Stability of Shredded Mozzarella Cheese Under Modified Atmospheres

S. C. Eliot, J.-C. Vuillemard, And J.-P. Emond

ABSTRACT

Stability of Mozzarella cheese was studied under 8 modified atmospheres (air, vacuum and mixtures carbon dioxide/nitrogen) during 8 wk. Samples, packaged in barrier bags and stored at 10°C, were periodically evaluated to investigate microbiological quality and composition of headspace gases. Both consumption of oxygen and production of carbon dioxide occurred in many packages. Modified atmospheres containing carbon dioxide efficiently stabilized lactic and mesophilic flora, while inhibiting staphylococci, molds and yeast. Psychrotrophs grew in all samples but were less numerous in high CO_2 atmospheres. Levels of 75% CO_2 were optimal to repress undesirable organisms and reduce gas formation.

Key words: Mozzarella cheese, modified atmosphere, carbon dioxide, preservation, microorganisms

INTRODUCTION

MAINTAINING CHEESE QUALITY DURING STORAGE REQUIRES protection against dehydration and reduction of undesirable microorganisms, especially pathogens. Protection against dehydration can be achieved by using packaging films with low water vapor transmission: semi-barrier (polypropylene, low density polyethylene) or barrier films (aluminum, polyvinylidene chloride, polyvinyl chloride, orientated polypropylene, high density polyethylene) (Day, 1992). Many types of undesirable microorganisms cause odor and flavor changes (Kornacki and Gabis, 1990). Yeast and molds may also modify texture and appearance (Fedio et al., 1994). Many processes can slow microbial proliferation, but those which affect sensory qualities of the product may not be suitable. Preservative addition (e.g., sorbate) may impart flavor modifications, and thermal processes such as pasteurization may interfere with textural properties (Chen and Hotchkiss, 1991). Physical processes aimed at decreasing oxygen in packages are more effective. The development of vacuum-packages has been very successful but they may not be appropriate for fragile products such as shredded cheese.

Modified Atmosphere Packaging (MAP) is the enclosure of food products in barrier materials, in which the gaseous environment has been modified. The modified atmosphere (MA) slows respiration rates, reduces microbial growth and delays enzymatic changes, extending shelf-life (Young et al., 1988). This technology has expanded considerably since the report of inhibitory properties of carbon dioxide (Daniels et al., 1985). Atmospheres containing CO_2 combined with high barrier packaging films have been effective in inhibiting growth of Gram-negative psychrotrophic organisms in a wide range of food products (Chen and Hotchkiss, 1991). Shelf-life of MAP products is influenced by initial quality of the product, composition of the gas atmosphere, types of packaging materials and storage temperatures (Hotchkiss, 1988). The gaseous mixture must be optimized for each product.

Published reports on storage of cheese under MAP is, however, restricted and inconsistent. Scott and Smith (1971) reported that packaging cottage cheese in nitrogen or carbon dioxide did not greatly increase its shelf-life (measured by flavor scores or bacteria counts). However, its storage in carbon dioxide led to flavor changes, which were judged to be either positive or negative depending on regional preferences. Kosikowski and Brown (1973) demonstrated the effectiveness of storing creamed cottage cheese in a carbon dioxide atmosphere or in a nitrogen atmosphere to repress spoilage organisms (psychrotrophic bacteria, yeasts and molds). Flavor and texture were maintained for 45 days at 4°C. Storage in air led to deterioration in flavor after 11–18 days and in texture after 32–45 days. Rosenthal et al. (1991) reported complete inhibition of yeast, molds and pseudomonads in storage of quark and cottage cheese under atmospheres composed of 67.1% CO_2, 26.3% N_2 and 6.6% O_2. This effect disappeared when CO_2 was replaced by N_2. Chen and Hotchkiss (1991) showed that dissolved CO_2 inhibited growth of Gram-negative bacteria and improved keeping quality and shelf-life of creamed cottage cheese (Chen et al., 1992).

Carbon dioxide effects depend upon its concentration, water activity, pH, number, age and kind of the microorganisms and temperature (Day, 1992). Different compositions of modified atmospheres have been tested to evaluate effects during cheese storage. MAP preserved sensory qualities of various cheeses and inhibited growth of psychrotrophic bacteria, yeasts and molds (Maniar et al., 1994; Fedio et al., 1994).

Piergiovanni et al. (1993) compared Tallegio cheese packaged under four modified atmospheres, and stored at 6°C, to traditional paper wrapping and found that samples packaged in MAP had satisfactory quality. MAP caused significant differences in sensory , chemical and color properties, but not in microbiological analysis. Alves et al. (1996) found the microbial growth in MAP sliced Mozzarella cheese at 7°C was retarded with high concentrations of CO_2 and verified the bacteriostatic and fungistatic properties of CO_2. Shelf-life increased under CO_2 atmospheres compared with air (385% under 100% CO_2, 246% under 50% CO_2/50% N_2). But the optimal composition of MA for cheese preservation varied depending on type of cheese: e.g., 10% CO_2/90% N_2 for Tallegio or 100% CO_2 for cottage cheese and sliced Mozzarella cheese. Nitrogen is the main gas employed in industrial packaging of cheeses under modified atmospheres.

The objectives of our study were to investigate the effects of a wide range of modified atmosphere packaging on growth of microorganisms in shredded Mozzarella cheese and to determine the optimal gas composition to improve cheese preservation under retail simulated conditions.

MATERIALS & METHODS

Cheese

Mozzarella cheese was studied because of its importance in the Northern American market. The shredded form is exposed to post contamination and not adapted to vacuum packaging. Furthermore, because of the increased surface area of shredded products, contact with gases would be considerably increased.

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Sample preparation and packaging

Three batches of shredded Mozzarella cheese (size of shred: 3.2 × 10–80 mm) manufactured by Fromage Côté Inc. (Kingsey, Warwick, Québec) were obtained a few days before the beginning of experiments. Samples of 50±2g were packaged in high gas barrier bags (B530 model, Cryovac Division of W.R. Grace, Mississauga, Ontario) under eight modified atmospheres (Table 1). The packages, dimensions ≈ 200 × 200 mm, were multilayers of polyolefins with a polyvinylidene chloride barrier layer, and oxygen permeability of 30–50 $cm^3/m^2/24h$ at 1 atm (101 kPa), 23°C and 0% R.H. They were evacuated, flushed and sealed on a Sipromac 350 machine with gas injection (Drummondville, Québec) after a vacuum of 711 mm Hg (95 Pa) was established. The gases used were industrial mixtures: air, nitrogen and atmospheres 3 to 6 (Table 1) were purchased from Prodair (Montréal, Québec) while carbon dioxide was purchased from Praxair (Mississauga, Ontario).

For each batch of cheese 64 packages were prepared and stored in the dark at 10±1°C and 90% R.H. This high storage temperature was studied to reproduce storage conditions of cheeses in retail stores. Samples (24) constituting the three repetitions of the eight treatments were analyzed each week for headspace gas composition and microbiological quality.

Headspace gas composition

This analysis was performed to verify quality of packages and seals. About 5 mL of package headspace was withdrawn with a gastight syringe. Its composition in N_2, O_2, CO_2 and Ar (in %) was determined using a gas chromatograph (HP 5890 Series II, Toronto, Ontario) with a thermal conductivity detector. The columns were a 5A molecular sieve and a poraplot U (10m × 0.53 mm), and conditions were 30°C for 1.6 min, decrease to 0°C in 1.25 min, hold for 1.15 min, increase to 30°C and hold for 2 min; injector and detector temperatures: 250°C (Eliot, 1997). The chromatograph calibration and standard curves were established with two reference gases, of compositions: 20.00%CO_2 + 3.01%O_2 + 2.00% Ar in 74.99%N_2 and 2.02%CO_2 + 19.10%O_2 in 78.88%N_2.

Microbiological analyses

These analyses were performed on week 0 on fresh, unpacked product, and every week during the study. Packages were aseptically opened and samples of 11g of cheese were dispersed in 99 mL of a 2% (w/v) sterile sodium citrate solution (BDH, Toronto, Ontario) and blended in a stomacher 400 (Seward Chemicals, London, UK) for 2 min. Serial dilutions of this solution were prepared in sterile sodium citrate. To improve homogeneity, $1/10^1$ and $1/10^5$ dilutions were mixed with a model T25-S1 ultra-turrax (Janke & Kundel, Staufen, Germany) for 20s at 13500 rpm.

Eight microorganisms were enumerated by cultures of appropriate dilution on specific broth or agar. Lactic acid bacteria (LAB) were plated on MRS Agar (Rosell Institute Inc, Montréal, Québec) and incubated at 37°C for 48h (Gerhardt et al., 1994). Total mesophilics were counted on Plate Count Agar (Difco Laboratories, Detroit, MI) after incubation at 30°C for 48h (APHA, 1985). Psychrotrophics were enumerated on Plate Count Agar after incubation at 4°C for 10 days (APHA, 1978). Enterococci were estimated on Enterococcosel Agar (Becton Dickinson, Cockeysville, MD, USA) after incubation at 37°C for 48h (Atlas and Parks, 1993). Presumptive Staphylococci were counted on Mannitol Salt phenol red Agar (Merck, Darmstadt, Germany) after incubation at 37°C for 48h (Speck, 1976). Yeasts and molds were enumerated with Dichloran-Rose-Bengal-Chloramphenicol Agar (Difco) after incubation at 22°C for 3 to 5 days (APHA, 1992). Yeasts formed yellowish circular curved opaque colonies and molds formed white or green downy colonies. Total presumptive coliforms were counted on Violet Red Bile Agar (Difco) after 24h at 37°C (APHA, 1978). All plates were done in duplicate and the level of detection was 1 log CFU/g. Results presented below this level indicate that at least one colony was detected in at least one of the six plates counted.

Table 1—Composition of atmospheres (vol/vol)

Atmosphere	1 (air)	2	3	4	5	6	7	8 (vacuum)
% CO_2	0	0	10	25	50	75	100	0
% O_2	21	0	0	0	0	0	0	0
% N_2	79	100	90	75	50	25	0	0

Statistical analyses

Experiments were repeated with three cheese batches constituting three repetitions using a randomized complete block design. A full factorial design of treatments involving eight levels of composition of atmosphere (Table 1) and eight levels of storage time (week 1 to week 8) was employed. Statistical analyses were performed on data using a multifactor ANOVA procedure with the *Statistical Analysis System® (Release 6.08 for VM*, SAS Institute Inc., Cary, NC), to test effects of the two factors and their interaction. Multiple range tests (LSD) were performed on data to determine the influence of atmosphere composition. Significant interactions between factors were examined by comparing the composition of atmospheres at each level of storage time (Mize and Schultz, 1985). Significance was defined as $p \leq 0.05$. A Bartlett test was conducted to verify the homogeneity of variances and logarithmic transformations of data were used when needed to improve homogeneity.

RESULTS & DISCUSSION

Headspace gas composition

The evolution of gas composition in packages was studied during the 8 wk of storage. A decrease in nitrogen concentrations occurred (Fig. 1) in samples of atmospheres 1 to 3 (initial nitrogen concentration ≥79%). Nitrogen slightly decreased in atmospheres 4 and 5 (25/75 and 50/50, CO_2/N_2) but remained stable in atmosphere 6 (75/25, CO_2/N_2). An increase in nitrogen was detected in atmosphere 7 (100% CO_2) where it reached 8.5% on week 8. Air contains 78% nitrogen and the difference should favor the influx of nitrogen into packages with initial concentrations lower than 78%. The ingress of nitrogen in such high barrier bags may however be very slow at 10°C.

Carbon dioxide increased in atmospheres 1 to 5 (initial carbon dioxide concentration ≤50%). The increase was important in samples initially packaged with air (atmosphere 1) where concentrations increased from 0.03% on week 0 to 29.3% on week 8 (Fig. 2). The

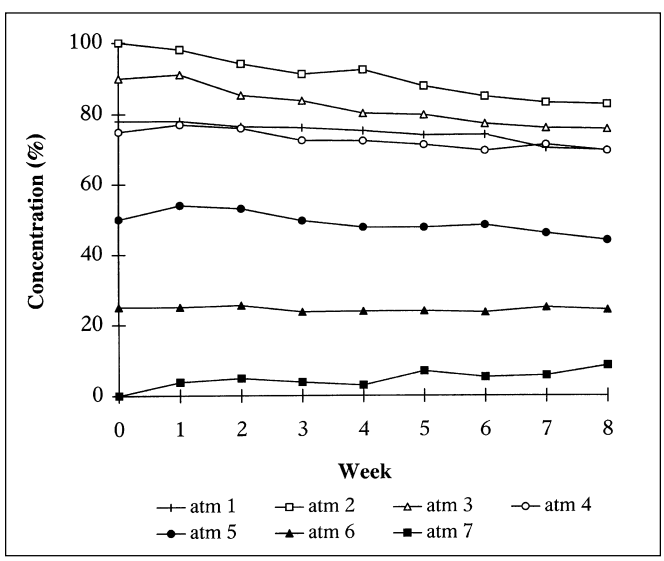

Fig. 1—Evolution of N_2 concentrations in the packages: initial compositions of the modified atmospheres were: atm 1, air; atm 2,100% N_2; atm 3, 10% CO_2/90% N_2; atm 4, 25% CO_2/75% N_2; atm 5, 50% CO_2/50% N_2; atm 6, 75% CO_2/25% N_2; atm 7, 100% CO_2. Means of three replicates.

carbon dioxide which accumulated in these packages probably did not come from the exterior but was apparently produced inside the packages. Piergiovanni et al. (1993) reported a similar phenomenon. This increase in CO_2 concentrations could explain the apparent decrease in N_2 concentrations reported as a percentage of gas content. The composition of atmosphere 6 (75/25, CO_2/N_2) remained stable but CO_2 concentrations in atmosphere 7 dropped to 90.6% on week 8. The detection sensitivity was lower at higher concentrations of CO_2 or N_2, thus the variations observed may not be dependably reliable.

Oxygen concentrations continuously declined as CO_2 increased in atmosphere 1 (Fig. 3). Residual oxygen was 2.8% on week 3. Oxygen was probably consumed inside these packages as Fedio et al. (1994) had noted. Oxygen concentrations increased slightly in other packages and then stabilized after several weeks. An equilibrium state was reached around 0.2% for atmospheres 2, 3 and 4 (respectively 0/100, 10/90 and 25/75, CO_2/N_2), around 0.5% for atmospheres 5 and 6 (50/50 and 75/25, CO_2/N_2) and around 0.8% for atmosphere 7 (100% CO_2). Concentrations of O_2 stabilized at very low levels ($<1\%$ O_2), and thus apparently after week 6, the rate of O_2 permeation was equal to the metabolism rate (Maniar et al., 1994).

Both CO_2 production and O_2 consumption seem to be linked to aerobic microbial metabolism since changes in CO_2 and O_2 were reduced at higher CO_2 concentrations. Carbon dioxide was not further produced under high levels of CO_2 ($\geq 75\%$ CO_2). The rate of O_2 consumption was reduced in these atmospheres and residual oxygen concentrations were higher. Oxygen consumption and CO_2 production in samples of atmosphere 1 (air) were probably due to respiration by aerobic microflora (Fedio et al., 1994) or to oxidative and enzymatic reactions involving oxygen.

Carbon dioxide was produced in significant quantities under anaerobic conditions. A great part of this production was independent of oxygen respiration. Carbon dioxide concentration reached (on week 8) 17% in packages of atmosphere 2 and 24% in atmosphere 3 (initially 0/100 and 10/90, CO_2/N_2). The growth of aerobic and anaerobic microorganisms was probably responsible for this increase (Alves et al., 1996). This CO_2 production may have been caused by heterofermentative Lactobacilli (Lee et al., 1990; Bellengier et al., 1993) or by yeasts (Belin, 1990).

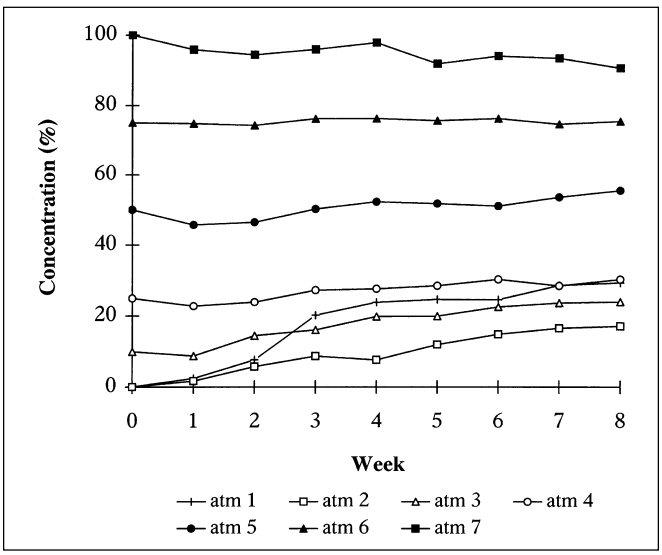

Fig. 2—Evolution of CO_2 concentrations in the packages: atm 1, air; atm 2,100% N_2; atm 3, 10% CO_2/90% N_2; atm 4, 25% CO_2/75% N_2; atm 5, 50% CO_2/50% N_2; atm 6, 75% CO_2/25% N_2; atm 7, 100% CO_2. Means of three replicates.

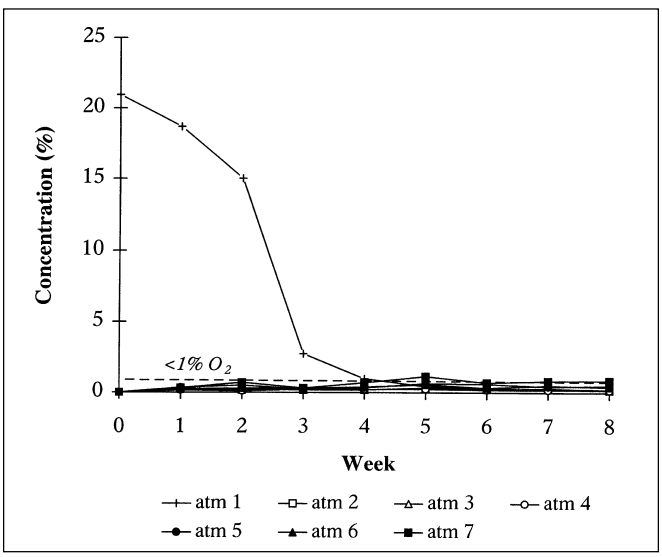

Fig. 3—Evolution of O_2 concentrations in the packages: atm 1, air; atm 2,100% N_2; atm 3, 10% CO_2/90% N_2; atm 4, 25% CO_2/75% N_2; atm 5, 50% CO_2/50% N_2; atm 6, 75% CO_2/25% N_2; atm 7, 100% CO_2. Means of three replicates.

Microbiological analyses

Lactic acid bacteria (LAB) counts varied little (Fig. 4) with all counts within a 1 log range, between 6.73 log CFU/g (atm 1: air, week 1) and 7.70 log CFU/g (atm 4: 50/50 CO_2/N_2, week 4). Means of each treatment were similar: 7.09 for atm 1, 7.34 for atm 7 (100% CO_2) and the others between 7.41 and 7.49 log CFU/g (Table 2). Oxygen sensitivity of anaerobic LAB would explain the decrease observed the first week in the aerobic atmosphere (atmosphere 1). However, LAB counts stabilized and slightly increased after week 4, probably because of oxygen disappearance. Counts remained stable and no growth was observed for cheeses packaged with the other modified atmospheres. Modified atmospheres slightly affected LAB compared to air, but they have reduced development and no growth was observed during the experiment. No significant differences were found between MA containing CO_2 and MA not containing CO_2 (nitrogen and vacuum).

Growth of psychrotrophics was found (Fig. 5) during the first weeks of storage for each modified atmosphere studied. The initial count (4.36 log CFU/g) reached 7 log CFU/g after 3 wk and then stabilized around 7.2 log CFU/g. Means of MAP were also similar

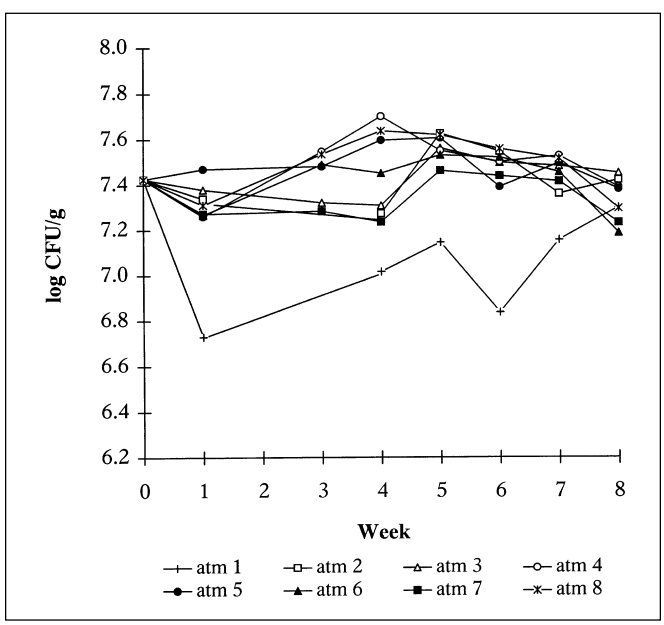

Fig. 4—LAB growth in MAP Mozzarella cheese stored at 10°C: Mean log value (n=3), atm 1, air; atm 2, 100% N_2; atm 3, 10% CO_2/90% N_2; atm 4, 25% CO_2/75% N_2; atm 5, 50% CO_2/50% N_2; atm 6, 75% CO_2/25% N_2; atm 7, 100% CO_2; atm 8, vacuum.

Table 2—Mean counts (standard deviations) in MAP Mozzarella cheese stored at 10°C (log CFU/g of cheese): means were calculated with counts from week 0 to week 8.

Microorganism	atm 1 air	atm 2 100% N_2	atm 3[a] 10% CO_2	atm 4[a] 25% CO_2	atm 5[a] 50% CO_2	atm 6[a] 75% CO_2	atm 7 100% CO_2	atm 8 vacuum
LAB	7.09	7.43	7.43	7.49	7.48	7.41	7.34	7.49
	(0.25)	(0.12)	(0.09)	(0.13)	(0.08)	(0.12)	(0.10)	(0.13)
Psychrotrophs	6.45	6.79	6.79	6.78	6.71	6.69	6.68	6.82
	(0.93)	(1.04)	(1.03)	(1.05)	(1.01)	(1.00)	(0.97)	(1.02)
Staphylococci	3.94	0.74	0.62	0.47	0.65	0.55	0.39	0.76
	(1.43)	(0.48)	(0.43)	(0.41)	(0.41)	(0.35)	(0.42)	(0.54)
Yeast	5.56	1.47	1.59	1.59	0.97	1.10	0.80	2.96
	(1.46)	(0.66)	(0.53)	(0.51)	(0.74)	(0.59)	(0.76)	(0.73)
Mesophilic bacteria	7.12	7.56	7.53	7.52	7.50	7.46	7.42	7.56
	(0.18)	(0.16)	(0.12)	(0.16)	(0.10)	(0.15)	(0.10)	(0.21)

[a]The composition in carbon dioxide is given, the complement is nitrogen.

(Table 2): between 6.68 and 6.82 log CFU/g in atm 7 and 8 respectively (100% CO_2 and vacuum). These results did not confirm the inhibitory effect of CO_2 on psychrotrophics, which had been demonstrated (Scott and Smith, 1971; Kosikowski and Brown, 1973; Chen and Hotchkiss, 1991; Rosenthal et al., 1991; Fedio et al., 1994; Maniar et al., 1994). They confirmed the results of Alves et al. (1996) that growth of psychrophiles also occurred in presence of CO_2 in sliced Mozzarella cheese. They demonstrated that the beginning of growth was retarded with higher CO_2, and their counts stabilized around 7.1 log CFU/g after 25 days in the 50% CO_2 /50% N_2 mixture (vs 7.2 log CFU/g on week 4), and they reached more than 6.5 log CFU/g on the 58th storage day under 100% CO_2 (vs 7 log CFU/g on week 8). The flora making up psychrophiles are complex and the species implicated in Mozzarella cheese may not be as susceptible to CO_2 inhibition.

The relatively high temperature we used may have greatly reduced the CO_2 inhibitory effect. This hypothesis is supported by research which demonstrated that inhibition of *Pseudomonas* by carbon dioxide in cottage cheese was greater at 5°C than at 15°C because of the increased solubility of CO_2 at lower temperatures (Moir et al., 1993). Psychrotrophic organisms were less numerous in samples of atm 1, with a mean count of 6.45 log CFU/g. This contradiction may be attributed to the competition induced by the proliferation of other aerobic microorganisms, such as staphylococci, yeast and molds. In these packages, residual oxygen quickly became a limiting factor of psychrotrophic growth.

The behavior of presumptive staphylococci, yeasts and molds was closely related to the type of atmosphere present in the packages. Each type exhibited the same pattern. Growth occurred in atm 1 (air) until week 3. Staphylococci and yeasts counts increased by 4 logs (Fig. 6 and 7) and low initial molds counts reached 3.92 log CFU/g on week 2 (Table 3). Staphylococci counts stabilized around 4.7 log CFU/g from week 4, yeasts counts varied in a 6–6.9 log CFU/g range and molds in a 3.4–4.3 log CFU/g range. Residual oxygen favored growth but eventual O_2 disappearance and CO_2 accumulation slowed growth after week 3. Counts had a tendency to decrease on weeks 7 and 8, precisely when CO_2 concentration reached 28%.

Staphylococci survived at low levels in atm 2 (100% N_2) and atm 8 (vacuum) where counts reached 1.89 and 1.72 log CFU/g on week 4. Yeasts grew slightly in vacuum packages with a mean count of 2.96 log CFU/g (Table 2) and a final count of 3.5 log CFU/g. Mold detection was sporadic in these packages with final counts at 2.26 and 1.56 log CFU/g on weeks 4 and 8.

The growth of staphylococci, yeasts and molds was completely inhibited under modified atmospheres containing CO_2, except on-week 4 in atm 3 and 4 (10/90 and 25/75, CO_2/N_2) for molds. The counts of staphylococci were <1 log CFU/g. High concentrations of carbon dioxide ($\geq$50% CO_2) also maintained yeast counts $\leq$1 log CFU/g and were more efficient than vacuum to inhibit yeast growth. The decrease of staphylococci and yeast counts in atmospheres containing high levels of CO_2 suggested a destructive effect of carbon

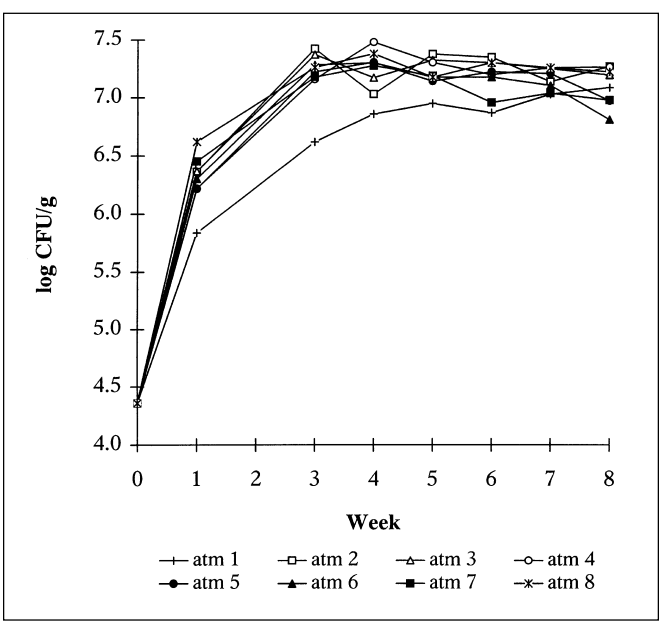

Fig. 5—Psychrotrophic bacteria growth in MAP Mozzarella cheese stored at 10°C: Mean log value (n=3), atm 1, air; atm 2, 100% N_2; atm 3, 10% CO_2/90% N_2; atm 4, 25% CO_2/75% N_2; atm 5, 50% CO_2/50% N_2; atm 6, 75% CO_2/25% N_2; atm 7, 100% CO_2; atm 8, vacuum.

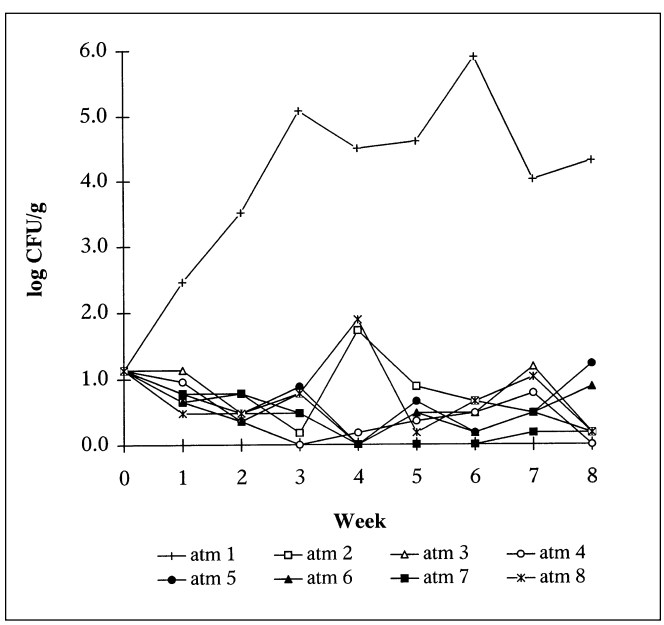

Fig. 6—Staphylococci growth in MAP Mozzarella cheese stored at 10°C: Mean log value (n=3), atm 1, air; atm 2, 100% N_2; atm 3, 10% CO_2/90% N_2; atm 4, 25% CO_2/75% N_2; atm 5, 50% CO_2/50% N_2; atm 6, 75% CO_2/25% N_2; atm 7, 100% CO_2; atm 8, vacuum.

Table 3—Molds growth in MAP Mozzarella cheese stored at 10°C (log CFU/g of cheese): mean of 3 replicates (standard deviation)[a]

Week	atm 1 air	atm 2 100% N_2	atm 3[b] 10% CO_2	atm 4[b] 25% CO_2	atm 5[b] 50% CO_2	atm 6[b] 75% CO_2	atm 7 100% CO_2	atm 8 vacuum
0	0.40 (0.52)	0.40 (0.52)	0.40 (0.52)	0.40 (0.52)	0.40 (0.52)	0.40 (0.52)	0.40 (0.52)	0.40 (0.52)
1	ND	0.18 (0.42)	0.18 (0.42)	ND	ND	ND	ND	ND
2	3.92 (4.16)	ND	0.18 (0.42)	ND	0.18 (0.42)	ND	ND	ND
3	3.41 (3.65)	ND	ND	ND	ND	0.18 (0.42)	ND	ND
4	3.99 (4.01)	ND	1.33 (1.57)	0.78 (1.02)	ND	ND	ND	0.18 (0.42)
5	3.36 (3.55)	ND	ND	ND	ND	ND	ND	2.26 (2.49)
6	4.34 (4.58)	ND	ND	ND	ND	ND	ND	0.18 (0.42)
7	3.50 (3.44)	ND	ND	ND	ND	ND	ND	ND
8	3.38 (3.44)	ND	0.18 (0.42)	0.48 (0.72)	1.03 (1.26)	0.18 (0.42)	ND	1.56 (1.80)

[a]The majority of the results are below the level of detection (1 log CFU/g), but they are given if at least one colony was detected in at least one of the 6 plates.
[b]The composition in carbon dioxide is given, the complement is nitrogen.
ND: not detected in samples (no colony detected in 6 plates).

dioxide. Final counts on week 8 were lower than initial counts in most cases, and equal to initial counts in the others (atm 5:50/50 CO_2/N_2 for yeast). Molds were not detected in the majority of samples containing CO_2 (atm 3 to 7:10–100% CO_2). The inhibitory properties of carbon dioxide have been clearly demonstrated (Kosikowski and Brown, 1973; Chen and Hotchkiss, 1991; Rosenthal et al., 1991; Day, 1992; Fedio et al., 1994; Alves et al., 1996).

Mesophilic bacteria counts remained relatively stable and varied between 6.84 log CFU/g (atm 1, week 1) and 7.82 log CFU/g (atm 2:100% N_2, week 2) within a 1 log range (Fig. 8). Their behavior was nevertheless dependent on the composition of the atmosphere. Mesophilic counts of cheeses packaged under atmosphere 1 (air) decreased during the first weeks, confirming the inhibition of lactic bacteria by oxygen. Then, they progressively increased because of the multiplication of yeasts, molds and staphylococci. However, mesophilic counts increased under the other MAP (development of psychrotrophic bacteria) and then stabilized with a tendency to decrease after week 4.

Conclusions about a specific effect of CO_2 on mesophilic bacteria may not be reliable because this class contains many different populations. The composition of atmosphere had a significant effect on counts of mesophilic bacteria. The multiple range comparison test (LSD) demonstrated that counts were lower under higher concentrations of CO_2 (≥75%) than under vacuum or nitrogen (Table 4: d vs a). High CO_2 concentrations were more effective than vacuum or nitrogen in reducing growth of mesophilic bacteria. Enterococci and coliforms were not detected in most samples (data not shown). This indicated that manipulation of samples and our sanitation conditions were appropriate and that no post-contamination occurred.

Table 4—Multiple range comparison of various compositions of modified atmospheres for Mean[a] Mesophilic Bacteria counts (CFU/g of cheese)[b]

Atmosphere	Mean Count	$LSD_{0.05}$				
8 (Vacuum)	42609848	a				
2 (100%N_2)	40914773	a	b			
4 (25%CO_2)	36938735		b	c		
3 (10%CO_2)	36386364		b	c		
5 (50%CO_2)	33753788			c		
6 (75%CO_2)	31450758			c	d	
7 (100%CO_2)	27472332				d	
1 (Air)	13649621					e

[a]Counts are the mean of three replicates for 9 wk of experiment (from week 0 to week 8)
[b]Means with different letters are significantly different (p≤0.05)

CONCLUSIONS

CARBON DIOXIDE WAS EFFECTIVE IN REPRESSING UNDESIRABLE microorganisms such as staphylococci, yeasts and molds. Carbon dioxide was not as effective in repressing psychrotrophic bacteria but it reduced growth of lactics and mesophilics. Two hypotheses may explain the small effect of carbon dioxide on psychrotrophic flora: a high temperature of storage, or a reduced sensitivity to CO_2 in Mozzarella

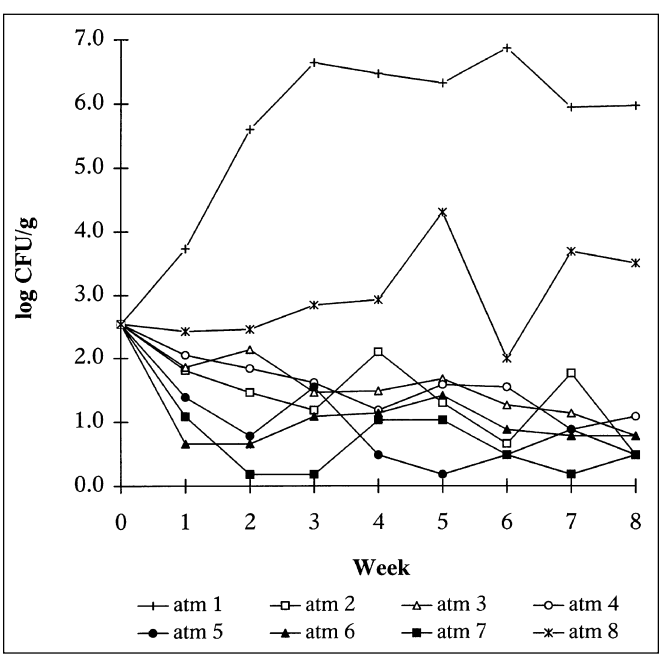

Fig. 7—Yeast growth in MAP Mozzarella cheese stored at 10°C: Mean log value (n=3),atm 1, air; atm 2, 100% N_2; atm 3, 10% CO_2/90% N_2; atm 4, 25% CO_2/75% N_2; atm 5, 50% CO_2/50% N_2; atm 6, 75% CO_2/25% N_2; atm 7, 100% CO_2; atm 8, vacuum.

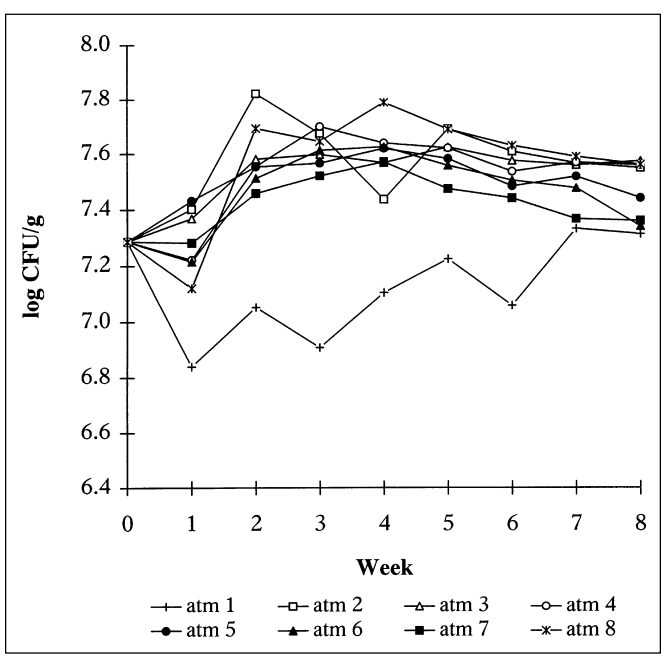

Fig. 8—Mesophilic bacteria growth in MAP Mozzarella cheese stored at 10°C: Mean log value (n=3), atm 1, air; atm 2, 100% N_2; atm 3, 10% CO_2/90% N_2; atm 4, 25% CO_2/75% N_2; atm 5, 50% CO_2/50% N_2; atm 6, 75% CO_2/25% N_2; atm 7, 100% CO_2; atm 8, vacuum.

cheese. Higher CO_2 concentrations were more effective than nitrogen to control mesophilics, and were also more effective than vacuum packaging in inhibiting yeasts and molds. CO_2 levels $\geq$75% were the most appropriate for maintaining microbiological quality and safety of shredded Mozzarella cheese during 8 wk and for reducing carbon dioxide production inside packages, hence minimizing package distention.

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