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The use of dissolved carbon dioxide to extend the shelf-life of dairy products

C. R. Loss and J. H. Hotchkiss, Cornell University, USA

18.1 Introduction: factors limiting the shelf-life of dairy products

The shelf-life of refrigerated dairy products is limited to 1 to 3 weeks (Muir, 1996). A number of factors contribute to this limited shelf-life: microbial quality of the raw milk (Muir, 1996), bacterial enzymes (Champagne *et al*., 1994), thermal processing conditions (Lewis, 1999), and distribution/storage temperatures (Henyon, 1999). Carbon dioxide (CO_2) can be used to influence these factors and improve the quality of a variety of dairy products. Growth and metabolism of a wide range of bacteria (Dixon and Kell, 1989), particularly those found in the dairy processing environment (Roberts and Torrey, 1988; Ruas-Madiedo *et al*., 1996), are inhibited in the presence of added CO_2. Combined inhibitory effects of CO_2 and other preservation techniques (refrigeration, pasteurisation or high barrier packaging) on bacterial growth and survival have been demonstrated.

18.2 The effects of CO_2 on bacterial growth

When CO_2 is dissolved in an aqueous medium it can retard the growth of Gram-positive and Gram-negative organisms. The magnitude of the effect on the different phases of growth depends upon the organism (see Table 18.1). For example, the lag phase of growth for *Pseudomonas fluorescens* increases with increasing concentrations of CO_2 (Fig. 18.1) (Hendricks and Hotchkiss, 1997). Other organisms are similarly affected but to different degrees: *Listeria monocytogenes* (Hendricks and Hotchkiss, 1997; Fernandez *et al*., 1997),

Table 18.1 Effects of CO_2 on bacterial growth (measured by conductance) described with the Gompertz model (adapted from Martin *et al.*, 2003)

Organism(s)	$[CO_2]$ (mM)	R^2	Growth rate (μS/h)	Time to max. growth rate (h)	Max. change in conductance (μS)	Doubling time (h)	Lag time (h)
Raw milk microflora	0.6	1.0	0.200[a]	26.0[a]	88.9[a]	1.8	20.0
	15.4	1.0	0.132[b]	33.0[b]	92.2[b]	2.3	25.4
	27.9	1.0	0.135[c]	40.2[c]	98.0[c]	2.2	32.8
	38.6	0.99	0.133[d]	44.3[d]	80.5[d]	2.3	37.7
	44.5	1.0	0.113[e]	52.9[e]	87.9[e]	2.7	44.1
P. fluorescens	0.4	0.99	0.112[a]	11.7[a]	78.2[a]	2.7	3.3
	11.2	0.99	0.128[b]	21.1[b]	69.2[b]	2.4	13.3
	27.1	0.99	0.130[b]	22.7[c]	59.9[c]	2.3	15.0
	33.6	0.99	0.088[c]	27.3[d]	61.9[d]	3.4	16.0
	46.3	0.99	0.088[c]	37.5[e]	65.6[e]	3.4	26.1
E. coli	0.5	0.99	0.064[a]	47.6[a]	56.0[a]	4.7	29.4
	49.4	0.97	0.055[b]	53.8[b]	22.0[b]	5.5	38.1
L. monocytogenes	0.5	0.98	0.136[a]	22.6[a]	118.0[a]	2.2	15.2
	48.9	0.99	0.100[b]	44.4[b]	71.5[b]	3.0	34.4
Enterococcus faecalis	0.5	0.99	0.055[a]	51.8[a]	69.0[a]	5.5	33.6
	51	0.98	0.076[b]	50.7[b]	40.7[b]	4.0	37.6
B. cereus	0.5	1.00	0.128[a]	33.9[a]	79.3[a]	2.4	26.1
	47.1	0.99	0.105[b]	37.6[b]	77.9[b]	2.9	28.1
	61.4	0.99	0.057[c]	44.4[c]	74.8[c]	5.3	26.7
B. licheniformis	0.5	0.99	0.057[a]	48.4[a]	51.4[a]	5.3	30.9
	49.4	0.96	0.057[a]	54.1[b]	31.2[b]	5.2	36.7

[a–e] For each organism, different superscript letters denote that parameters are statistically different from each other ($\alpha = 0.05$).

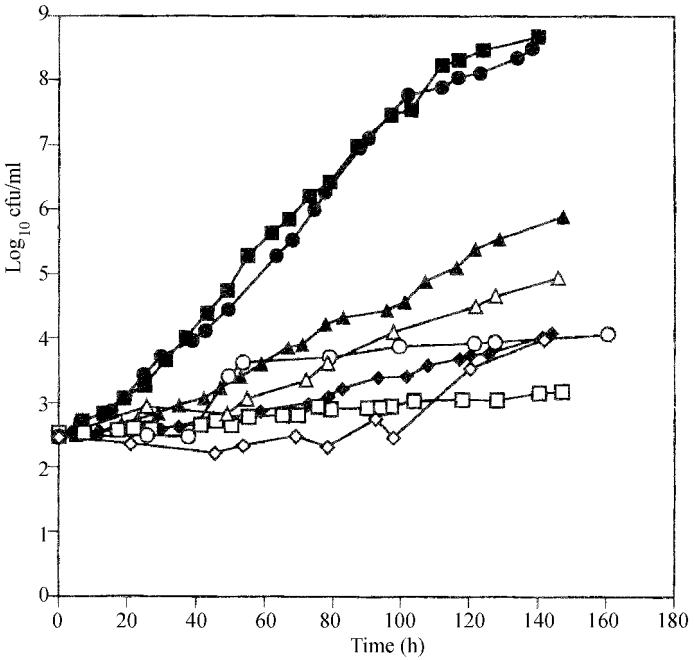

Fig. 18.1 Effects of CO_2 concentrations in modified atmospheres on growth of *Pseudomonas fluorescens* in media at 7.5ºC. $O_2/CO_2/N_2$: 20/0/80 ■; 20/5/75 ●; 20/20/60 ▲; 20/40/40 ◆; 20/80/0 ❒; 10/30/60 ❍; 20/30/50 Δ; 40/30/30 ◇ (Hendricks and Hotchkiss, 1997, with permission).

Escherichia coli (Martin *et al*., 2003), *Bacillus licheniformis* (Martin *et al*., 2003), SPC, and milk-borne psychrotrophs (Roberts and Torrey, 1988).

The log phase of growth is also altered by CO_2. An atmosphere containing 70% CO_2 doubled the generation time of *Pseudomonas aeruginosa* when grown in defined media at 24ºC compared to controls grown in the presence of air (King and Nagel, 1967). The maximum specific growth rate (per hour) of *Bacillus cereus*, in broth, decreased from 0.46 at 0.0 atm CO_2 pressure to 0.37, 0.13, and 0.0 (no growth) at 0.5, 2.0, and 3.0 atm CO_2 respectively (Enfors and Molin, 1980). A simultaneous increase in the lag phase and decrease in the growth rate due to CO_2 has been demonstrated in experimental growth medium for *P. fluorescens* (Devlieghere *et al*., 1998a) and in UHT milk for *L. monocytogenes* (Martin *et al*., 2003).

The growth of fungi is also inhibited by CO_2 (McIntyre and McNeil, 1998). Inhibitory effects of CO_2 on the growth of yeast and moulds in dairy products have been demonstrated (Alves *et al*., 1996; Choi and Kosikowski, 1985; Eliot *et al*., 1998). The growth of anaerobes in media (Reilly, 1980) and milk (Roberts and Torrey, 1988) are inhibited in the presence of CO_2.

18.2.1 Effects of CO_2 on spores

Carbon dioxide affects spore germination in laboratory media (Hambleton and Rigby, 1970). *Clostridia* species germinate at a faster rate in the presence of CO_2 (Foegeding and Busta, 1983) in peptone yeast extract broth, but germination of *B. cereus* spores in phosphate buffer is inhibited (Enfors and Molin, 1978b). Even different strains respond differently to CO_2. In milk treated with CO_2 to a pH of 5.86 activation of *B. cereus* spores was increased by 26% while *B. cereus* var. *mycoides* was not activated by the CO_2 (Guirguis *et al*., 1984).

Carbon dioxide (11.9 mM) dissolved in sterile milk packaged in glass jars and stored at 6ºC for 35 days had no effect on the germination and outgrowth of *B. cereus* spores (Werner and Hotchkiss, 2002). It is suggested that moderate concentrations of CO_2 will not increase the risk of *B. cereus* spores growing in milk stored for extended periods of time.

Concerns with the possibility of *Clostridium botulinum* germination, outgrowth and toxin production in CO_2-treated milk prompted researchers to measure toxin production in milk inoculated with *C. botulinum* spores (Glass *et al*., 1999). After a heat shock treatment, a cocktail of proteolytic and nonproteolytic strains of *C. botulinum* spores was inoculated into pasteurised milk containing 9.1 and 18.2 mM CO_2 or no added CO_2 (control) and stored for 6 days at abusive temperatures (21ºC) and for 60 days at 6ºC. Controls and CO_2-treated milks stored at 21ºC were grossly spoiled (SPC reaching 10^7 cfu/ml) at day 2 before *botulinum* toxin was detectable. Milk stored at 6ºC, regardless of treatment, did not contain toxin over the 60-day storage period, leading to the conclusion that dissolved CO_2 as high as 18.2 mM in milk does not increase the risk of botulism.

18.2.2 Effects of CO_2 on enzyme production and activity in milk

The effects of CO_2 on extracellular enzyme production by *P. fluorescens* in a simulated milk medium have been reported (Rowe, 1988). Carbon dioxide dissolved at 30 mM resulted in a 50% reduction in protease production at 7ºC. After 5 days, lipase production was 85% greater in controls than in CO_2-treated milk. This may have something to do with the increased solubility of CO_2 in lipids. More recently Habulin and Knez demonstrated that supercritical CO_2 (100 bar) can also significantly decrease the activity of a *P. fluorescens* lipase by 50% (Habulin and Knez, 2001).

18.2.3 Inhibitory mechanisms of CO_2

The direct and indirect mechanisms by which CO_2 affects microbial growth and metabolism are not entirely clear even though the topic has been studied in detail (Daniels *et al*., 1985; Dixon and Kell, 1989; McIntyre and McNeil, 1998; Stretton and Goodman, 1998). Four major theories on the inhibitory mechanism of CO_2 have been proposed:

1. Solubility of CO_2 in lipids may adversely affect membrane stability (Nilsson *et al*., 2000; Ballestra *et al*., 1996).

2. Hydration reactions of CO_2 result in reduced pH creating intracellular and environmental stress (Wolfe, 1980).
3. As a metabolite in many biochemical pathways, CO_2 can cause futile expenditure of cell energy (Dixon *et al*., 1987).
4. CO_2 can cause physiochemical alteration and regulation of enzymes (King and Nagel, 1975; Pichard *et al*., 1984).

Depending upon the growth medium, the organism, and its physiological state, a combination of these mechanisms is probably responsible for the observed effects.

It has been demonstrated that O_2 displacement is not the only inhibitory mechanism affecting growth (Enfors and Molin, 1980; King and Nagel, 1967). For example, when O_2 concentrations are kept constant the lag phase for *P. fluorescens* increases as CO_2 concentration increases (Hendricks and Hotchkiss, 1997) (Fig. 18.1). The inhibitory effect of CO_2 on anaerobes (Reilly, 1980) supports these conclusions.

The significance of solubility

Carbon dioxide must first dissolve to have an inhibitory effect on microorganisms. When the gas dissolves into an aqueous environment a series of hydration reactions occur (equation 18.1):

$$CO_2 + H_2O \longleftrightarrow H_2CO_3 \longleftrightarrow HCO_3^- + H^+ \longleftrightarrow CO_3^{2-} + 2H^+ \qquad 18.1$$

Upon solvating, CO_2 forms carbonic acid, which dissociates to form the bicarbonate anion and H^+. Carbon dioxide and the H^+ ions are largely responsible for the inhibitory mechanisms mentioned above; however, bicarbonate and carbonate ions have also been shown to have an inhibitory effect (Diez-Gonzalez *et al*., 2000; Corral *et al*., 1988).

The equilibrium of the reaction (equation 18.1) will be determined by the pH of the aqueous phase. At lower pH, the reaction will be pushed to the left. Therefore the pH of the medium in which the CO_2 is dissolved or its buffering capacity may affect the mechanism that is at work.

Carbon dioxide molecules are nonpolar and therefore more soluble in lipids than in water. When CO_2 comes into contact with a bacterial membrane it will prefer to dissolve into the lipid bilayer. In doing this, CO_2 increases the fluidity of the membrane (Sears and Eisenberg, 1961; Nilsson *et al*., 2000) and exposes the cytoplasm of the cell to its toxic environment. Depending upon concentration and pressures, some CO_2 will eventually solubilise in the cytoplasm, which is an aqueous environment with a neutral pH. These conditions will permit the reduction in the pH of the cytoplasm and a change in the pH gradient that will stress the cell.

The mechanism by which CO_2 affects spores is not clear. Spores have a thin membrane embedded beneath a series of protein coats. These coats are porous (Setlow and Johnson, 1997) and it may be that CO_2 solubilises within the spore membrane, rendering the spore more sensitive to environmental stress such as

heat either by triggering germination or by increasing the fluidity of the membrane (Enfors and Molin, 1978a).

The effects of CO_2 on phenotypic characteristics of microorganisms suggest that there are changes in gene expression due to CO_2 levels in the environment. Stretton and Goodman (1998) have reviewed the literature describing the effects of CO_2 on gene expression in a wide range of microorganisms. It is proposed that CO_2 as a ubiquitous environmental signal is part of a global regulatory system.

The inhibitory effect of CO_2 is also dependent upon temperature, as it is more soluble at lower temperatures (Tomasula and Boswell, 1999). The effect of temperature on CO_2 solubility and its inhibitory effect on the growth rate of *Lactobacillus sake* demonstrated that growth rates in laboratory media are inversely correlated with CO_2 solubility (Devlieghere *et al*., 1998b). Carbon dioxide is soluble in milk even at temperatures of 38ºC (Ma *et al*., 2001) and therefore can be added and retained in refrigerated products during storage and distribution.

18.3 The effects of CO_2 on raw milk quality

The inhibitory effect of dissolved CO_2 and storage temperature on total plate counts (TPC), psychrotrophic plate counts (PPC), coliforms, anaerobic plate counts, lactobacilli and *Bacillus* spp. have been demonstrated. The effects of CO_2 on spores and their outgrowth have been studied to a lesser extent. Different species and strains have varying resistance to CO_2. Physiochemical changes in the raw milk resulting from dissolved CO_2 have been observed to be minimal or reversible depending upon the concentrations added.

18.3.1 Standard plate counts

Carbon dioxide dissolved in refrigerated (4–7ºC) raw milk can reduce the growth of TPC organisms by 1–1.5 log compared to untreated controls that do not contain added CO_2 (Ruas-Madiedo *et al*., 1996, 1998b). The growth of spoilage organisms in raw milk of both good and poor quality can be slowed with the addition of CO_2 (Espie and Madden, 1997; Roberts and Torrey, 1988). For example, raw milk stored at 4ºC with high (1.6×10^5 cfu/ml) and low (7.8×10^3 cfu/ml) initial TPC prior to CO_2 addition (30 mM) remained below 10^6 cfu/ml for an additional 2.2 days and >3 days respectively compared to controls (King and Mabbitt, 1982). Aerobic plate count organisms in raw milk stored at 7ºC had an increased lag phase (72 h) in the presence of 25–27 mM CO_2 compared to controls (24 h) (Roberts and Torrey, 1988).

18.3.2 Psychrotrophs

Psychrotrophs in raw milk are sensitive to CO_2 (King and Mabbitt, 1982; Sierra *et al*., 1996). For example, after 6 days of storage at 7ºC raw milk containing 25

mM CO_2 had 3.4 log cfu/ml fewer psychrotrophs compared to controls (Roberts and Torrey, 1988). The lag phase of psychrotrophs in raw milk stored at 4ºC was 6 days in the presence of 30 mM CO_2 compared to 2 days in untreated milk (King and Mabbitt, 1982).

Some researchers have not measured CO_2 concentrations directly but instead have looked at the effects of pH reduction due to CO_2 addition on PPC. Reducing the pH from 6.8 to 6.0 held the PPC to 5.89 log cfu/ml after 4 days' storage at 7ºC, compared to controls that had 7.30 log cfu/ml (Sierra *et al*., 1996). The same pH reduction reduced lipolytic PPC by approx 1 log cfu/ml after 4 days at 4ºC compared to controls that had reached 3.5 log cfu/ml (Ruas-Madiedo *et al*., 1996). This bactericidal effect of CO_2 on lipolytic psychrotrophs was not discussed. Nonpolar characteristics of CO_2 allow it to be more soluble in the lipid fraction. It is possible that higher concentrations in the milk fat rendered this particular carbon source inaccessible to the lipolytic psychrotrophs, putting them at a disadvantage compared to the other microbial populations.

18.3.3 Coliforms

Growth of coliforms in raw milk is inhibited by CO_2. After 4 days' storage at 4ºC raw milk acidified to pH 6.0 with CO_2 had coliform counts of 2.5 log cfu/ml compared to untreated controls that had 3.5 log cfu/ml (Ruas-Madiedo *et al*., 1996). The greater the amount of CO_2 dissolved in raw milk the greater the magnitude of inhibition. For example, raw milk containing 45 mM CO_2 stored at 6ºC for 6 days had 4.7 log cfu/ml coliforms compared to controls that had 7 log cfu/ml (Espie and Madden, 1997). Milk containing lower levels of CO_2 (25–28 mM) and stored at a slightly higher temperature (7ºC) for 6 days inhibited coliform growth by 1–1.5 log cfu/ml compared to controls (Roberts and Torrey, 1988).

18.3.4 Anaerobes

Carbon dioxide has an inhibitory effect on anaerobically grown microorganisms (Enfors and Molin, 1980). Raw milk containing 26.5 mM CO_2 stored at 7ºC for 6 days had 5.7 log cfu/ml anaerobic counts compared to 8.5 cfu/ml in controls (Roberts and Torrey, 1988).

18.3.5 *Pseudomonas* species

Pseudomonas species, in general, are significantly inhibited by CO_2 (Roberts and Torrey, 1988; King and Mabbitt, 1982). Counts of pseudomonads were 3 log cycles lower in raw milk containing 30 mM CO_2 stored at 4ºC for 4 days compared to controls (Espie and Madden, 1997).

18.3.6 Biochemical and physical changes in raw milk due to CO_2 addition

The biochemical effects of CO_2 on raw milk have been studied. Carbon dioxide does not alter the stability of fat-soluble vitamins in raw milk stored at 7ºC for seven days (Sierra *et al*., 1996). Organic acid profiles and the casein and whey

protein ratios in refrigerated (4ºC) raw milk were unaltered by CO_2 addition that reduced the pH to 6.0 (Ruas-Madiedo *et al*., 1996). High-temperature short-time (HTST) treatments of raw milk, post-CO_2 removal, did not alter the protein ratios or composition of volatile compounds (Ruas-Madiedo *et al*., 1996) compared to untreated controls. Standard analytical tests such as alkaline phosphatase detection, freezing point, pH, antibiotic tests, and FTIR to measure fat, protein, and lactose, were performed in raw milk with and without CO_2 (Ma *et al*., 2001). Alkaline phosphatase and antibiotic tests were unaltered due to CO_2 addition. Fat and protein content as measured by FTIR were also unaltered. An absorbance increase in the lactose wavelengths due to CO_2 was measured. The reduction of pH due to CO_2 (Fig. 18.2) was noted to be the likely cause of the concurrent reduction in freezing point of raw milk with added CO_2. Reductions in pH cause a dissociation of casein micelles, resulting in a release of calcium and phosphate salts into the aqueous portion (Gevaudan *et al*., 1996). This increase in solutes likely causes the linear decrease in freezing point (FP) from 0.55ºHortvet (ºH) in raw milk without added CO_2 to 0.59ºH in milk containing 1000 ppm CO_2 ($FP = -0.5434 - 0.0000510 \times [CO_2]$ (ppm); $R^2 = 0.98$) (Ma *et al*., 2001).

Destabilisation of the casein micelle due to CO_2 addition may be of concern with respect to increased fouling on plate heat exchangers during thermal processing. It is known that decreasing the pH of milk during thermal treatment can increase the amount of fouling (Patil and Reuter, 1988; Skudder *et al*., 1986) and CO_2 acidification of milk could cause an increase in scaling on the heat

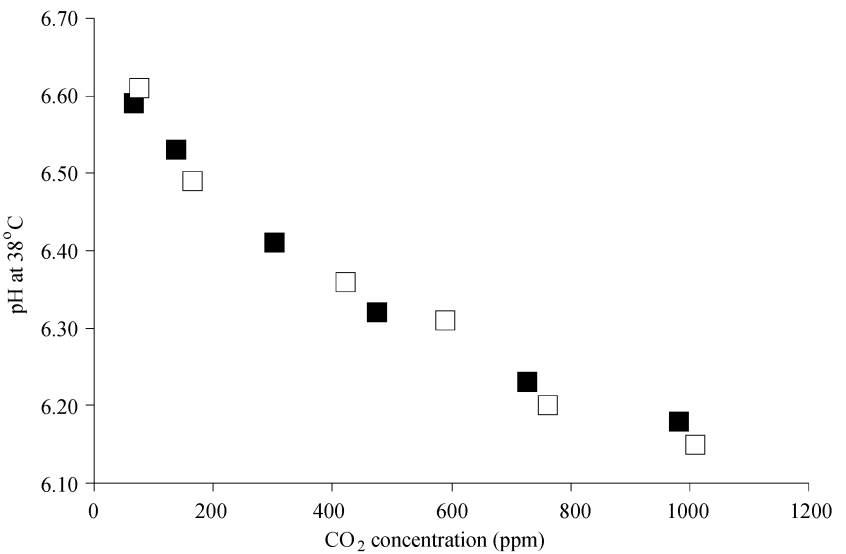

Fig. 18.2 Effect of dissolved CO_2 on pH of raw milk (38ºC) from two different farms (distinguished by open and closed symbols) (Ma *et al*., 2001, with permission).

exchangers (Calvo and de Rafael, 1995). Twenty litres of raw milk treated with CO_2 sufficient to reduce the pH to 6.0 and heated for 20 s at 80ºC formed deposits of mostly protein and minerals on pasteuriser plates. The lipid content of the deposits on heat exchanger plates exposed to the CO_2-treated milk was dramatically lower (45 ± 0.72 g/kg dry matter) than in untreated controls (234 ± 0.79 g/kg dry matter). The concentration of CO_2 dissolved in the milk was not measured. Milk acidified to the same pH with hydrochloric acid completely clogged the pasteuriser (Calvo and de Rafael, 1995).

Recent work (Guillaume *et al*., 2002) concluded that the effects of acidification of reconstituted milk to pH 5.8 were completely reversible after CO_2 was removed by vacuum treatment. Both the composition and structure of the micellar calcium phosphate were unaltered by CO_2 addition and removal.

18.4 The effects of CO_2 on dairy product quality

Carbon dioxide is 'Generally Recognized As Safe' (GRAS; FDA, 2000). It can be used to extend the shelf-life of a variety of dairy products including concentrated raw milk, pasteurised milk, yogurt, cottage cheese, ice cream mixes, and aged cheeses. For fluid products CO_2 can be directly sparged in-line (DAC) but for cheeses CO_2 is incorporated into a modified atmosphere surrounding the product. Important to both of these approaches is a low CO_2/O_2 permeability of the package barrier.

18.4.1 Concentrated raw milk

Growth of Gram-negative and TPC can be reduced in raw milk that has been concentrated by reverse osmosis (RO) or ultrafiltration (UF) techniques (Hotchkiss, unpublished data). UF whole milk containing 25.3 mM CO_2 had TPC of 10^5 cfu/ml after 14 days of storage at 7ºC, compared to untreated controls that had 10^8 cfu/ml. RO concentrated whole milk containing 25 mM CO_2 had 10^3 and 10^5 cfu/ml of Gram-negatives and TPC respectively after 7 days compared to 10^6 cfu/ml for both Gram-negative and TPC in controls.

18.4.2 Fluid milk

Carbon dioxide can improve the microbial quality of milk packaged in paperboard cartons (Duthie, 1985), glass containers (Glass *et al*., 1999), and plastic pouches (Hotchkiss *et al*., 1999). Depending upon packaging material and CO_2 concentration, the shelf-life can be increased by as much as 200% (Hotchkiss *et al*., 1999). Table 18.2 describes the relationship between package barrier permeability, CO_2 concentration in the milk, and microbial growth. Shelf-life, defined as the days to reach 10^6 cfu/ml, increases as CO_2 concentration increases and package barrier permeability to CO_2 decreases. Shelf-life of milk packaged in a low-barrier film increased by 65% from 9.6 days without added CO_2 to 15.9

Table 18.2 Combined effects of dissolved CO_2 and packaging permeability on shelf-life of milk (adapted from Hotchkiss *et al*., 1999)

Film permeability[a]	Days at 6.1ºC for SPC to reach 10^6 cfu/ml[b] $[CO_2]$ (mM)			
	0	8.7	14.2	21.5
3801	9.6	11.8	12.5	15.9
2040	–	9.9	14.0	14.6
110	–	13.4	18.4	19.6
<0.5	–	13.3	19.1	19.1

[a] (cm^3/m^2/24 h at 7ºC).
[b] Initial counts = 1 cfu/ml.

days at 21.5 mM CO_2. Milk packaged in higher-barrier films had a shelf-life of 19 days (Hotchkiss *et al*., 1999).

The growth of psychrotrophs (Duthie, 1985), pseudomonads (Hotchkiss *et al*., 1999; King and Mabbitt, 1982; Shipe *et al*., 1982), and SPC (Glass *et al*., 1999) is inhibited in pasteurised milk that has been treated with CO_2. Safety issues associated with the effect of CO_2 on toxin-producing anaerobes, such as *Clostridium botulinum* (Glass *et al*., 1999) and pathogenic spore formers such as *Bacillus cereus* (Werner and Hotchkiss, 2002) have been addressed. Carbon dioxide does not appear to increase the risk of toxin production by *C. botulinum* or the outgrowth of *B. cereus* in inoculated refrigerated milks.

The sensory threshold, as determined by a trained panel, for CO_2 in 2% pasteurised milk is approximately 9.0 mM. At levels just below the threshold (8.7 mM) a high-barrier package increased the shelf-life by nearly 40% (Table 18.2). The organoleptic properties of CO_2-treated milk are improved after 14 days (Duthie, 1985) and 21 days (Glass *et al*., 1999) of refrigerated storage compared to controls not containing CO_2.

Recently, conductance, which correlates with plate counts, has been used to monitor the growth at 15ºC of inoculated spoilage organisms (in mixed and pure cultures) and pathogens in milk containing dissolved CO_2 at levels ranging from 0.4 to 61.4 mM (Martin *et al*., 2003). Conductance data accurately fit the Gompertz model with R^2 ranging from 0.96 to 1.00, permitting a quantifiable effect of CO_2 on the lag, exponential and stationary phases of growth (Table 18.1). The doubling time for native microflora in raw milk was 1.8 h in controls containing 0.6 mM CO_2 and increased to 2.3 and 2.7 h in milk containing 15.4 and 44.5 mM CO_2 respectively. Lag times (h) for raw milk microflora were 20, 25.4 and 44.1 respectively for the same CO_2 concentrations mentioned above. The doubling time for *B. cereus* increased from 2.4 to 2.9 and 5.3 h as the CO_2 concentration (mM) increased from 0.5 to 47.1 and 61.4 respectively. The doubling time for *Listeria monocytogenes* increased from 2.2 to 3.0 h and the lag time more than doubled from 15.2 to 34.4 h in milk containing 0.5 and 48.9 mM CO_2 respectively (Martin *et al*., 2003).

18.4.3 Effects of CO_2 on the microbial quality of cottage cheese

Several commercial cottage cheese manufacturers in the United States use CO_2 to improve the quality of their product (DMI, 1998) as well as in other parts of the world. When CO_2 is flushed into the headspace (Kosikowski and Brown, 1972; Maniar *et al*., 1994), bubbled into the cream dressing (Lee, 1996; Chen and Hotchkiss, 1991, 1993), or bubbled directly through the finished product (Moir *et al*., 1993), the lag phase of spoilage organisms increases and product quality improves.

A simple and effective approach to incorporating CO_2 into cottage cheese is to add it to the cream dressing prior to mixing with the curds. An inline sparging apparatus and flow diagram for this process has been described (Hotchkiss and Lee, 1996). Cottage cheese prepared in this way can have a shelf-life as long as 80 days (Fig. 18.3). A mixture of three Gram-negative psychrotrophic spoilage organisms inoculated at a level of 10^3 cfu/ml did not grow in CO_2-treated (40% in head space) cottage cheese over a 70-day period when stored at 4ºC in glass jars (Chen and Hotchkiss, 1991). In controls not containing CO_2 bacterial levels reached 10^6 cfu/ml within 15 days. A storage temperature of 7ºC combined with CO_2 addition held counts below 10^4 cfu/ml for 30 days, and in controls, SPC reached 10^6 cfu/ml within 5 days.

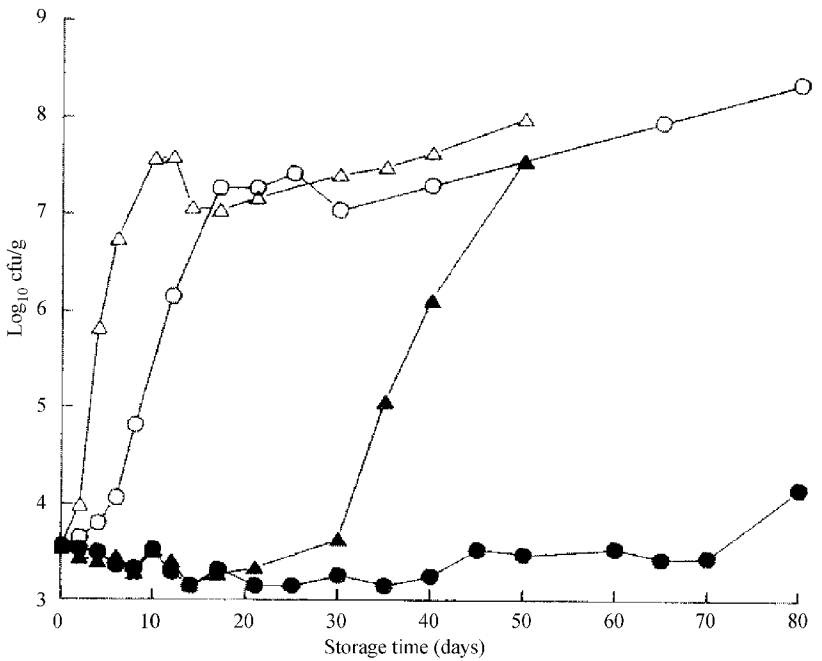

Fig. 18.3 Standard plate counts in cottage cheese packaged with (solid symbols) and without (open symbols) added dissolved CO_2 and stored at 4ºC (●, ○) and 7ºC (▲, △) (Chen and Hotchkiss, 1991, with permission).

Gram-negative organisms, particularly *Pseudomonas* spp. (Moir *et al*., 1993), psychrotrophs (Maniar *et al*., 1994), yeasts and moulds (Kosikowski and Brown, 1972; Chen and Hotchkiss, 1991) and lactic acid bacteria (Maniar *et al*., 1994), are inhibited by CO_2 in cottage cheese. Microscopic examination of CO_2-treated cottage cheese showed that Gram-positive bacilli and cocci predominated compared to controls (Chen and Hotchkiss, 1991). Carbon dioxide seemed to have an inhibitory effect on the yeast and mould populations in cottage cheese, as they were undetectable in CO_2-treated samples but found in the controls at levels of 10 cfu/g (colony forming units per gram). These effects are not attributed to the pH-reducing effect of CO_2 as the controls and treated samples had similar pH (5.2–5.25) (Chen and Hotchkiss, 1991).

Improved flavour scores for cottage cheese parallel the improved microbial quality in CO_2-treated samples (Maniar *et al*., 1994; Kosikowski and Brown, 1972; Lee, 1996). The amount of CO_2 added is below the taste threshold and cannot be detected by the consumer.

Concerns with outgrowth of facultative and obligate anaerobes, such as *L. monocytogenes* and *C. sporogenes* have been addressed (Chen and Hotchkiss, 1993). Over a 63-day storage period at 4ºC and 7ºC, *C. sporogenes* did not grow in either CO_2-treated (35% of the volume in the headspace) or control cottage cheeses. *Listeria monocytogenes* inoculated at 10^4 cfu/ml in CO_2-treated cottage cheese did not increase during 63 days of storage at 4ºC, but did increase by 1 log when stored at 7ºC. In the control products without added CO_2, counts reached 10^7 cfu/ml in 28 days and 7 days when stored at 4ºC and 7ºC respectively.

18.4.4 Carbonated milk-based beverages

Milk-based beverages with sufficient CO_2 to be detectable by taste have a refrigerated shelf-life of more than 6 weeks. Such carbonated flavoured dairy beverages have equal or better nutritional quality than that of milk. The amount of CO_2 added and the type of flavouring system used in these products are critical to their acceptance.

18.4.5 Yogurt

When using CO_2 to improve the quality of fermented dairy products, the growth of beneficial bacteria such as lactic acid producers or probiotics cannot be inhibited and at the same time spoilage organisms must be inhibited in order to extend shelf-life. Two approaches have been taken to improve the quality of yogurts and cheeses:

1. Incorporate CO_2 into the raw milk to provide starting ingredients with good microbial quality.
2. Incorporate CO_2 into the final product or atmosphere surrounding the product to inhibit spoilage.

To evaluate these strategies, researchers have monitored the growth and metabolism of fermentative bacteria in CO_2-treated products and evaluated their quality compared to controls made by conventional methods.

Yogurt made from CO_2-treated raw milk (to a pH of either 6.0, 6.2, or 6.4) had similar sensory properties and viscosity as control yogurt but lower pH values after 7 days of storage at 7ºC (Calvo *et al*., 1999). In a different study growth and metabolism of two combinations of yogurt starter cultures in carbonated milk were monitored over a 49-day storage period at 4ºC (Vinderola *et al*., 2000). Carbon dioxide was added to the milk after the heat treatment of the raw milk and prior to inoculation with either of two starter culture blends:

- *Lactobacillus acidophilus* and *Streptococcus thermophilus*
- *L. acidophilus, S. thermophilus* and *Bifidobacteria bifidum*.

The growth of the first culture mixture was unaltered by the addition of CO_2 which reduced the pH from 6.84 to 6.31. In the presence of *B. bifidum* and CO_2 the counts of *L. acidophilus* were lower towards the latter part of the storage period. Concentrations of organic acids (pyruvic, lactic, and acetic) were the same in both CO_2-treated and control milks for both culture combinations at the end of the storage period. However, acetic acid was lower in the CO_2-treated milk containing *B. bifidum* during the first 4 weeks of storage, which may have been related to the lower counts of *L. acidophilus* in these samples later on. After 24 days the sensory properties of the yogurts, including mouthfeel, odour, acidity, and overall acceptability, were slightly improved in the CO_2-treated yogurts, but statistically indistinguishable from controls. Carbon dioxide-treated milks reached the break point pH of 5.0 sooner than untreated milk (Vinderola *et al*., 2000). Gueimonde *et al*. (2002) found that CO_2 dissolved in milk did not have a negative effect on the growth of probiotic bacteria.

When CO_2 was dissolved directly into finished Swiss-style yogurt, the growth and viability of inoculated pathogens (*L. monocytogenes, E. coli*) and typical starter cultures were unaltered (Karagul-Yuceer *et al*., 2001). In this study the CO_2 content was not measured directly so the actual amount dissolved is unclear.

A consumer acceptance test demonstrated that the shelf-life of a yogurt beverage could be extended to 4 months with the addition of CO_2 (5 kg/cm^2 at 4ºC) compared to uncarbonated controls that were spoiled at 30 days (Kosikowski and Choi, 1985). The yogurt beverages (fermented with *L. bulgaricus* and *S. thermophilus*) were packaged in glass containers and stored at 4.4ºC and 10ºC. After 40 days yeast and mould counts increased from 10 cfu/g to 100 and 200 cfu/g in uncarbonated yogurt beverages stored at 4.4 and 10ºC respectively, whereas in the carbonated product they remained below 10 cfu/g over an 80-day period at both storage temperatures. The soluble protein and volatile fatty acid content of the control yogurts increased at a faster rate than the carbonated samples, an indication that spoilage was occurring more rapidly, though unfortunately SPC were not measured. The noncarbonated yogurt pH dropped faster than the carbonated yogurt pH, indicating slowed metabolism of

the lactic acid bacteria (LAB), but their growth was not measured in the different treatments.

18.4.6 Ice cream and unfrozen mixes

Early attempts to incorporate CO_2 into ice-cream processing did not demonstrate a significant effect on microbial growth in the frozen product (Prucha *et al*., 1922; Rettger *et al*., 1922; Valley and Rettger, 1927). Extending the shelf-life of the unfrozen mix using dissolved CO_2 may be more useful and feasible. Total aerobic plate and Gram-negative counts were measured in chocolate ice-cream mix containing 0, 690, and 1080 ppm CO_2, packaged in high-barrier pouches and stored at 6.1ºC for 43 days (Hotchkiss, unpublished data). Total aerobic plate counts reached 6.0 log cfu/ml at 20, 35 and 41 days in mixes containing 0, 690 and 1080 ppm CO_2 respectively. Gram-negative counts reached 6.0 log cfu/ml in control mix at 30 days, whereas the CO_2-treated samples took longer than 40 days to reach this level. Sensory tests on soft-serve ice cream made from treated mixes indicated that the threshold for CO_2 was 800 to 1400 ppm.

18.4.7 Aged cheeses

Carbon dioxide added to raw milk to be used for cheese production can decrease the processing time (Montilla *et al*., 1995; McCarney *et al*., 1995; Ruas-Madiedo *et al*., 2002), reduce the amount of rennet necessary for coagulation (Montilla *et al*., 1995; McCarney *et al*., 1995; Calvo *et al*., 1993), and increase yields (Ruas-Madiedo *et al*., 1998a, 2002). Organoleptic properties of cheese made from CO_2-treated milk are as good as (Ruas-Madiedo *et al*., 2002) or better than (McCarney *et al*., 1995) controls made from untreated milk.

The amount of rennet necessary to make Cheddar cheese can be decreased by 50% when added to milk that has been treated with CO_2 to a concentration of 30.6 mM (McCarney *et al*., 1995). Lipolysis and proteolysis in cheese made from CO_2-treated milk was significantly lower compared to controls after 3 months of storage at 7ºC. The cheese made from CO_2-treated milk received a higher sensory score than control cheese as determined by a commercial grader (McCarney *et al*., 1995).

When raw milk with high TPC (5×10^5 cfu/ml) was sparged with CO_2 (to pH 6.2) and refrigerated (4ºC) prior to being used for production of a short ripened cheese, the resulting cheese had a higher yield compared to cheese made from uncarbonated milk (Ruas-Madiedo *et al*., 1998a). After 7 days of ripening at 17ºC the CO_2-treated cheese had a yield of 10.4% compared to 5.9% in the control. After 3 and 7 days of ripening the non-casein nitrogen fraction of the cheese made from CO_2-treated milk was significantly lower than in the untreated controls, an indication of reduced proteolysis. Alpha, beta, and gamma casein fractions were not affected by the CO_2 treatment, and at 15 days of ripening the proteolytic activity in both cheeses was the same. Treating milk

with CO_2 did not alter the sensory properties of the cheeses compared to controls (Ruas-Madiedo *et al*., 1998a).

Carbon dioxide dissolved in milk does not affect the growth and metabolism of cheese starter cultures (Van Hekken *et al*., 2000; Ruas-Madiedo *et al*., 1998a; Calvo *et al*., 1993). The growth of individual strains and mixed cultures of *Lactococcus* ssp. and *Leuconostoc citreum* at 22ºC over an 18 h period was the same in pasteurised milk treated with CO_2 (to a pH of 6.2) and untreated controls (pH 6.7). After 8 hours of incubation the concentrations of organic acids (citric, pyruvic, lactic, formic, acetic, and hippuric) were identical in both CO_2-treated and control milks (Ruas-Madiedo *et al*., 1998a).

Once cheese has been manufactured, modified atmospheres containing CO_2 can be used to extend shelf-life (Alves *et al*., 1996; Eliot *et al*., 1998; Piergiovanni *et al*., 1993; Pintado and Malcata, 2000; Gonzalez-Fandos *et al*., 2000; Olarte *et al*., 2001, 2002). Fluorescent light exposure may need to be considered in order to prevent defects in colour that are enhanced by high CO_2 concentrations (Colchin *et al*., 2001).

Pintado and Malcata (2000) compared the growth of psychrotrophs, mesophiles, lactococci, lactobacilli, *Bacillus*, and spore-forming clostridia, amongst others, in Requeijão, a whey cheese, packaged under 100% CO_2 to that packaged under 100% N_2. After 15 days at 4ºC storage under CO_2, none of the populations tested had reached 10^6 log cfu/g, whereas under N_2 most had exceeded this level. For example, PPC reached 5.97 and 8.08 log cfu/g under CO_2 and N_2 atmospheres respectively. *Pseudomonas* spp. reached 4.32 log cfu/g under 100% CO_2 compared to 8.81 log cfu/g under N_2-packaged cheese. At 18ºC the two packaging configurations had less effect on the outgrowth of the microbial populations tested, emphasising the need for refrigerated storage together with MAP to hinder microbial growth. Yeasts and moulds were not detected in cheeses stored in 100% CO_2 at day 15 (at 4ºC) compared to 4 log cfu/g in 100% N_2. *Bacillus* spp. were not detected at days 2 and 4 in CO_2-packaged cheese but reached 5.6 log cfu/g by day 15, the same as that found in N_2-packaged cheese (Pintado and Malcata, 2000).

Atmospheres containing 20–100% CO_2 reduced proteolysis and lipolysis in Cameros, a fresh goat's milk cheese, and inhibited the growth of psychrotrophs, mesophiles, Enterobacteriaceae, and coliforms. After 28 days of storage at 4ºC control cheeses, packaged in the presence of air, had psychrotrophic levels reaching eight log cfu/g whereas in an atmosphere containing 100% CO_2 counts had not exceeded 2.7 log cfu/g (Gonzalez-Fandos *et al*., 2000). Yeasts were undetectable in the CO_2-stored cheese, but in controls counts reached 3.34 log cfu/g in 28 days. Packaging Taleggio cheese in an atmosphere containing 10% CO_2 resulted in a 1 log reduction in moulds compared to controls packaged in the presence of air (Piergiovanni *et al*., 1993). Anaerobes decreased in Cameros cheese packaged in 100% CO_2 atmospheres from 3 log cfu/g to 1 log cfu/g after 28 days. In controls (air), 20 and 40% CO_2 atmospheres (balance N_2) there was a 4 log cfu/g increase over the same time period (Olarte *et al*., 2002). Sensory analysis of Cameros cheeses suggests that 100% CO_2 atmospheres can be

deleterious to quality but that a 50/50 (CO_2/N_2) atmosphere is optimal for improving flavour and reducing microbial growth (Gonzalez-Fandos *et al*., 2000; Olarte *et al*., 2001).

The microbial quality of Mozzarella shreds (Eliot *et al*., 1998) and slices (Alves *et al*., 1996) stored under modified atmospheres containing CO_2 was improved over those packaged under air or N_2, and 100% CO_2 atmospheres inhibited the growth of yeast and moulds in both products. Over a 58-day storage period at 7ºC no growth of yeast and moulds was detected on Mozzarella slices packaged in 100% CO_2 compared to greater than 10^6 cfu/ml in controls after only 10 days.

Shredded Cheddar cheese stored at 4ºC under 100% CO_2 (barrier permeability: O_2 transmission of 2 cm^3/m^2/day forming layer; 4 cm^3/m^2/day – non-forming layer) contained lower levels of volatiles associated with mould growth (Colchin *et al*., 2001). *L**, *a** and *b** colour-values for N_2- and CO_2-packaged cheeses stored under fluorescent lighting were measured. *L** values were significantly higher, and *a** and *b** values were significantly lower, in cheeses stored under CO_2 compared to N_2. It is suggested that CO_2 generates free radicals in the presence of fluorescent light, oxidising bixin (the carotenoid compound in anatto responsible for the orange colour) and resulting in a bleached appearance. Perhaps opaque packaging material could hinder this defect.

18.5 Bactericidal and sporicidal effects of dissolved CO_2 during thermal processing

Bacteria and their spores are more sensitive to thermal treatments under acidic conditions (Jay, 1992). When CO_2 is dissolved in an aqueous solution, such as milk, the pH decreases (equation 18.1, Fig. 18.2). The effects of CO_2 on the thermal resistance of vegetative cells and spores have been studied. The majority of work has been conducted in media at high pressures or near-supercritical conditions and at moderate to ambient temperatures.

D-values (min) for *L. monocytogenes* in a solution of physiological saline and media (1%) under 15 atm CO_2 pressure were 35.8, 22.3 and 14.3 at 25ºC, 35ºC and 45ºC respectively (Erkmen, 2000). At 60 atm CO_2 the *D*-values at these temperatures decreased to 13.4, 8.8 and 7.3 respectively, demonstrating that increased CO_2 pressures can increase the thermal sensitivity of a common facultative pathogen. A similar pattern was observed for the inactivation of *E. coli* in ringer solution (Ballestra *et al*., 1996). The $D_{35ºC}$-values (min) were 496, 30.3 and 1.9 at 1.2, 2.5 and 5 MPa CO_2 respectively.

At 45ºC in the absence of CO_2 the viability of *E. coli* cells was unchanged over a 1 h period. As the CO_2 pressure was increased from 1.2 to 5 MPa the inactivation curves became biphasic. An initial shoulder portion during the first 30 min of exposure became shorter and the slope of the second phase became more negative as CO_2 pressure increased (Ballestra *et al*., 1996). Similar patterns were observed for *L. monocytogenes* (Erkmen, 2000). It is hypothesised

that during the initial phase CO_2 penetrates the cell, and that the second phase represents the point at which a critical amount of CO_2 has collected in the cell, resulting in a more dramatic drop in viability. Scanning electron micrographs (SEM) of CO_2-treated cells revealed deformed cell walls of some cells but the percentage of damaged cells did not correlate with the loss in viability. The activity of seven of eight enzymes assayed was reduced in the CO_2-treated cells compared to untreated (Ballestra *et al*., 1996). It is hypothesised that CO_2 passes through the cell membrane and acidifies the cell cytoplasm below the isoelectric point of the enzymes, rendering them inactive. It has also been proposed that the chemical composition of the bacterial cell wall and the surface-to-volume ratio of the cell may be important factors determining the sensitivity of a particular bacterial strain (Dillow *et al*., 1999).

The amount of heat and the concentration of CO_2 influence the degree of increased thermal sensitivity of bacteria in milk. Microbial survivors (enumerated by SPC) in heat-treated raw milk were significantly lower in milk containing 44–58 mM CO_2 compared to controls that had natural levels of CO_2 ranging from 2 to 4 mM (Loss and Hotchkiss, 2002). For example, CO_2-treated milk heated for 5 min at 67ºC, 72ºC and 90ºC had 257, 89 and 25 cfu/ml respectively compared to controls that had 338, 282 and 44 cfu/ml in untreated milk heated for the same time and temperatures (Loss and Hotchkiss, 2002). Aerobic plate counts and *L. monocytogenes* in whole milk heated to 45ºC under 60 atm of CO_2 both decreased by 3 log cycles after 12 hours (Erkmen, 2000). Unfortunately, reductions in control milks without added CO_2 are not mentioned in this study.

Thermal inactivation rates of *P. fluorescens* in whole milk treated with CO_2 (0–35 mM) increase as concentration of CO_2 increases (Fig. 18.4). There is a negative linear correlation between $D_{50ºC}$-value (min) and CO_2 concentration ($D_{50ºC} = -0.20x + 13.74$; $r^2 = 0.90$). $D_{50ºC}$-values in the untreated control, 15 and 35 mM CO_2-treated milk were 13.4, 10.5 and 7.2 min respectively, representing reductions of 22% and 46% (Loss and Hotchkiss, 2002). Although this organism is sensitive to HTST treatments, its lipolytic and proteolytic enzymes are not (Champagne *et al*., 1994). Perhaps on-farm thermisation combined with CO_2 treatments could reduce the numbers of psychrotrophs that can proliferate during bulk milk collection and transportation.

Carbon dioxide added to fluid foods in the form of carbonate can reduce the pH of the food and increase the thermal sensitivity of spores (Alderton, 1969). Unlike vegetative cells, spores are essentially metabolically inactive and support little enzyme activity. It has been proposed that the increased acidity results in a desorption of cations from the spore causing an increase in hydration of the core of the spore (Lynch, 1988). Dehydration of the spore is its main defence against thermal treatments as wet heat is more severe than dry heat (Setlow and Johnson, 1997).

Carbon dioxide at 5 MPa can increase the thermal sensitivity of bacterial and fungal spores in Ringer solution (Ballestra and Cuq, 1998). *Bacillus subtilis* spores heated at 80ºC under 5 MPa CO_2 for 1 h were reduced by 3 logs, but in

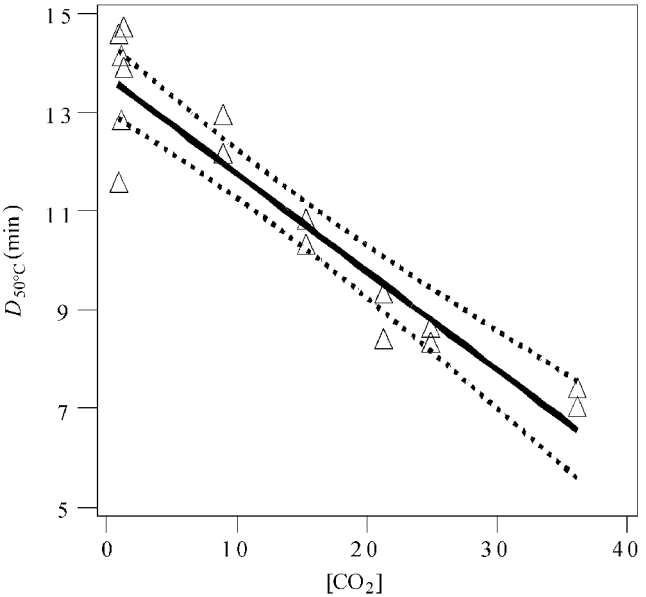

Fig. 18.4 Effect of dissolved CO_2 on $D_{50°C}$-values for *P. fluorescens* R1-232 in milk ($y = -0.20x + 13.74, R^2 = 0.90$). Dotted lines represent 95% confidence bands ($n = 1$).

the absence of CO_2 no reduction in viability was observed at this temperature. $D_{90°C}$-values for *B. subtilis* spores were 14 min at 5 MPa CO_2 and 66 min in the absence of CO_2. $D_{80°C}$-values for *Byssochlamys fulva* ascospores decreased from 350 min in controls to 85.5 min in the presence of 5 MPa CO_2. $D_{50°C}$-values for *Aspergillus niger* conidia decreased from >200 min in controls to 11 min under 5 MPa CO_2. At 60ºC and 85ºC 5 MPa CO_2 had no effect on the thermal sensitivity of *A. niger* conidia and *B. fulva* ascopores, suggesting that the lethal effects of the heat treatment are masking the sporicidal effects of the CO_2 (Ballestra and Cuq, 1998).

The $D_{89°C}$-value for *Bacillus cereus* spores was significantly decreased from 5.56 min in control milks (no added CO_2) to 5.24 min in milk containing 33 mM CO_2 (Loss and Hotchkiss, 2002). A higher concentration of dissolved CO_2 (37 mM) in milk containing an initial inoculum of 8.7 log cfu/ml also resulted in fewer survivors (4.25 log cfu/ml) after a 15-minute treatment at 89ºC compared to controls that had 4.73 log cfu/ml survivors (Loss, 2001). After a 40-second treatment at 105ºC, CO_2-treated TSB (pH reduced to 6.3) had 1 log fewer survivors of *B. cereus* spores compared to untreated (pH 7.2) media heated for the same amount of time (Loss, unpublished data).

The effect of CO_2 on spore germination at higher heat treatments for shorter durations depends upon species and strain (Guirguis *et al*., 1984). For example, 100% of spores of *B. subtilis* in reconstituted milk with pH adjusted to 5.86 with CO_2, heated at 120ºC for 2 s, survived compared to 0.1% survival of spores

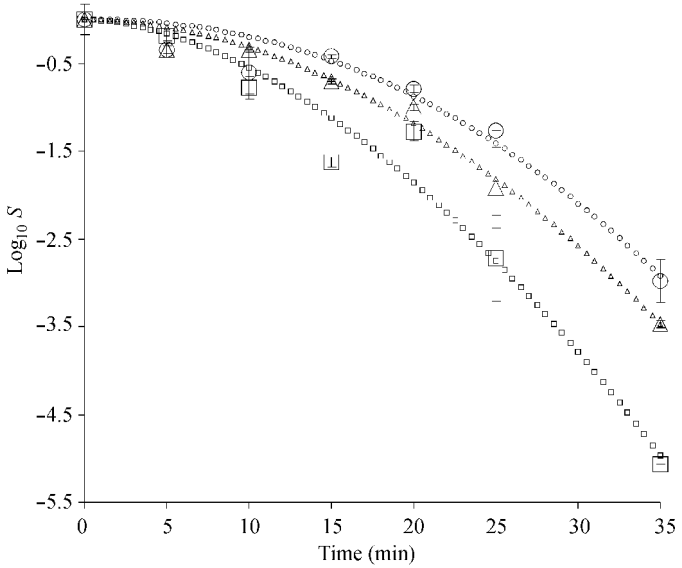

Fig. 18.5 Nonlinear modelling of thermal death rate of *P. fluorescens* R1-232 at 50ºC in milk. Curves represent nonlinear regressions of the survival data in milk containing 1 (❍ actual, ❍ predicted), 15 (Δ actual, Δ predicted) and 36 mM (❒ actual, ❒ predicted) dissolved CO_2, respectively. $R^2 = 0.96$, 0.99 and 0.97 respectively ($n = 2$, ±1 s.d.).

suspended in control milk that had no CO_2. On the other hand, 2% of *B. cereus* spores heated at 125ºC for 2 s in the CO_2-treated milk survived compared to 100% survival in control milk.

The combined effects of dissolved CO_2 and thermal treatment on inactivation of spores and vegetative cells have been modelled using the Weibull function (Loss and Hotchkiss, 2002) (Fig. 18.5). Thermal inactivation curves for *P. fluorescens* at 50ºC in the presence and absence of CO_2 are distinctly biphasic in nature, as is the case for *B. subtilis* spores (Ballestra and Cuq, 1998) and *E. coli* (Ballestra *et al*., 1996). The Weibull model more accurately describes the combined effects of dissolved CO_2 and thermal treatments on survival of common milk spoilage organisms ($R^2 = 0.96$, 0.99 and 0.97 for 1, 15 and 36 mM CO_2 treatments respectively) than does the linear model ($R^2 = 0.83$, 0.89 and 0.90 respectively). Given a useful model like the Weibull, nonthermal treatments such as CO_2 combined with thermal processing can be used to optimise hurdle preservation approaches.

Two major obstacles to producing raw milk cheese on a large scale are the potential for survival of pathogens (such as *L. monocytogenes*) and the deleterious effects of psychrotrophic spoilage organisms. Current data suggest that CO_2 combined with heat treatments may decrease the number of pathogens in the cheese at the beginning of the ageing process and also improve sensory quality by reducing the number of surviving spoilage organisms.

18.6 Conclusions

Carbon dioxide is a unique natural antimicrobial and processing aid that has several potential uses in the dairy industry. It is unique because it can be added to and removed from dairy products with no deleterious effects. It is GRAS, and at the present time does not need to be declared on an ingredient label. The physiochemical properties of CO_2, i.e. ease of solubility in aqueous and lipid phases, its ability to reduce pH, and temperature-dependent solubility, make it ideal for use in dairy products. It can be dissolved into fluid dairy products as a preservative to inhibit growth of pathogens and spoilage organisms and/or to alter the functionality of the casein micelle, and then it can be removed with a simple vacuum or by agitation and mild heating.

The benefits of CO_2 to the cottage cheese industry are quite clear. There is also an abundance of data supporting the use of CO_2 to improve the microbial quality of raw milk, but the benefits of this technology have not transferred to the farm and milk collection sector of the industry. Safe raw milk cheeses are highly desirable in the US and Europe, and CO_2 has the potential to reduce the risk of pathogen survival in these products without altering their unique flavour characteristics.

Quantifying the effects of CO_2 on growth of spoilage organisms and pathogens through the use of statistical modelling will be critical for optimising its use and ensuring safe and wholesome products. Work in this area is just beginning but thus far has demonstrated that the effects of CO_2 can be accurately described using the Gompertz growth model (Martin *et al*, 2003). All preservation technologies from refrigeration to pasteurisation have altered the microbial ecology of dairy products. Refrigeration has selected for Gram-negative psychrotrophs, and pasteurisation has created a niche for psychrotrophic spore formers. Undoubtedly, CO_2 will also effect the microbial ecological balance in dairy foods. Modelling the effects of CO_2 on a wide spectrum of bacteria in pure and mixed cultures, in a variety of dairy products, will give us a better understanding of the changes due to CO_2 and allow us to protect and preserve our nutritious supply of dairy foods.

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