15% cream (−0.544°C), and 30% cream (−0.544°C) were similar and the values were typical for milk (Packard and Ginn, 1990). The pH (0°C and 40°C) and FP of the skim milk and two creams from the same batch of whole milk were expected to be the same because they had the same skim portion.

Because the skim milk and the unhomogenized creams containing 15% and 30% fat were prepared from a single batch of pasteurized whole milk, the properties of both the skim portion and the fat portion (unhomogenized milk fat globules) of the skim milk and the two creams within a replication were the same. This ensured that the buffering capacity and mineral content in the skim portion of the milk and the creams were the same and after CO₂ injection, any differences observed in the extent of pH decrease and FP depression among the skim milk and the two creams was due to differences in their fat content.

pH at 0°C. Treatment (i.e., fat concentration) had a significant (*P* < 0.05, Table 2) impact on the pH of milk when CO₂ was injected at 0°C, a temperature at which the milk fat was mostly solid. There were significant (*P* < 0.01) linear and quadratic effects of CO₂ concentration on milk pH and there was a significant (*P* < 0.05) CO₂ quadratic by treatment interaction effect (Table 2). The treatment effect can be seen clearly in Figure

1: at the same total CO₂ concentration, milk containing a higher fat content achieved a lower pH when CO₂ was injected at 0°C. This occurred because the CO₂ could not penetrate the solid fat in the fat globules and therefore the amount of CO₂ dissolved in the skim portion increased with increasing fat content. For all milks, pH decreased with increasing CO₂ concentration and the rate of pH decrease was reduced at higher CO₂ concentration (Figure 1). Second order polynomial regression curves that model the pH of the skim milk and the two creams when CO₂ was injected at 0°C are plotted in Figure 1 (dotted lines) and the regression coefficients are shown in Table 3.

Freezing point. FP were measured on skim milk and creams with CO₂ injection at 0°C. Treatment (i.e., fat content of milk) had an impact (*P* = 0.06) on milk FP, which was manifested as a significant CO₂ linear × treatment interaction (Table 2). The interaction effect of CO₂ linear by treatment can be seen clearly from the divergence of the linear regression lines in Figure 2. When CO₂ was injected into milk at 0°C, at the same total CO₂ concentration, the higher the fat content the lower the FP. Unlike the impact of CO₂ concentration on milk pH (Figure 1), the effect of CO₂ on FP was linear (Figure 2).

The regression coefficients shown in Table 3 were calculated using the observed FP. Only the changes of FP were plotted (Figure 2), that is, each of the regression lines was shifted to have a zero intercept on the y-axis while keeping the slope the same. The reason for doing this adjustment was that only the slopes of the regression lines or the changes of FP as a function of CO₂ concentration were of interest for comparison of treatments. Increasing fat content resulted in a more negative slope for FP when CO₂ was injected into milk at 0°C (Table 3 and Figure 2).

Figure 3. pH (0°C) of skim milk ($\square$, $\blacksquare$), 15% fat unhomogenized cream ($\bullet$, $\circ$), and 30% fat unhomogenized cream ($\blacktriangle$, $\triangle$) after CO₂ injection at 0°C as a function of the estimated CO₂ content (ppm) in the skim portion of the milk or cream. The solid and open symbols represent data from replication 1 and 2, respectively.

Figure 4. Change of freezing point (FP) in degrees Hortvet of skim ($\square$, $\blacksquare$), 15% fat unhomogenized cream ($\bullet$, $\circ$), and 30% fat unhomogenized cream ($\blacktriangle$, $\triangle$) after CO₂ injection at 0°C as a function of the estimated CO₂ content (ppm) in the skim portion of the milk or cream. The solid and open symbols represent data from replication 1 and 2, respectively.

Effect of fat concentration at 0°C. Because both pH and FP reflect the amount of CO₂ dissolved in the aqueous portion of milk, it can be concluded that at the same total CO₂ concentration, the concentration of CO₂ in the skim portion was highest for the 30% cream, followed by the 15% cream, and then the skim milk. If it can be assumed that at 0°C, solid milk fat dissolves little if any CO₂, then the concentration of CO₂ in the skim portion of the milk and creams can be estimated as [total CO₂ concentration in milk or cream/(1 minus (percent fat/100))].

A new ANOVA was conducted using the estimated CO₂ concentration in the skim portion as one of the model terms (Table 4). After the correction for the fat content, the estimated CO₂ concentrations in the skim portion of the creams increased substantially, espe-

cially for the 30% cream and at a higher total CO₂ concentration. For example, a 30% cream with 2000 ppm CO₂ would have an estimated 2857 ppm CO₂ in its skim portion if all the CO₂ was dissolved in the skim portion. No observations on pH and FP were made on skim milk at such high CO₂ concentration (i.e., 2857 ppm). Thus, the highest estimated CO₂ concentrations in the skim portion of the 15% and 30% creams (> 2300 ppm, Figure 3 and 4) were not included in the ANOVA (Table 4) so that the skim portion of the skim milk, 15% cream, and 30% cream were being compared over the same range of CO₂ concentration. In contrast to the results of the ANOVA (Table 2) using the total CO₂ concentration, the ANOVA (Table 4) using the estimated CO₂ concentration in the skim portion found no

Figure 5. pH (40°C) of skim ($\square$, $\blacksquare$), 15% fat unhomogenized cream ($\bullet$, $\circ$), and 30% fat unhomogenized cream ($\blacktriangle$, $\triangle$) after CO₂ injection at 40°C as a function of total CO₂ content (ppm) of the milk or cream. The solid and open symbols represent data from replication 1 and 2, respectively. The dotted line is the least square second order polynomial regression line.

significant effect ($P > 0.05$) of treatment, CO₂ linear $\times$ treatment, or CO₂ quadratic $\times$ treatment in the model for pH and no significant effect ($P > 0.05$) of treatment or CO₂ linear $\times$ treatment in the model for FP. When pH and FP were plotted as a function of the estimated CO₂ concentration in the skim portion of the milk and creams, the extent of pH reduction (Figure 3) and FP depression (Figure 4) became similar for the skim milk, 15% unhomogenized cream, and 30% unhomogenized cream. Therefore, at a CO₂ injection temperature of 0°C, almost all of the CO₂ was solubilized in the skim portion and very little was dissolved in the solid milk fat.

pH at 40°C. When CO₂ injection and pH measurement were conducted at 40°C, treatment (i.e., fat con-

centration) did not have a significant ($P > 0.05$) impact on the pH of milks of different fat contents (i.e., 0%, 15%, and 30%) (Table 2). At the same total CO₂ concentration, the extent of pH reduction did not differ among the skim milk, 15% unhomogenized cream, and 30% unhomogenized cream (Figure 5). A single second order polynomial regression curve was calculated to predict the pH of milk and creams at various CO₂ concentrations when CO₂ injection was done at 40°C (Figure 5). The regression coefficients are shown in Table 3. Therefore, when CO₂ was injected into milk or cream at 40°C, at which milk fat was liquid, the concentration of fat in the milk did not have an influence on the pH of the milk. This is different than what happened when CO₂ was injected into milk at 0°C. It can be concluded that the CO₂ was dissolved in both the skim and fat portions of milk when CO₂ was injected into milk at 40°C.

Experiment 2: Effect of Temperature of CO₂ Injection on pH and FP of Creams with Different Fat Characteristics

Noncarbonated creams. Mean ($n = 2$) fat contents of the unhomogenized cream, the vegetable oil emulsion, and the butter oil emulsion were 15.19, 15.23, and 15.22%, respectively. The vegetable oil and butter oil emulsions had similar fat globule size distribution and their volume mean diameters were 1.19 and 1.18 μm , respectively. The CO₂ concentrations in the noncarbonated 15% butter oil emulsion (16 ppm) and 15% vegetable oil emulsion (13 ppm) were similar but both were lower than the CO₂ concentration in the noncarbonated 15% unhomogenized cream (66 ppm). During the production of the two emulsions, a substantial amount of the naturally dissolved CO₂ was lost when the creams were heated (71°C) and agitated in the steam kettle.

At 0°C, the pH of the noncarbonated 15% fat cream (6.95), 15% vegetable oil emulsion (6.95), and 15% butter oil emulsion (6.95) were similar and increasing temperature to 40°C decreased each of their pH to 6.60. This decrease in pH was consistent with observations in experiment 1 and also with literature values (Chaplin and Lyster, 1988). Before carbonation, the FP of the 15% unhomogenized cream (-0.544°H), 15% vegetable oil emulsion (-0.543°H), and 15% butter oil emulsion (-0.546°H) did not differ.

pH at 0°C. The ANOVA (Table 5) found a significant CO₂ linear ($P < 0.01$), CO₂ quadratic ($P < 0.01$), treatment ($P < 0.05$), and CO₂ linear $\times$ treatment ($P < 0.01$) effect but not CO₂ quadratic $\times$ treatment effect ($P > 0.05$). Comparison (lsd = 0.0094 pH unit) among the least square means of the pH for the three creams calculated across CO₂ concentrations found that the mean pH of the 15% butter oil emulsion (6.54) and the 15%

Table 5. Sum of squares (SS) and probabilities (*P*) from the ANOVA of pH (with CO₂ injection at 0 and 40°C) and freezing point (FP, measured using creams with CO₂ injection at 0°C) data in experiment 2. The treatments were 15% fat unhomogenized cream, 15% vegetable oil emulsion homogenized in skim milk, and 15% butter oil emulsion homogenized in skim milk.

	pH at 0°C SSM ¹ = 2.0603 Model R ² = 0.999		FP (CO ₂ injection at 0°C) SSM = 0.03043 Model R ² = 0.995		pH at 40°C SSM = 1.9164 Model R ² = 0.999	
	SS ²	<i>P</i>	SS	<i>P</i>	SS	<i>P</i>
Replicate	0.0001		0.0001		0.0005	
Treatment	0.0023	<0.05	0.0001	NS ³	0.0005	NS
Replicate × treatment	0.0001		0.0000		0.0000	
CO ₂ linear	2.0417	<0.01	0.0304	<0.01	1.6623	<0.01
CO ₂ quadratic	0.0662	<0.01	...	...	0.0461	<0.01
CO ₂ linear × treatment	0.0045	<0.01	0.0000	NS	0.0001	NS
CO ₂ quadratic × treatment	0.0004	NS	...	...	0.0001	NS
Error (E)	0.0030		0.0002		0.0034	

¹SSM = Sum squares of model.

²SS = Sum squares of individual factors.

³NS = Not significant, *P* > 0.05.

unhomogenized cream (6.54) were similar (*P* > 0.05) and were both lower (*P* < 0.05) than the mean pH of the 15% vegetable oil emulsion (6.59). It can be concluded that when CO₂ was injected at 0°C, more CO₂ was dissolved in the liquid vegetable oil than in the partially solid milk fat or butter oil and therefore the content of CO₂ in the skim portion of the vegetable oil emulsion was lower than the two milk fat emulsions.

The effect of treatment (i.e., fat types) can be seen in Figure 6. It is easiest to see the differences in pH at 0°C among the three creams at CO₂ > 1000 ppm (Figure 6). Based on the ANOVA results (Table 5), two second order polynomial regression lines were produced: one for the 15% cream and 15% butter oil emulsion, and one for the 15% vegetable oil emulsion (Figure 6) and the regression coefficients are shown in Table 3.

A separate ANOVA (not shown) analyzing the decrease of pH at 0°C for only the 15% homogenized butter oil emulsion and the 15% unhomogenized cream found no significant (*P* > 0.05) effect of treatment (i.e., homogenization). Therefore, when CO₂ was injected at 0°C into the two milk fat based creams with the same fat content, the difference in the physical dispersion of the fat globules due to homogenization had no influence on the uptake of CO₂ by milk fat when the fat was partially solid.

The results of experiment 2 were consistent with those of experiment 1, suggesting that for any potential solvent (i.e., water or fat) to dissolve CO₂, the solvent needs to be in a liquid form. When CO₂ was injected at 0°C into creams that contained the same fat content (15%) but that differed in their amount of solid fat at 0°C, the cream with more solid fat achieved a lower pH in its skim portion because the solid fat was not

dissolving CO₂ and more of the total amount of CO₂ was dissolved in the skim portion.

Freezing point. No effect of treatment (i.e., fat type) on the FP of creams was detected (Table 5). While a statistically significant, but small absolute difference in pH at 0°C was detected, this difference in pH was not sufficient to produce a detectable change in FP under the conditions in this experiment. At the same total CO₂ concentration, the changes of FP were similar (*P* > 0.05) among the three creams when CO₂ injection and pH measurement were conducted at 0°C (Figure 7). A single linear regression line that modeled the FP as a function of CO₂ concentration at 0°C for the three creams with 15% fat (Figure 7) was calculated and the regression coefficients are shown in Table 3.

pH at 40°C. No effect (*P* > 0.05) of treatment (i.e., fat type) on the pH of creams was detected when CO₂ was injected into the creams at 40°C (Table 5). At the same carbonation level, there was no difference (*P* > 0.05) in the extent of pH reduction for the three creams with 15% fat (Figure 8). At 40°C, the butter oil, native milk fat, and vegetable oil were all in a liquid state. This suggested that on a unit weight base, an equal amount of CO₂ was dissolved in the skim portion, the vegetable oil, the butter oil, and milk fat when CO₂ was injected into creams at 40°C. Based on the ANOVA, a single second order polynomial regression line was calculated to predict the pH of the three creams upon CO₂ injection at 40°C (Figure 8) and the regression coefficients are shown in Table 3.

Practical Implications for Extended Shelf Life of Cream

When CO₂ is injected into milk at 0°C, milk with a high fat content will have a higher CO₂ concentration

Figure 6. pH (0°C) of 15% fat unhomogenized cream (●, ○), 15% vegetable oil emulsion in skim milk (◆, ◇), and 15% butter oil emulsion in skim milk (▲, △) after CO₂ injection at 0°C as a function of total CO₂ content (ppm) of the cream. The solid and open symbols represent data from replication 1 and 2, respectively. The dotted lines are the least square second order polynomial regression lines.

in its skim portion compared with milk with a lower fat content. For example, assuming that little if any CO₂ is dissolved in milk fat at 0°C, when CO₂ is injected at 0°C into milk with 0, 15% and 30% fat to achieve a total concentration of 1000 ppm, the predicted CO₂ concentration in the skim portion of the three milks would be approximately 1000, 1176, and 1425 ppm, respectively. Using the regression coefficients in Table 3, the expected pH of the three milks would be 6.51, 6.46, and 6.40, respectively. Therefore, at a CO₂ injection temperature of 0°C, even at the same total CO₂ concentration, the CO₂ concentration in the skim portion can be substantially different between milks with very different milk fat contents.

Because the antimicrobial effect of CO₂ is dependent on the concentration of CO₂ in the aqueous portion of

Figure 7. Change of freezing point (FP) in degrees Hortvet of 15% fat unhomogenized cream (●, ○), 15% vegetable oil emulsion in skim milk (◆, ◇), and 15% butter oil emulsion in skim milk (▲, △) after CO₂ injection at 0°C as a functional of total CO₂ content (ppm) of the cream. The solid and open symbols represent data from replication 1 and 2, respectively. The dotted line is the least square linear regression line.

a food (Fernandez et al., 1997; Devlieghere et al., 1998), and because the higher the CO₂ concentration, the greater the antimicrobial effect (King and Mabbitt, 1982 and 1987), the same total CO₂ content may be more effective in controlling microbial growth and extending product shelf life in a high fat dairy food than in a low fat dairy food when CO₂ is injected at low temperatures (i.e., ≤ 4°C). This could be of great practical importance in extending the refrigerated shelf life of creams.

CONCLUSIONS

When CO₂ was injected into milks and creams at 0°C, the amount of CO₂ dissolved in the skim portion was

Figure 8. pH (40°C) of unhomogenized 15% fat cream (●, ○), 15% vegetable oil emulsion in skim (◆, ◇), and 15% butter oil emulsion in skim milk (▲, △) after CO₂ injection at 40°C as a functional of total CO₂ content (ppm) of the cream. The solid and open symbols represent data from replication 1 and 2, respectively. The dotted line is the second order polynomial least square regression line.

dependent on the fat content and the melting characteristics of the fat. At the same total CO₂ concentration with a CO₂ injection temperature of 0°C, milk with a higher fat content had a lower pH and a lower FP than milk with a lower fat content. This indicated that with CO₂ injection at 0°C, when milk fat was mostly solid, very little CO₂ was dissolved in the fat portion and most of the total dissolved CO₂ was in the skim portion. At a CO₂ injection temperature of 0°C, the 15% vegetable oil emulsion had a slightly higher pH than the 15% butter oil emulsion and the 15% unhomogenized cream, indicating that the liquid vegetable oil dissolved more CO₂ than the mostly solid milk fat and butter oil. No effect of homogenization on pH and FP was observed in creams with 15% fat when CO₂ was injected at 0°C.

At a CO₂ injection temperature of 40°C, similar pH was observed in milk with 0%, 15%, and 30% fat and in the three 15% creams with different fat characteristics at the same total CO₂ concentration because CO₂ dissolved in both the fat and the skim portions. Injection of CO₂ into cream at a low temperature (i.e., ≤ 4°C) will result in a higher CO₂ concentration in the skim portion of the cream and thus a better antimicrobial effect during storage at the same total CO₂ concentration than if the CO₂ is injected at a higher temperature (i.e., ≥ 40°C).

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REFERENCES

- Association of Official Analytical Chemists, International. 2000. Official Methods of Analysis. 17th ed. AOAC, Arlington, VA.
- Brunner, J. R. 1978. Physical equilibria in milk: the lipid phase. Pages 474–602 in *Fundamentals of Dairy Chemistry*. 2nd ed. B. H. Webb, A. H. Johnson, and J. A. Alford ed. The AVI Publishing Company, Inc., Westport, CT.
- Buchheim, W. 1970. Der verlauf der fettkristallisation in den fettkügelchen der milch. elektronenmikroskopische untersuchungen mit hilfe der gefrierätztechnik. *Milchwissenschaft* 25:65–70.
- Chaplin, L. C., and R. L. J. Lyster. 1988. Effect of temperature on the pH of skim milk. *J. Dairy Res.* 55:277–280.
- Corredig, M., and D. G. Dalgleish. 1996. Effect of temperature and pH on the interactions of whey proteins with casein micelles in skim milk. *Food Res. Int.* 59:49–55.
- Devlieghere, F., J. Debevere, and J. van Impe. 1998. Concentration of carbon dioxide in the water-phase as a parameter to model the effect of a modified atmosphere on microorganisms. *Int. J. Food Microbiol.* 43:105–113.
- Dixon, B. 1963. The effect of temperature on the pH of dairy products. *Austra. J. Dairy Technol.* 18:141–144.
- Fernandez, P., S. M. George, C. C. Sills, and M. W. Peck. 1997. Predictive model of the effect of CO₂, pH, temperature and NaCl on the growth of *Listeria monocytogenes*. *Int. J. Food Microbiol.* 37:37–45.
- Fogg, P., and W. Gerrard. 1991. Solubility of carbon dioxide. Pages 241–264 in *Solubility of Gases in Liquids. A Critical Evaluation of Gas/Liquid Systems in Theory and Practice*. John Wiley & Sons, West Sussex, England.
- Fox, P. F., and P. L. H. McSweeney. 1998. Salts of milk. Pages 239–264 in *Dairy Chemistry and Biochemistry*. Blackie Academic & Professional, New York, NY.
- Gill, C. O. 1988. The solubility of carbon dioxide in meat. *Meat Sci.* 22:65–71.
- Glantz, S. A., and B. K. Slinker. 2001. Multicollinearity and what to do about it. Pages 185–187 in *Primer of Applied Regression & Analysis of Variance*. 2nd ed. McGraw-Hill, Inc. New York, NY.
- Hege, W. U., and H. G. Kessler. 1986. Deposit formation of protein containing dairy liquids. *Milchwissenschaft* 41:356–360.
- Hotchkiss, J. H., and E. Lee. 1996. Extending shelf-life of dairy products with dissolved carbon dioxide. *Eur. Dairy Mag.* 8(3):16, 18–19.
- Hotchkiss, J. H., J. H. Chen, and H. T. Lawless. 1999. Combined effects of carbon dioxide addition and barrier films on microbial

- and sensory changes in pasteurized milk. *J. Dairy Sci.* 82:690–695.
- King, J., and L. A. Mabbitt. 1982. Preservation of raw milk by the addition of carbon dioxide. *J. Dairy Res.* 49:439–447.
- King, J. S., and L. A. Mabbitt. 1987. The use of carbon dioxide for the preservation of milk. Pages 35–43 in *Preservatives in the Food, Pharmaceutical, and Environmental Industries*. Technical Series, Society for Applied Bacteriology. No. 22. Blackwell Scientific Publications, Boston, MA.
- Löwenadler, J., and U. Rönner. 1994. Determination of dissolved carbon dioxide by coulometric titration in modified atmosphere systems. *Lett. Appl. Microbiol.* 18:285–288.
- Ma, Y., D. M. Barbano, J. H. Hotchkiss, S. Murphy, and J. M. Lynch. 2001. Impact of CO₂ addition to milk on selected analytical testing methods. *J. Dairy Sci.* 84:1959–1968.
- Ma, Y., D. M. Barbano, and M. Santos. 2003. Effect of CO₂ addition to raw milk on proteolysis and lipolysis at 4°C. *J. Dairy Sci.* 85:1616–1631.
- Marshall, R. T. ed. 1993. *Standard Methods for the Examination of Dairy Products*. 16th ed. Am. Publ. Health Assoc., Inc., Washington, D.C.
- Metzger, L. E., and D. M. Barbano. 1996. Effect of fat content and protein type on milk fat globule size and protein adsorption produced by homogenization. *J. Dairy Sci.* 79(Suppl. 1):88.
- Packard, V., and R. Ginn. 1990. An evaluation of freezing point changes in raw milk analyzed by Dairy Quality Control Institute, Inc. over ten years, 1979–1988. *Dairy Food Environ. Sanit.* 10:347–351.
- SAS. 2001. Version 8.02 Edition. SAS Inst., Inc., Cary, NC.
- Singh, H., M. S. Roberts, P. A. Munro, and C. T. Teo. 1996. Acid-induced dissociation of casein micelles in milk: Effect of heat treatment. *J. Dairy Sci.* 79:1340–1346.
- Smith, E. B., D. M. Barbano, and J. M. Lynch. 1995. Infrared analysis of milk: Effect of homogenizer and optical filter selection on apparent homogenization efficiency and repeatability. *J. AOAC Int.* 78:1225–1233.