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Addition of Carbon Dioxide to Dairy Products to Improve Quality: A Comprehensive Review

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ABSTRACT: Changes in distribution patterns and demand for increased food quality have resulted in a desire to improve the shelf life of nonsterile dairy products. Refrigerated shelf life extension typically requires, at a minimum, reductions in the growth rate of spoilage microorganisms and subsequent product deterioration. Reducing initial bacterial loads, increasing pasteurization regimes, and reducing postprocessing contamination have all been employed with measured success. The use of antimicrobial additives has been discouraged primarily due to labeling requirements and perceived toxicity risks. Carbon dioxide (CO_2) is a naturally occurring milk component and inhibitory toward select dairy spoilage microorganisms; however, the precise mechanism is not fully understood. CO_2 addition through modified atmosphere packaging or direct injection as a cost-effective shelf life extension strategy is used commercially worldwide for some dairy products and is being considered for others as well. New CO_2 technologies are being developed for improvements in the shelf life, quality, and yield of a diversity of dairy products, including raw and pasteurized milk, cheeses, cottage cheese, yogurt, and fermented dairy beverages. Here we present a comprehensive review of past and present research related to quality improvement of such dairy products using CO_2.

Introduction

The shelf life of nonsterile dairy products, including pasteurized milk, cottage cheese, and some types of yogurt and fermented milk products, is generally limited to 1 to 3 wk (Labuza 1982; Salvador and Fiszman 2004), depending upon the quality of the raw ingredients, processing conditions, and postprocessing handling. Spoilage results primarily from the growth of organisms surviving pasteurization and/or postprocessing microbial contamination and degradative (proteolytic, lipolytic) enzymes surviving the high temperature short time (HTST) process (Gebre-Egziabher and others 1980). In the United States, HTST pasteurized milk has a shelf life of approximately 10 to 14 d (Cousin 1982; Schroder and others 1982). Ultrapasteurization (UP) processing can extend shelf life up to 90 d under refrigeration temperatures while ultra high temperature (UHT) processed products achieve a shelf life of 3 mo to 1 y with no refrigeration (Boor and Murphy 2002). Packaging milk using extended shelf life (ESL) equipment increases shelf life by a week or more. In North America, cottage cheese has been typically code dated for 21 to 28 d, although there often is considerable loss of quality during this period (Chen and Hotchkiss 1991a, 1991b). Dairy products such as cottage cheese and pasteurized milk also vary from day to day in initial quality, which affects the ultimate shelf life. For these and other reasons there is an interest in extending shelf life and reducing variability in the quality of dairy foods.

In addition to extending the shelf life of processed dairy products, there are advantages to reducing microbial growth rates in raw milk. The yield and quality of milk products, including cheeses, ice cream and yogurt mixes, cultured products, and related products, can be affected by the condition of raw milk (Ma and others 2000). Significant changes occur as a result of microbial growth in raw milk during transport and holding. The most detrimental result from the release of lipolytic and proteolytic enzymes is the breakdown of the protein structure of the micelles and/or hydrolysis of lipids (Espie and Madden 1997; Boor and Murphy 2002). These enzymes are not completely inactivated by HTST pasteurization and may be heat stable under HTST conditions (Gebre-Egziabher and others 1980) and active at

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refrigeration temperatures. For these reasons, potential improvements in yield and quality provide incentive to retard raw milk degradation even though the milk will be pasteurized prior to manufacture into other dairy foods.

Microbiological Effects of Carbon Dioxide

Milk and dairy products are excellent growth media for pathogenic and spoilage microorganisms, hence the major (but not only) mechanisms of the deterioration of dairy foods are directly or indirectly microbiological (Muir 1996a, 1996b, 1996c). The composition of most dairy products provides a favorable physical and chemical environment for the growth and propagation of a broad spectrum of microorganisms. Microbiological deterioration of refrigerated raw and pasteurized milk, cottage cheese, and similar products is often caused by the growth of psychrotrophic gram-negative bacteria species (*Pseudomonas, Acinetobacter, Flavobacterium, Enterobacter, Klebsiella, Aerobacter, Escherichia, Serratia, Proteus, Aeromonas,* and *Alcaligenes*), yeasts, and molds (*Geotrichum, Scopulariopsis, Mucor, Alternaria* and *Penicillium*) (Ternstrom and others 1993; Jay 2000; Boor and Murphy 2002; Chambers 2002), resulting in flavor, textural, and visual spoilage. In a study of pasteurized milk samples from 3 commercial dairy plants, Fromm and Boor (2004) identified the heat-resistant psychrotrophic gram-positive rods *Paenibacillus, Bacillus,* and *Microbacterium* as the predominant spoilage organisms. It has been estimated that 25% of all milk shelf life problems are due to thermoduric psychrotrophs, primarily *Bacillus* spp. (Ternstrom and others 1993; Sorhaug and Stepaniak 1997). These organisms produce extracellular protease and lipase activity, which reduces the functionality of milk proteins, and often produce undesirable aromas, many of which can be described as "fruity." Gram-positive organisms, particularly those producing lactic and acetic acids, can spoil dairy foods, but the numbers of organisms required are generally higher than for gram-negative bacteria and the changes can be less noticeable. The growth of heat-resistant lactic acid-producing cocci is responsible for the depression of pasteurized milk pH to the point where curdling occurs (Jay 2000).

Over the last 4 decades several investigators have demonstrated that adding CO_2 to the atmosphere surrounding a product reduces the rate of growth of many food spoilage and pathogenic microorganisms (Farber 1991; Hanlin and others 1995; Devlieghere and others 1998; Devlieghere and Debevere 2000). The largest inhibition occurs with gram-negative psychrotrophs, particularly *Pseudomonas* spp., and the least inhibition effect generally observed with gram-positive psychrotrophs, particularly *Lactobacillus* spp. (King and Nagel 1967, 1975; Molin 1983; Hendricks and Hotchkiss 1997). Factors such as species, substrate, and CO_2 concentration influence the effect on pathogenic psychrotrophs (Bennik and others 1995).

There are at least 3 general mechanisms by which CO_2 inhibits microorganisms. The 1st and simplest is by the displacement of O_2. The 2nd mechanism is a lowering of the pH in the medium or food due to the dissolution of CO_2 and formation of carbonic acid in the aqueous phase of the food in the following equilibrium (Butler 1982):

$$CO_2 \text{ (dissolved)} + H_2O \longleftrightarrow H_2CO_3$$

$$K_{hyd}(298\ ^\circ K, 1\ atm) = 2.6 \times 10^{-3} = \frac{[H_2CO_3]}{[CO_2]}$$

$$H_2CO_3 \longleftrightarrow HCO_3^- + H^+$$

$$K'_a(298\ ^\circ K, 1\ atm) = 4.3 \times 10^{-7} = \frac{[H^+][HCO_3^{-2}]}{[CO_2]}$$

$$HCO_3^{-2} \longleftrightarrow CO_3^- + H^+$$

$$K''_a(298\ ^\circ K, 1\ atm) = 5.61 \times 10^{-11} = \frac{[H^+][CO_3^{-2}]}{[CO_2]}$$

The 3rd mechanism is a direct effect on the metabolism of microorganisms as opposed to the indirect effects of pH reduction and displacement of O_2 (Daniels and others 1985).

Several reports on the effect of CO_2 on microbial growth and survival have appeared in recent years. The most common experimental design in defined media replaces some portion of the air surrounding the growth media with CO_2. Unfortunately, the media has not always been buffered to negate large shifts in pH due to CO_2 dissolution and formation of carbonic acid in the media, so it is unclear if the effect is simply due to a reduction in pH or if CO_2 has an inhibitory effect not associated with reduced pH. Furthermore, these experiments are often conducted in film pouches that allow permeation of both O_2 and CO_2 and the composition of the atmospheric changes over the course of the experiment. Microbial and/or fruit and vegetable respiration also contributes to the atmospheric changes. Thus, the relative importance of each of these factors in inhibiting growth or respiration may not be apparent due to several factors changing at the same time.

The effects of CO_2-modified atmospheres on the growth of *Pseudomonas fluorescens* and *Listeria monocytogenes* in highly buffered nutrient solution under either constant O_2 (20%) and varying concentrations of CO_2 (0 to 80%) or constant CO_2 (20%) and varying concentrations of O_2 (0 to 40%) (balance N_2) have been investigated. Bacterial suspensions were incubated at 7 °C under a continuous flowing atmosphere of each gas mixture in order to better understand the relative significance of pH, O_2 depletion, and direct effects of CO_2 on growth (Hendricks and Hotchkiss 1997). The results showed that CO_2 suppresses growth, even when the amount of O_2 in the atmosphere is held constant at 20% and the media does not change pH. This agrees with previous workers who concluded that CO_2 directly inhibits microbial growth in dairy products as opposed to the indirect effects of pH and O_2 displacement (King and Mabbitt 1982). Using empirical data and modeling, Devlieghere and others (1998) have concluded that the main variable controlling microbial growth in modified atmosphere packaging (MAP) is the dissolved CO_2 concentration.

While these experiments show that CO_2 has a direct effect on the metabolic processes of certain microorganisms, the mechanism through which this effect is manifested is not well characterized. There is evidence to support at least 3 mechanisms, including changes in membrane fluidity due to CO_2 dissolution (Sears and Eisenberg 1961), reductions in intracellular pH, and direct inhibition of metabolic pathways, including decarboxylation reactions and DNA replication (Dixon and Kell 1989; Hong and Pyun 2001).

Because CO_2 is highly soluble in hydrophobic materials such as lipids, it may be that the CO_2 concentrates in the lipid bacterial cell membrane, disrupting the physiochemical properties of the membrane. It may also be that the lipophilic nature of CO_2 allows it to pass through membranes and concentrate inside the cell, lowering intracellular pH. Intracellular CO_2 could stimulate "futile cycles"; carboxylation and decarboxylation reactions, which are common to all cells, could be stimulated without beneficial outcomes, resulting in a net energy expenditure and loss of ATP. Lastly, CO_2 may interfere directly with required enzymatic processes within cells, including gene expression (Stretton and others 1996; Stretton and Goodman 1998).

CO_2 Processing and Packaging Technology

Modified atmosphere packaging compared to direct addition of CO_2

One of several general approaches to extending the shelf life of refrigerated nonsterile food products is MAP technology (Farber and Dodds 1995). MAP is defined as the replacement of the headspace gas surrounding a food product with a gas mixture different from air. The objective of this technology is to slow the growth of spoilage microorganisms and/or inhibit senescence and respiration of fruits and vegetables. MAP has become widely practiced in food storage and distribution (Brody 1995). In addition to altering the gas composition surrounding the food, a barrier packaging material is often employed to retard the dissipation of the modified atmosphere through the package material. The shelf life of MAP products often directly correlates with the barrier properties of the package. Unfortunately, higher barrier materials are more costly and a cost-benefit trade-off must be determined. Surrounding a food with a gas mixture is an indirect method of adding the gas to the product due to solubilization of the gas in the water phase. This is particularly true for CO_2, which dissolves rapidly in foods and can create a vacuum inside of rigid packages containing high moisture foods (Parry 1993).

MAP of dairy products, including cottage cheese and fluid milk, has been reported to retard microbial growth (Kosikowski and Brown 1973; Mannheim and Soffer 1996) but requires substantial changes in the form of the traditional package. In most cases, packaged dairy foods do not have sufficient headspace to serve as a reservoir for the active gases (for example, CO_2) and insufficient CO_2 may be available to retard microbial growth. In the case of dairy products, MAP may not provide sufficient control and the shelf life of the product may be inconsistent (Moir and others 1993). However, flushing packages with CO_2 before sealing is commonly used to inhibit mold growth in certain cheeses (Farkye and Vedamuthu 2002).

The direct injection of 5.68 to 22.7 mM CO_2[1] directly into products coupled with high barrier packaging has been developed as a method to inhibit undesirable microorganisms in dairy products and thus extend shelf life (Chen and Hotchkiss 1991a, 1991b). Liquefied or compressed CO_2 gas can be incorporated directly into a flowing stream of product via a gas-sparging unit, a process commercially practiced in several areas of the world. The device that is most often employed consists of a sintered stainless steel frit with porosity in the range of 7 to 30 μm. The process has been termed "direct addition of carbon dioxide" in order to distinguish it from conventional MAP. The net effect is similar to MAP; the gas is added to the product for the purpose of increasing shelf life by inhibiting microbial activity. The cost of the addition of CO_2 to dairy foods via this method is generally economically feasible, and the incorporation of CO_2 typically occurs within the normal stream of product in a production system. Only a minimal one-time investment is required for equipment, and the cost of CO_2 gas is low; the most significant and recurring cost involved is in barrier packaging.

Several authors have pointed out that in extending shelf life, atmospheric CO_2 first dissolves in the undissociated form into the liquid phase of the product before inhibiting respiratory and microbial systems (Barnett and others 1971; Daniels and others 1985). Thus, CO_2 in the atmosphere in MAP is not the effective agent *per se* in the inhibition of microorganisms. The CO_2 must first dissolve into the product and eventually into microbial cells. The amount of CO_2 dissolved in water is governed by the partial pressure of the CO_2 above the water as well as the amount of CO_2 available, which is determined by both the volume of the headspace and the concentration of CO_2 in that headspace.

Rather than rely on an equilibrium being established between the headspace in a package and the product, it has been suggested that the direct addition of CO_2 into products may result in improved microbial control by ensuring a constant low concentration of dissolved CO_2 (Gorski 1996). Henry's law illustrates that as the aqueous concentration of CO_2 increases, the partial pressure of CO_2 (pCO_2) increases accordingly at a fixed temperature. If the temperature of the product is controlled, the concentration of CO_2 within the aqueous liquid will remain constant, assuming a closed system and no loss of CO_2. This process has advantages over conventional MAP in that no headspace is required and the amount of dissolved CO_2 can be carefully controlled.

Fluid whole or reduced fat milk comprises both hydrophilic aqueous and hydrophobic fat portions. Both the temperature at which direct CO_2 injection occurs into milk and the milk fat content influence the degree to which CO_2 is dissolved in the skim portion of the milk. Ma and Barbano (2003a) looked at freezing point and pH of milk with different fat contents in response to CO_2 injection at 0 and 40 °C. Their data showed that at the low injection temperature the CO_2 content in the skim portion can be very different between milks having different fat contents. Data also indicated that CO_2 injection into milk at low temperatures results in more gas dissolved in the skim portion of the cream, suggesting that the antimicrobial effect of CO_2 would be maximized as more of the gas is available in the aqueous phase.

Effect of packaging materials

One of the most important factors affecting the use of direct addition of CO_2 to dairy products has been the lack of sufficient barrier in the packaging materials. There is little benefit to adding CO_2 to a product if the gas is allowed to dissipate. Packaging

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[1]mM concentration can be converted into mg/kg (parts per million, ppm) by multiplying the concentration (mM) by 44 (mg per millimole).

The concentration of CO_2 in carbonated soft drinks is often given in "volumes." Volumes are the ratio of volume of CO_2 gas at atmospheric pressure to volume of beverage at 15.6 °C (60 °F). Thus, a beverage containing 1 L of CO_2 (at 1 atmosphere) dissolved in 1 L of beverage at 15.6 °C would be carbonated to 1 volume (Woodroof and Phillips 1981). The amount of CO_2 (in moles or mg) contained in 1 L of water held at 60 °F (15.5 °C, 288.5 °K) at a pressure 1 atmosphere (i.e., held under pure CO_2 at 1 atmosphere absolute pressure) can be calculated using the gas laws as follows:

$$PV = nRT \text{ or } n/V = P/RT,$$

where n = moles, V = volume in liters, P = absolute pressure in atmospheres, R = gas constant = 0.0821 (L.atm per deg.mole), T = temperature Kelvin.

For mmol CO_2/L, the equation is multiplied by 44 g/mole and 1000 mg/g and reduces to

$$\text{mg } CO_2\ L^{-1} = 44{,}000 \times P/0.0821 \times T.$$

Thus, the mg of CO_2 in 1 L of water at 60 °F and 1 atmosphere = 44000/0.0821 × 288.5 = 1,858 mg CO_2 L^{-1}. At 40 °F (4.4 °C, 277.4 °F) and 1 atmosphere absolute pressure, 1 L of water will contain 44000/0.0821 × 277.4 = 1932 mg CO_2. This represents 1.04 volumes (1932/1858).

Conversely, water containing 2,500 mg CO_2 L^{-1} represents 1.34 volumes of CO_2.

Alternatively, CO_2 concentration can be estimated based on Henry's law (Butler 1982) as follows:

$$[CO_2]aq = K_H pCO_2$$

where K_H = Henry's law constant in mol L per atm and pCO_2 = partial pressure of CO_2 in atm. At 60 °F (15.5 °C) $K_H = 10^{-1.33}$ in moles. In mmoles $K_H = 10^{1.67}$.

Thus,

$$[CO_2]\text{aq} = pCO_2 \times 46.8 \text{ mmol.} \times 44 \text{ mg/mol} = pCO_2 \times 2058 = \text{mg } CO_2\ L^{-1}.$$

Henry's law gives somewhat higher estimates of $[CO_2]$ because it assumes a pH of < 5.

is the principal means of preserving the original concentration of CO_2 within the product. CO_2 has been found to decrease rapidly during storage when dissolved in cottage cheese samples and packaged in conventional polystyrene plastic tubs (Moir and others 1993). The conventional polyolefin tubs used in cottage cheese packaging are highly CO_2 permeable and the simple friction closure offers little resistance to outgassing. The CO_2 is lost through the gaps between the cover and the tub since the container is not airtight. A solution to this problem is to add a high CO_2 barrier foil/polyolefin laminant seal over the opening of a high CO_2 barrier tub or shrink-wrapping the standard polystyrene container with a high barrier film (Gorski 1996). Another solution is to package the cottage cheese in high CO_2 barrier film pouches. This could also facilitate disposal, especially at an institutional level.

Lee and Hotchkiss (1997) studied the increase in standard plate counts in CO_2-modified cottage cheese packaged in 2.3 kg high barrier polymer bags. As expected, the combination of the high barrier bags and the addition of CO_2 to the product reduced microbial growth rate and increased shelf life. CO_2 concentration did not decrease from initial levels over 29 d of storage, suggesting that the combination of temperature (4 °C) and high barrier material was successful at maintaining the residual CO_2 levels.

Safety issues

The safety risks associated with extending the shelf life of a nonsterile food must be understood. With refrigerated products, including MAP or CO_2-treated dairy foods, the major risk is that the increased shelf life will allow development of slow-growing pathogenic microorganisms that would not be manifest in products with shorter shelf lives; an additionally important risk is that certain pathogens will be stimulated. Understanding the effects of CO_2 on pathogenic psychrotrophs such as *L. monocytogenes* is of particular importance. CO_2 has the added concern that it could, in theory, enhance the outgrowth (germination) of pathogenic spore-forming organisms such as *Bacillus cereus* and *Clostridum botulinum* (Dixon and others 1988).

We have investigated the effect of added CO_2 on *B. cereus* and *C. botulinum* growth and toxigenesis in milk (Glass and others 1999; Werner and Hotchkiss 2002). While CO_2 at levels of <20 mM inhibits the growth of selected spoilage organisms and extends refrigerated shelf life, CO_2 could influence the risk of botulism from milk. In the latter study (Glass and others 1999), pasteurized 2% fat milk was modified with approximately 0, 9.1, or 18.2 mM CO_2 and inoculated with a 10-strain mixture of proteolytic and nonproteolytic *C. botulinum* spore strains to yield 10^1 to 10^2 spores mL^{-1}. The milk was stored at 6.1 °C for 60 d or 21 °C for 6 d in sealed glass jars or high-density polyethylene (HDPE) plastic bottles. Milk stored at 21 °C curdled and exhibited a yogurt-like odor at 2 d and was putrid at 4 d. Botulin was detected in milk containing 9.1 mM CO_2 after 4 d and in all treatments after 6 d of storage at 21 °C. All toxic samples were grossly spoiled based on visual evaluation at the time the toxin was detected. Although botulin appeared earlier in milk treated with 9.1 mM CO_2 compared to both the 18.2 mM and untreated milk, gross spoilage would act as a deterrent to consumption of toxic milk. No botulin was detected in any treatment stored at 6.1 °C for 60 d. At 6.1 °C, the standard plate counts (SPC) were generally lower in the CO_2-treated samples than in controls, with 18.2 mM CO_2 milk having the lowest SPC. These data indicate that the low-level addition of CO_2 retards spoilage of pasteurized milk at refrigeration temperatures and does not increase the risk of botulism from treated milk stored at refrigeration or abuse temperatures.

We have conducted similar studies of the effects of 11.9 mM CO_2 on the growth of *B. cereus* spores inoculated into sterile homogenized whole milk at 10^1 and 10^6 spores mL^{-1} and stored at 6.1 °C for 35 d (Werner and Hotchkiss 2002). *B. cereus* counts from CO_2-treated and control milk both decreased over 35 d. There was no consistency as to whether the control or test milk was higher in counts. Added CO_2 reduced the pH of the milk from an average value of 6.61 to an average value of 6.31; however, this drop did not correlate with changes in any other parameter measured. The data indicated that moderate levels of CO_2 neither enhance the outgrowth of *B. cereus* over long-term storage nor increase the risk of foodborne illness due to the organism.

The effects of CO_2 on growth of *L. monocytogenes* and *C. sporogenes* in cottage cheese have also been investigated (Chen and Hotchkiss 1993). *C. sporogenes* did not grow under any conditions tested while *L. monocytogenes* grew slowly in control cheese at 4 and 7 °C. The addition of CO_2 resulted in a slight inhibition of growth of *L. monocytogenes*. Other workers have shown that the addition of CO_2 does not promote growth and is likely to cause a small but significant inhibition. For example, Fedio and others (1994) found that CO_2 inhibited growth of *L. monocytogenes* in cottage cheese.

To our knowledge, no data to date indicate the use of CO_2 to extend the keeping quality of dairy products increases the risks from pathogenic microorganisms. The data generated from investigations of *L. monocytogenes* suggest that CO_2 treatment may result in enhanced safety for this pathogen, which has been the causative agent in human disease in which dairy foods were the vehicle.

Carbon Dioxide as a Natural Ingredient of Raw Milk

It has been known for over 100 y that milk as drawn from animals contains significant amounts of dissolved CO_2. However, early quantitative data must be viewed with caution, as analytical methods for dissolved gases were less reliable than those more currently employed. Early researchers were interested in how CO_2 affects processing (Noll and Supplee 1941), collection (Marshall 1902; Jackson 1936), freezing point (Moore and others 1961; Smith 1964), and the ability to distinguish pasteurized from raw milk (Van Slyke and Baker 1919; Van Slyke and Keeler 1920; Frayer 1941).

Van Slyke and Baker (1919) suggested that a third of the CO_2 in milk exists as carbonic acid and two-thirds as bicarbonate. However, modern physical chemistry shows that the fraction of carbonate, bicarbonate, and CO_2 ions varies in solution as a function of pH (Daniels and others 1985). In milk at pH 6.3 to 6.5, approximately 88% of CO_2 exists as dissolved CO_2 gas, 2% as carbonic acid, and the remaining 10% as bicarbonates. At 15 °C, concentrations of CO_2 added to raw milk in the range of 0.4 to 33.6 mM lowered the pH from 6.80 to approximately 6.1 (Martin 2002). In autoclaved raw milk similarly amended at lower temperatures (4 °C) with 0 to 35 mM CO_2, the pH was lowered from 6.70 to 5.9 (Loss 2001).

The CO_2 content of raw milk decreases when milk is exposed to air after milking while O_2 and N_2 levels increase (Marshall 1902). The loss in CO_2 results from gradual equilibration of the milk gas content with that of air, which has a lower atmospheric pCO_2. Jackson (1936) suggested that "anaerobically drawn" milk contained 11.1 to 12.5 mM CO_2. Noll and Supplee (1941) analyzed 63 samples of mixed raw milk as received at a commercial milk plant, reporting the CO_2 level to be 2.0 mM. This agreed with Frayer (1941) who showed that, prior to receipt at the processing plant, there is a significant loss in CO_2 from milk. Noll and Supplee (1941) showed that CO_2 levels are significantly reduced in several processing steps. Our limited analyses for CO_2 content of raw milk from a single local herd using modern methodology

averaged 5.5 mM, with a range of 3.9 to 7.5 mM ($n = 10$ animals) (Lee 1996).

CO_2 losses occur during the pasteurization treatment in response to temperature and pressure changes during processing. Smith (1963) found a drop in the CO_2 content from 1.6 mM in raw to 0.68 mM in processed milk. The CO_2 loss was attributed to the aeration of milk during pumping and nonhermetic storage of milk. Further significant decreases in CO_2 were observed for vacuum-treated and pasteurized milk. Moore and others (1961) reported an average loss of CO_2 from milk after pasteurization of 72% when a single vacuum chamber was placed after the raw regeneration loop.

Applications of CO_2 Addition to Dairy Products

Raw (unpasteurized) milk

Introduction. Reports on the use of CO_2 at elevated pressures as an antimicrobial agent in milk date back to the turn of the 20th century. Hoffman (1906) reported that the addition of 50 atmospheres[2] of CO_2 reduced the rate of increase in the microbial counts in milk. While untreated milk curdled within 24 h at room temperature, milk kept under 10 atm of CO_2 was not observed to curdle, even after 72 h. Van Slyke and Bosworth (1907) observed that elevated CO_2 pressures delayed lactic fermentation. They suggested that the best results in the preservation of milk were secured when newly pasteurized milk or "cleanly drawn" raw milk was treated with CO_2 in tanks such as is used in bottling establishments to prepare carbonated drinks. Similar discoveries regarding the relationship between pCO_2 and microbial activity in raw milk were reported by other laboratories in the early part of the century (Prucha and others 1922; Donald and others 1924; Valley and Rettger 1927).

Storage and transport. More recent investigations have documented the chemical and microbiological effects of low levels of added CO_2 in raw milk (Skudra 1983). In a series of pioneering studies, King and Mabbitt (1982), Mabbitt (1982), and Law and Mabbitt (1983) added 10 to 40 mM CO_2 to untreated whole and bacteria-inoculated sterilized skim milk stored at 4, 7, and 10 °C for up to 6 d. Increasing CO_2 concentrations and decreasing temperatures were shown to inhibit microbial growth rates; these effects were greatest when the 2 parameters were manipulated together. The enhanced effects were most likely due to the combination of reduced growth rate and increased solubility of CO_2 as the temperature is lowered. The lower the initial counts in the untreated milk and the lower the holding temperature, the greater the effect. They also demonstrated that the initial microbial quality of the raw milk influences the effect of CO_2. The difference between SPC in <4 log cfu mL^{-1} milk treated with CO_2 and the same milk left untreated was as much as 3 log units after 6 d, while the difference between >5 log cfu mL^{-1} milk treated with CO_2 and controls was less than 1 log cycle (Mabbitt and King 1982). Milk with an initial count >5 log cfu mL^{-1} held at 10 °C demonstrated only a small benefit from CO_2. Addition of 20 to 30 mM of CO_2 to milk collected from tankers unloading farm milk had approximately 3 log cfu mL^{-1} fewer counts than untreated milk after 4 d storage at 7 °C. Milk acidified with HCl to the same pH as the CO_2-treated milk failed to demonstrate the same degree of microbial inhibition. King and Mabbitt (1982) concluded that the effect was directly due to the presence of CO_2, not due to lowering the pH or to the displacement of oxygen. These data indicate that greater benefit from the addition of CO_2 is gained in a high-quality product as compared to a poor-quality product.

Roberts and Torrey (1988) inoculated sterile milk with several common proteolytic psychrotrophic bacteria isolated from milk and investigated the effects of 20 to 30 mM CO_2 on growth at 7 °C. They found that lag time increased and exponential growth rate decreased in the presence of increasing dissolved CO_2 for both inoculated and uninoculated raw milk. There was no evidence that CO_2 increased the growth of anaerobic and facultative organisms, including spore formers. They concluded that refrigerated storage of raw milk could be extended 1 to 3 d by the addition of low amounts of CO_2. Amigo and others (1995) investigated the effects of "acidification" of inoculated, sterilized, and raw milk as well as the effects on sensory properties; CO_2 was used to reduce an initial milk pH of 6.7 to 6.2 and 6.0. Unfortunately, the concentration of CO_2 required for these reductions in milk pH was not determined, making it difficult to directly compare results with other studies. CO_2 treatment increased generation times and decreased growth rates for several *Pseudomonas* spp. Sensory evaluation of degassed and pasteurized milk resulted in no detectable differences between treated and untreated samples. Samples that were not degassed scored significantly lower than controls, perhaps due to the tactile sensations associated with higher levels of dissolved CO_2.

Espie and Madden (1997) reported the effects of 30 and 45 mM CO_2 on the indigenous microbial populations in raw milk stored at 6 °C for up to 7 d. Sample analysis included enumeration of SPC, coliforms, psychrotrophic count, and lactobacillus. All with the exception of lactobacillus demonstrated inhibition with the addition of CO_2. The authors concluded that an extension in keeping quality could be achieved by the addition of CO_2 to the raw milk.

Martin and others (2003) examined the effects of 0.6 to 61.4 mM CO_2 on bacterial growth in both raw and inoculated sterile milks during storage at 15 °C and found that these concentrations significantly inhibited the growth of raw milk bacteria. SPC of natural populations in raw milk and populations of *Pseudomonas fluorescens*, *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Enterococcus faecalis*, and *Bacillus licheniformis* in inoculated sterile milk were examined. For raw milk SPC, as CO_2 concentrations increased, the time to maximum growth and lag time increased while the growth rate decreased. For each specific microorganism studied, CO_2 reduced the growth rate, with a greater effect toward gram-negative than toward gram-positive bacteria. The lag time for *P. fluorescens* incubated with 0.4 mM CO_2 was 3.3 h compared to 26.1 h with 46.3 mM CO_2. Similar effects were noted for *L. monocytogenes*. For *B. cereus*, slight decreases in growth rate and no change in lag time were noted with increasing CO_2 concentration; the growth rate of *B. licheniformis* did not change while the lag time increased. These results show that, even at above-refrigeration temperatures, CO_2 can reduce the growth of milk pathogens and spoilage organisms.

Rajagopal and others (2005) examined microbial growth in aged and fresh raw milks from a single herd after treatment with 68 to 689 kPa CO_2 at 5, 6.1, 10, and 20 °C, and storage for up to 9 d, parameters that did not result in protein precipitation. All treatments significantly reduced raw milk SPC, even those incorporating above-refrigeration temperatures. At the highest CO_2 pressure, a reduction in SPC, total gram-negative bacteria, and lactobacillus were measured at the end of storage. At 6.1 °C, the time to reach 4.30 log cfu mL^{-1} increased by 4 d as compared to the untreated control. Coliform levels remained unchanged in these treated samples while levels doubled in the control milk. The level of thermoduric bacteria was significantly lower after 9 d in the treated milk than in the control milk. In the United States, the Pasteurized Milk Ordinance (PMO) Grade A regulations specify an upper SPC limit for raw milk prior to pasteurization of 5 log cfu mL^{-1} (U.S. Department of Health and Human Services 1999); at 6.1 °C, this limit was reached in the control milk before 4 d of

[2] 1 atm = 14.7 psi = 1.01 Pa = 1.01 × 10^{-6} mPa = 760 mm Hg = 760 Torr.

storage while this limit was not reached in 689 kPa CO_2-treated milk until day 8. These data suggest that pressurized CO_2 might be an effective method of preserving raw bulk milk, adding to storage shelf life and overall milk quality.

A scaled-up field application trial of this work by our research group (unpublished data) supports these laboratory data, by preliminarily showing that application to 18,900 L raw bulk milk of 45 mM CO_2 under pressures of 138 to 345 kPa can significantly extend storage time. Milk was stored in a stainless steel liquid bulk tank used for rail shipment under ambient (20 to 25 °C) conditions outdoors. The temperature of the milk loaded was initially 2 °C, which slowly increased to about 10 °C by day 14 of storage. Standard plate count analysis shows that the treated milk did not reach the PMO quality limit until day 14, 4 d longer than that for the control milk, suggesting that moderate CO_2 pressures can be an effective method of storing bulk raw milk and extending possible transport time.

Processing. Calvo and De Rafael (1995) suggested that CO_2 should be removed prior to pasteurization to minimize buildup of deposits on the walls of the pasteurizer. Beaulieu and others (1999) showed that in a model milk system, increasing soluble protein content and decreasing pH increases aggregation of caseins under HTST pasteurization temperatures, a condition that could result in the fouling of heat processing equipment. The pH of milk treated with 0 to 54 mM CO_2 during pasteurization was found to decrease in response to increases in pressure and in CO_2 concentration; at a fixed CO_2 concentration, the effect of pressure on decreasing milk pH was greater at higher temperature treatments, while at a fixed temperature, the effect of pressure on decreasing milk pH was greater at higher CO_2 treatment concentrations (Ma and Barbano 2003c). Ma and others (2001) found that pH depression caused by modification of milk with up to 23 mM of CO_2 could be reversed by vacuum removal of CO_2. At 80 °C and 345 kPa pressure, the pH of 55 mM CO_2-modified milk can be as low as 5.63; thus, pasteurization temperatures and pressures as well as the initial CO_2 content of milk are important factors to regulate to prevent milk degradation during pasteurization (Ma and Barbano 2003c). If these factors are modulated, CO_2 may be used as a processing aid during pasteurization to increase microbial kill. Loss (2001) showed that increasing concentrations of dissolved CO_2 in raw milk between 1 and 36 mM linearily decreased the decimal reduction time at 50 °C (D_{50} values) for *P. fluorescens*, and CO_2 concentrations of 44 to 58 mM significantly reduced the *z* value for SPC (63 to 93 °C). A more comprehensive review of the bactericidal effects of dissolved CO_2 during pasteurization has been prepared by Loss and Hotchkiss (2003).

If left in the milk postpasteurization, 23 mM CO_2 was not found to significantly impact antibiotic residue, freezing point, infrared milk component, or alkaline phosphatase tests, important analysis used in the United States to determine antibiotic contamination, water adulteration, protein/fat/lactose content, and effectiveness of pasteurization in reducing microbial load (respectively) of fluid milk (Ma and others 2001). Ruas-Madiedo and others (1996) reported the results of a pilot-scale study in which sufficient CO_2 was added to 200-L batches of raw milk to lower the pH to 6.0 or 6.2 (CO_2 concentrations not reported). The milk was held at 4 °C for 4 d, vacuum treated to remove residual CO_2, and pasteurized. The milk samples were evaluated organoleptically, microbiologically, and chemically. Neither caseins nor whey proteins were affected by the combined treatment of CO_2 addition, vacuuming, and pasteurization. Generally, the organic acid content of the milk was not different, with the exception of lactic acid, which was slightly lower in CO_2-treated milk. The volatile organic compound concentration of the treated product was lower, presumably because of lower microbial activity. No significant differences in sensory properties were detected. The only major difference was that the CO_2-treated milk had lower coliform, psychrotrophic, proteolytic psychrotrophic, and lipolytic psychrotrophic counts compared to untreated raw milk after 4 d of storage. The authors concluded that CO_2 could be added to raw milk to inhibit microbiological deterioration during storage and easily removed during processing without detrimental effects. Later, this same group and others reported that the additional shelf life gained by the addition of CO_2 did not affect fat- or water-soluble vitamin (Ruas-Madiedo, Bada, and others 1998a, 1998b) or free monosaccharide (Ruas-Madiedo and others 2000) content of raw milk.

The effects of direct addition of CO_2 to raw milk on milk quality after CO_2 removal and pasteurization have been recently investigated (Thongoupakarn 2001). Carbon dioxide (14.8 to 22.7 mM) was added to raw milk, which was held at 4 °C for up to 10 d before CO_2 removal and subsequent HTST pasteurization and storage at 6 °C for 30 d in HDPE plastic bottles. Raw and pasteurized milks were assayed for SPC, gram-negative psychrotroph counts (Gm–), proteolysis, lipolysis, and pH. The percentage of casein nitrogen over total nitrogen (CN/TN) was used as an index for proteolysis, while acid degree value (ADV) was used as an index for lipolysis.

CO_2-treated raw milk had lower microbial counts prior to pasteurization, exhibiting lower growth rate and longer lag phase after pasteurization than non-CO_2-treated milk. The degree of proteolysis and lipolysis of pasteurized milk was also reduced by CO_2 addition. The differences in ADV and casein content of CO_2-treated pasteurized milk as compared to non-CO_2-treated pasteurized milk were greatest for raw milk, which had reached SPC values of >6 log cfu mL^{-1} prior to pasteurization (those stored raw for 10 d). The time to reach SPC of 6 log cfu mL^{-1} postpasteurization was also affected by CO_2 treatment. Similarly, Ma and others (2003) showed that raw milk stored under 34 mM CO_2 at 4 °C resulted in reduced growth of milk bacteria and subsequent reduced overall proteolysis and lipolysis.

It is clear that the addition of CO_2 to milk retards the growth of selected psychrotrophic gram-negative organisms as well as the deterioration of raw milk stored under refrigeration. Moreover, inhibition of microorganisms in raw milk improves the overall quality of pasteurized milk. While the use of CO_2 in refrigerated raw milk has been investigated, less work has focused on the potential effects of CO_2 on raw milk held at temperatures above normal refrigeration temperatures (7 to 10 °C) or under changing temperature conditions experienced during bulk transport. While the inhibitory effects of CO_2 are diminished as the temperature increases, relatively small reductions in growth rates could be important for raw milk that is not adequately refrigerated. CO_2 addition could be a low-cost means for improving milk quality in regions where low-temperature refrigeration is inadequate. Rashed and others (1986) reported that CO_2 had little effect on raw milk held at 20 °C compared to storage at 7 °C. However, the initial bacterial counts in the raw milk were high (approximately 10^6 cfu mL^{-1}) and it is possible that lower initial counts would have resulted in a significant difference in growth rates.

CO_2 removal. CO_2 removal from raw milk immediately prior to pasteurization is feasible by applying vacuum treatment. Such equipment is commercially available; for example, the Feldmeier Aro-Vac (Syracuse, N.Y.), to remove off-flavors or to deaerate milk. Moore and others (1961) used commercially available nonsteam flavor removal equipment to degas CO_2-treated raw milk prior to HTST pasteurization. Ruas-Madiedo, Bada-Gancedo, and others (1996) constructed a pilot-scale pasteurizer and vacuum degassifier system to remove CO_2 prior to subsequent HTST pasteurization. Raw milk was modified with CO_2 (quantities not reported) to reduce the milk pH to levels between 5.9 and 6.3.

CO_2- treated raw milk was first heated to 55 to 60 °C in a plate-pasteurizer, pumped into a secondary tank where a 300-mmHg vacuum was applied, pumped into another plate pasteurizer for a HTST treatment of 72 °C/15 sec, and finally cooled to 38 °C. Gevaudan and others (1996) applied 5.8 mmHg vacuum at room temperature to remove CO_2 from milk that had been similarly acidified. In the Bada-Gancedo and others (1996) study, analysis of the milk before and after pasteurization showed little difference in terms of sensory and biochemical properties. Similarly, results obtained from by Amigo and others (1995) showed that sensory properties of CO_2-treated milk after degasification and pasteurization were no different than for untreated pasteurized milk. Recent research reports laboratory-scale vacuum treatments combined with mild heating for the effective removal of CO_2 from raw milk (Ma and others 2001; Santos and others 2003; Rajagopal and others 2005).

Other methods have been used to successfully degasify CO_2-treated raw milk prior to pasteurization. In an early study (Noll and Supplee 1941), it was found that gas flushing, vacuum treatment, or the treatments in combination were effective in removing low levels of CO_2. More recently, Thongoupakarn (2002) used a flowing stream of nitrogen gas immediately prior to HTST pasteurization to reduce CO_2 levels from 14 to 19 mM to 1 to 2 mM. Rajagopal and others (2005) used a combination of depressurization and mild temperatures (30 to 35 °C) to degas CO_2 amended milks.

Pasteurized milk

The feasibility of using direct addition of CO_2 to pasteurized milk for shelf life extension has not been extensively investigated, probably due to the assumption that added CO_2 would detrimentally affect the organoleptic quality of milk (King and Mabbitt 1982). However, preliminary work has suggested that the levels of CO_2 below the organoleptic threshold are inhibitory for selected microbial growth (Shipe and others 1982; Duthie 1985; Duthie and others 1985). Carbon dioxide levels of 1.81 to 3.18 mM in full-fat pasteurized milk stored in paperboard cartons at 6 °C for up to 14 d improved keeping quality. Trained sensory panelists found no difference between the control (no CO_2) and CO_2-treated milk prior to 14 d. However, at day 14 the highest CO_2-treated samples scored significantly higher than untreated milk. Psychrotrophic and total bacterial counts were similarly lower in treated samples. Control milk coagulated during testing while the treated samples did not. The sensory threshold for CO_2 in this work was 740 mg L^{-1}, which was above the highest CO_2 level tested. These preliminary data suggested that the addition of CO_2 to pasteurized milk could significantly improve keeping quality (Duthie and others 1985).

The use of CO_2 in pasteurized milk has been investigated in more detail. Work with inoculated milk packaged in pouches with different CO_2 barrier properties showed that the addition of low levels of CO_2 inhibits the growth of psychrotrophic microorganisms and provides a moderate extension of shelf life (Chen and others 1992; Hotchkiss and others 1998). Lag-phase extension, growth rate reduction, and maximum bacterial counts in 0 to 21.5 mM CO_2-treated inoculated whole milk increased directly with increasing CO_2 content. The inhibitory effect of CO_2 was greater at 4 °C than at 7 °C.

Cheeses

CO_2 and storage. Hard and semi-hard cheeses, such as cheddar, are commonly packed in 100% CO_2 or mixtures of CO_2–N_2 using horizontal form-fill-seal (FFS) pouch-pack equipment. MAP cheese packed in polypropylene film has a shelf life of up to 4 wk, compared to only 14 to 15 d when packaged under normal conditions. The major effect of CO_2 on these cheeses is the inhibition of surface mold growth (Maniar and others 1994), although high CO_2 MAP atmospheres have been shown to inhibit growth of lactic and mesophilic bacteria as well as that of molds and yeasts on shredded mozzarella cheese (Eliot and others 1998). CO_2 acts both directly on molds and by indirectly displacing O_2; molds have an absolute requirement for O_2. Vacuum packaging does not remove all of the O_2 and thus mold and yeast growth can still occur (Hocking and Faedo 1992), particularly in regions of the food product–packaging interface where package wrinkling occurs. MAP with reduced O_2 combined with increased CO_2 concentration will allow mold growth but at a substantially reduced rate, thus extending shelf life. CO_2 also is absorbed into the cheese and creates a vacuum within the pouch. Sliced and grated cheeses can be pillow-packed under MAP (Fierheller 1991). The gas mixture typically used is 70% N_2:30% CO_2 to inhibit mold growth, to keep the package from collapsing around the shreds, and to prevent shred matting (Parry 1993). In this case, the N_2 acts as filler to prevent package collapse and formation of a vacuum as the CO_2 is absorbed. Alves and others (1996) have compared 100% N_2 and 100% CO_2 with 50% N_2:50% CO_2 for packaging sliced mozzarella cheese in high-barrier laminated films. They reported that atmospheres of $\geq$50% CO_2 were more effective than air or 100% N_2 in improving shelf life of sliced mozzarella cheese. Atmospheres of 100% N_2 had only a minor effect on sensory shelf life but atmospheres of 100% CO_2 increased shelf life by 385%. Molds, yeast, and psychrotrophic bacteria were all inhibited by the 100% CO_2. Eliot and others (1998) found similar benefits of CO_2 in shredded mozzarella cheese. Gonzalez-Fandos and others (2000) recently demonstrated that packaging in 50% CO_2:50% N_2 or 40% CO_2:60% N_2 effectively inhibited undesirable chemical and microbiological changes in cheese and extended shelf life. Juric and others (2003), however, found that packaging atmospheres of 100% CO_2 resulted in undesirable changes in texture and flavor of sliced Samso cheese stored under light. Elevated CO_2 packaged cheese became dry and crumbly, and developed off-flavors, colors, and aromas due to increased photooxidation.

CO_2 and cheese manufacture. The effect of CO_2 treatment of raw milk intended for manufacturing cheese has been investigated. Calvo and others (1993) found that acidification of raw milk with CO_2 to pH between 6.0 and 6.5 reduced psychrotrophic bacteria counts, resulting in improved cheese yields. However, the differences were small and the initial microbial counts were in the range of 10^5 to 10^7 cfu mL^{-1} in the controls, making it unclear if similar results would been seen with lower initial counts. Other studies (Ruas-Madiedo, Alonso, and others 1998; Ruas-Madiedo, Bada Gancedo, and others 1998) looked at milk of lower microbial load and found that cheese yields from CO_2-treated and -untreated stored milk did not differ significantly. In poor quality milk, however, yield of the control milk was significantly less than yield achieved in the CO_2-treated milk. In this study, CO_2 was removed prior to cheese making, and the cheese was acid coagulated. McCarney and others (1995) have also investigated the effects of CO_2 addition to milk used to make cheese. They concluded that the addition of 30 mM of CO_2 reduced the time to reach psychrotrophic counts of 10^6 cfu mL^{-1} and that this in turn improved grading scores. The cheese made from CO_2-treated milk showed fewer products of casein and lipid breakdown, presumably due to reduced proteolytic and lipolytic activity. Montilla and others (1995) showed a 75% reduction in the amount of rennet necessary for coagulation along with a small reduction in proteolysis in cheeses made with CO_2-treated milk. The effect of CO_2 on cheese yield was not clear from the data. There was no significant difference in the organoleptic properties of the cheeses. The authors suggested that use of CO_2-treated

milk would not have detrimental effects on cheese properties or yield and would extend the keeping quality of the raw milk.

In a later study, Ruas-Madiedo and others (2002) examined the effect of CO_2 addition to raw milk on the manufacture of rennet-coagulated Spanish hard cheeses, both made from pasteurized milk and aged for 30 d and from a 90:10 mixture of raw milk from cows and ewes and aged 75 d. CO_2 was removed from raw milk prior to pasteurization and/or the cheese-making process. Compared to cheese made with pasteurized milk, CO_2-treated milk showed slower initial growth of lactic acid bacteria with lower levels of acids. Compared to cheeses made from unpasteurized milk, both CO_2-treated cheeses exhibited no change in volatile compound production, a reduction in clotting time, a higher cheese yield, and an increase in cheese hardness. In a later study (Ruas-Madiedo and others 2003) the group extended this work by examining the effects of the treatments on proteolysis. Cheeses made from CO_2-treated milk exhibited lower amounts of hydrophilic peptides and no change in hydrophobic peptides at the end of ripening. β-casein breakdown was not affected while α_{s1}-casein breakdown was enhanced during aging; no difference in taste was detected, as measured by a sensory panel.

Nelson and others (2004a, 2004b) similarly found no change in β-casein breakdown and an increase in α-casein breakdown during the aging of cheese made with CO_2-treated milk. In this study, however, milk was preacidified with 35 mM CO_2, which was not removed prior to cheese making. A significant reduction in make time was observed compared to the control milk cheese. Cheese manufactured from CO_2-acidified milk had less total fat and calcium than the control cheese, and higher total salts, while total crude protein did not change. During aging, the use of starter and coagulant cultures was the same for both treated and untreated milks; however, proteolysis was found to be higher in the CO_2 treated cheese.

Ultrafiltration (UF) and microfiltration (MF) of raw milk to allow separation and concentration of milk components can produce a concentrated milk with optimized protein content; such modified milk can be used in cheesemaking. Ma and Barbano (2003b) examined the effect of protein concentration and type in CO_2-treated UF and MF milks on freezing point and pH, and found that increasing either casein or soluble protein increased the buffering capacity of milk. At low CO_2 injection temperatures, where the amount dissolved in the milk skim portion is maximized (Ma and Barbano 2003a), pH reduction was influenced by the protein concentration and type. Work by Gevaudan and others (1996) with skim milk modified with moderate pressures of CO_2 showed that the buffering capacity of the milk shifted to a slightly lower range, which was thought to be due to an irreversible increase in milk salts; pH change, however, was reversible. Thus, if a specific CO_2 level in the milk is desired, pH cannot be used as an estimate of CO_2 levels; direct measurement of CO_2 content should be performed for more accurate analysis.

Cottage cheese

The use of CO_2 has been found to be commercially beneficial in the preservation of cottage cheese. Creamed cottage cheese sealed in flexible containers following CO_2 flushing and storage at 4 °C showed repressed growth of psychrotrophs, yeast, and molds (Kosikowski and Brown 1973). Fresh flavor was maintained for 73 d, but due to the high level of CO_2 the cottage cheese had a "fizzy" character. Other laboratories have subsequently investigated gas flushing of the headspace for the preservation of cottage cheese (Rosenthal and others 1991; Moir and others 1993; Fedio and others 1994). Maniar and others (1994) reported that gas flushing with 100% CO_2 was preferred over other gas mixtures for maintaining the microbiological and sensory quality.

Gas flushing is reportedly used commercially in Germany (Honer 1987). Cups are flushed with CO_2 before filling with cottage cheese and at the end of the filling the headspace is again flushed with CO_2. The tubs are sealed with aluminum foil and capped.

Moir and others (1993) suggested that addition of CO_2 throughout the cheese before packaging was necessary to inhibit psychrotrophs both on the surface and within the depth of the cheese. They reported a significant difference in the microbial counts between the surface and the interior of cottage cheese packaged in conventional, thermoformed, high-impact polystyrene cups. CO_2 concentrations were found to have decreased throughout the storage period, as the CO_2 permeability of the containers was high.

Many of the problems associated with CO_2 in cottage cheese have been overcome by the direct addition of CO_2 into the cream dressing prior to mixing with the curd to form cream-style cottage cheese (Chen and Hotchkiss 1991a, 1991b, 1993). The quality of CO_2-containing cottage cheese packaged in polystyrene tubs overwrapped with a high-barrier heat-shrinkable film can be maintained for 63 and 42 d at 4 °C and 7 °C, respectively (Chen and Hotchkiss 1991b).

The commercial procedure for manufacturing cottage cheese with a low level of CO_2 involves injecting CO_2 into the cream dressing via an in-line sparging unit designed for food applications. CO_2 gas is injected into the moving stream of cream dressing at a controlled rate in a pressurized line. The treated dressing is mixed with the curd and filled into containers. Several parameters should be controlled, including the size of the CO_2 bubbles, backpressure within the line, residence time in the line, temperature, and the filling process (Hotchkiss and Lee 1996).

The question of the "taste" (or more precisely, mouth feel) of CO_2 in cottage cheese and other products is often raised. The amount of CO_2 used is often below that which is capable of producing the common tactile sensation associated with CO_2-containing beverages or sodas. Using trained sensory panelists in triangle tests we have found that the lowest threshold for CO_2 in milk is between 4.54 and 9.10 mM (Chen and others 1992; Lee 1996). The flavor threshold for untrained "consumer" panels is likely to be higher. Moir and others (1993) found that 10 mM CO_2 injected into cottage cheese cream dressing and package headspace could significantly increase shelf life while not affecting pH or flavor.

Yogurt and fermented dairy beverages

Mold and yeast growth and development of off-flavors can be a major determinant of shelf life of yogurts (Robinson and others 2002; Viljoen and others 2003). In addition, the survival of probiotic organisms is of importance in some yogurt products. Technologies that extend shelf life must therefore take into account the effect on both spoilage and desirable organisms in the product. As with cottage cheese, headspace flushing of yogurt packages with CO_2 can extend shelf life by inhibiting spoilage organisms (Tamime and Deeth 1980), and it is possible that direct incorporation of CO_2 into the product may also beneficially impact shelf life. A method whereby spoonable yogurt could be carbonated has been patented and modifications of a more optimized method and model for carbonation of viscous fluids have been published (Taylor and Ogden 2002). As with production of CO_2-injected cottage cheese, the economic investment in equipment and supplies is minimal, with packaging costs the most significant expense. Karagul-Yuceer and others (2001) recently reported that high levels (1.1 to 1.2 volumes) of dissolved CO_2 incorporated into yogurt had little effect on desirable (typical or nontypical starter cultures) or undesirable (spoilage and pathogenic) microorganisms. It had been hypothesized that the addition of CO_2 to the product could feasibly stimulate growth

of starter bacteria, reducing production time. The growth of only 3 different spoilage and pathogenic microorganisms, *E. coli*, *L. monocytogenes*, and *B. licheniformis*, was monitored. *L. monocytogenes* was not affected by dissolved CO_2 and populations slowly declined in both CO_2-treated and -untreated product during storage. Populations of *E. coli* decreased to nondetectable levels in the CO_2-treated yogurt during 60 d storage, while *B. licheniformis* was reported not to grow under any conditions.

In an earlier study, Karagul-Yuceer and others (1999) showed no differences in shelf life sensory properties or consumer acceptance between CO_2-modified and nonmodified yogurts. Wright and others (2003) determined that the sensory carbonation threshold in yogurt is on average 5.97 mM, at considerably lower levels than those tested in the previous study. The threshold could be used by manufacturers to develop carbonated yogurt products or to make CO_2 amendments to yogurt to extend shelf life without changing sensory properties.

As previously discussed, raw milk modified with CO_2 during storage prior to dairy product manufacture can result in improved microbial quality with no noticeable changes to the finished product characteristics. Calvo and others (1999) reported that yogurt made from CO_2-amended skim milk was not significantly different, including lactic acid production, from control yogurts made from nontreated milk. They concluded that the addition of CO_2 to raw milk destined for yogurt production would be feasible. Gueimonde and others (2003) also found no difference in the evolution of organic acids between yogurts made with CO_2-treated and -untreated milk. These authors also found no difference in sensory properties and in the growth of starter cultures used. Neither study, however, reported the levels of CO_2 applied.

Carbon dioxide-modified raw milk has also been evaluated for its use in the manufacture of fermented milk beverages. Vinderola and others (2000) found that CO_2 modification decreased milk fermentation time in both *Streptococcus thermophilus/Lactobacillus acidophilus* (AT) and *S. thermophilus/ L. acidophilus/Bifidobacterium bifidum* (ABT)-fermented milk products; no negative impact on sensory characteristics of the milks was noted. Similarly, Gueimonde and de los Reyes-Gavilan (2004) found shortened incubation times in carbonated fermented milks using a variety of *L. acidophilus* and *S. thermophilus* starter strains. Noriega and others (2003) later examined *B. cereus*-inoculated ABT milk, finding significant inhibition of growth of the pathogen in CO_2-modified milk during incubation at 37 °C. During storage at 4 °C, proteolysis and acid production were reduced in inoculated milk. The authors conclude that CO_2 can be an effective method of reducing the risk of *B. cereus* contamination in ABT milk during the required prolonged incubation period. In both studies, no impact on the growth of the probiotic *Bifidobacterium* was noted.

Butter

Addition of CO_2 to butter during the churning stage has been investigated (Hunziker 1924; Prucha and others 1925). The gas was allowed to flow into the cream during the entire churning operation. No pronounced effect on microbial growth was observed. The "sourish" taste (undoubtedly due to residual CO_2 levels that were above the taste threshold) of the butter immediately after carbonation disappeared during storage. This latter phenomenon suggests that the CO_2 level was not maintained within the butter sufficiently to have an inhibitory effect. Prucha and others (1925) observed that bacterial growth was suppressed only when carbonated butter was packaged in airtight vessels. It is unclear why, in these studies, CO_2 did not remain dissolved in the butter; it is generally recognized that CO_2 is highly soluble in nonpolar lipids (Fogg and Gerrard 1991). However, the injection temperatures used may have influenced overall solubility. In a study of the effect of CO_2 injection temperature on CO_2 solubility in milk and cream, Ma and Barbano (2003a) found that at higher temperatures where milk fat is liquid, CO_2 solubility in the fat increased; at temperatures where milk fat was in a solid phase, CO_2 solubility decreased. To the best of our knowledge, more recent work on CO_2 in butter has not been reported.

Dry milk powders

Dry milk powders packaged in cans or drums for long-term storage are commonly commercially packaged in modifed atmospheres, including mixed CO_2 and N_2, 100% N_2, or reduced O_2 atmospheres. Packaging strategies for dry milk powders seek to improve shelf life through elimination or reduction of O_2 to prevent or slow fat oxidation that can cause undesirable off-flavors and odors, particularly in whole-fat milk powders. Gas flushing as well as insertion of oxygen absorbers can be used to achieve the desired in-package storage atmosphere. In an early study by Driscoll and others (1985), the sensory quality of instant and regular nonfat dry milk after 4 years of storage in cans or polybags at 10, 21, and 32 °C and modified atmospheres (air, 100% CO_2, 100% N_2) was measured. At 21 °C, milk stored in cans or in polybags under air was less desirable in sensory qualities (off-flavors) than milk stored under either N_2 or CO_2 at the same temperature. The 100% CO_2 atmosphere was created by addition of a pellet of dry ice to the package, which was allowed to sublime. Holm and others (1927) found that dry whole milk stored under air or vacuum developed off-flavors and odors sooner than milk stored in CO_2. Neither group reported the effect of high CO_2 and O_2-depleted storage environments on pathogens or spoilage organisms. Quality variations in dry milk powder stored in cans with modified atmospheres can occur, particularly over very long-term storage. A survey by Lloyd and others (2004) of 10 brands of nonfat dry milk stored in No. 10 cans obtained from 7 different manufacturers within a broad distribution area showed wide variability in headspace oxygen content, water activity, and sensory ratings (aroma, flavor, and overall acceptance) as well as package integrity. This variability was attributed to differences in packaging and manufacturing processes occurring between manufacturers and inconsistencies in initial quality control measures.

Summary and Conclusions

The relatively short shelf life and rapid loss of quality coupled with the desire to consolidate manufacturing in larger plants has necessitated the requirement of an increased shelf life for many dairy products. Thermal processes such as ultra-high temperature (UHT) and pasteurization of cottage cheese have been developed to meet this need; however, in many cases these processes alter the organoleptic properties of the products. Use of antimicrobial agents such as sorbic acid and nisin has been adopted but there are concerns over labeling these additives. The direct addition of CO_2 to dairy products coupled with increasing the barrier properties of the containers has been commercially successful and economically feasible with cottage cheese and other fluid products. Shelf life extensions of 200% to 400% have been achieved. Substantial research exists to show that direct addition of CO_2 to raw bulk milk during storage prior to processing or further manufacturing of different dairy products can significantly improve and extend the shelf life of the products, increase product safety, and in some cases improve product quality. Additionally, increases in shelf life can enable longer distance transport of fluid raw milk than what is currently achievable, leading to the opening up of new markets. Milk intended for consumption as a pasteurized fluid product would require that the CO_2 be reduced to a level that was similar to the levels found in untreated raw milk; vacuum treatment is one available technology to achieve this reduction.

Additional research can increase the efficiency of the process and contribute to a better understanding of the fundamental basis of the biostatic action of CO_2.

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