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Effect of protective atmospheres on physicochemical, microbiological and rheological characteristics of sliced Mozzarella cheese

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ABSTRACT

Mozzarella cheese slices packaged in bags of polyvinylidene chloride under vacuum (V) and using a gas mixture of equal parts of CO_2 and N_2 (G) were stored at 4 °C for 8 weeks and analysed at different storage times by physicochemical, microbiological and rheological characterization. Expected values of moisture, total nitrogen, soluble nitrogen at pH 4.6, and chloride contents and of pH were observed. The degradation of α_{s1}-casein was greater than β-casein degradation, while no significant differences were observed due to the packaging methods. Coliform microorganisms were not detected, while levels of moulds and yeasts counts were acceptable. Expected values of total mesophile counts were obtained. The temperature at crossover moduli (T_c) was determined from temperature sweeps carried out by rheometry. Greater values of T_c were observed in samples G. The influence of temperature on complex viscosity was studied by an Arrhenius-type equation. Activation energy values were obtained from the solid-like region (20–40 °C) and liquid-like region (40–60 °C). A more rapid change in viscosity with temperature was observed when storage time increased and when storage method V was used.

1. Introduction

Modified atmosphere packaging (MAP) technologies increase commercial life of cheeses because they combine the protection against oxidation and dehydration with the inhibition of undesirable microorganisms (Eliot, Vuillemard, & Emond, 1998; Juric, Bertelsen, Mortensen, & Petersen, 2003). For the application of these technologies, films with low water vapour transmission and low oxygen permeability, and modified gaseous environment are used. In this sense, atmospheres containing CO_2 and N_2 have been effective to inhibit the proliferation of undesirable microorganisms (García Iglesias, Gago Cabezas, & Fernández Nuevo, 2006; Mortensen, Bertelsen, & Nielsen, 2004; Ooraikul, 2003). These gases are common and readily available, safe, economical and not considered as chemical additives (Mastromatteo, Conte, & Del Nobile, 2010). Products also may be packaged under vacuum but it may not be appropriate for fragile products such as shredded cheese (Eliot et al., 1998; García Iglesias et al., 2006). Furthermore, surface area of shredded products increases making them more susceptible to degradative changes; therefore MAP technology should be more convenient (Juric et al., 2003).

The potential of MAP for extending commercial life of cheese has been clearly demonstrated, although cheese packaging is dependent on the type of cheese, the starter used during manufacturing and storage conditions, among very important parameters (Gammariello, Conte, Di Giulio, Attanasio, & Del Nobile, 2009). In the last decades, MAP of different cheese varieties has been studied (Rodriguez-Aguilera, Oliveira, Montanez, & Mahajan, 2011), but scarce information related to MAP of Mozzarella cheese or similar *pasta filata* type cheese is available.

Alves, Sarantópoulos, Van Dender, and Faria (1996) studied the stability of sliced Mozzarella cheese in modified atmosphere packaging. The authors verified the bacteriostatic and fungistatic properties of CO_2 and found a significant shelf life increase under atmospheres containing that gas compared with air (385% under CO_2, 246% under equal parts of CO_2 and N_2). Eliot et al. (1998) investigated the effect of a wide range of MAP on growth of microorganisms in shredded Mozzarella cheese and determined the optimal gas composition to improve cheese preservation under retail simulated conditions. The authors found that CO_2 was effective in inhibiting undesirable microorganisms such as staphylococci, yeast and moulds; it was not as effective in inhibiting

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psychrotrophic bacteria but it reduced growth of lactics and mesophilics. Carbon dioxide levels ≥ 0.75 were the most appropriate for maintaining microbiological quality and safety of shredded Mozzarella cheese during 8 weeks. Gammariello et al. (2009) determined the microbiological, pH, and sensory changes in Stracciatella cheese, stored under MAP conditions at 8 °C. The authors found that microbial stability limits the shelf life of all samples, whereas the samples under MAP have a better quality from a sensorial point of view when compared with the traditional packaging. In particular, the mixture 0.5 CO_2/0.5 N_2 and 0.95 CO_2/0.05 N_2 were the most effective in retaining good sensory characteristics.

Although MAP of Mozzarella cheese is a promising method for packaging, there is a lack of information related to adequate packaging conditions and the effects of MAP on microstructural characteristics of Mozzarella cheese (Eliot et al., 1998). Moreover, literature related to the effect of MAP and vacuum packaging on melting properties of Mozzarella cheese was not found, when they are the most important functional properties of this type of cheese. Therefore, the aim of this work was to study the effect of MAP and vacuum on physicochemical, microbiological and rheological characteristics of sliced Mozzarella cheese.

2. Materials and methods

2.1. Cheese sampling and treatments

Seven blocks of fresh Mozzarella cheese ($28 \times 10 \times 10\ cm^3$ in size), which were provided by a local factory and stored at 4 °C for 15 days, were used. Cheese manufacture was carried out according to the Código Alimentario Argentino specifications (CAA, 2010). Slices of 2 mm thickness were obtained and packaged in bags of polyvinylidene chloride barrier layer (BC40LA model, Cryovac Division, Sealed Air, Argentina). Bags have an oxygen permeance of 0.1–0.18 $cm^3\ m^{-2}\ day^{-1}\ kPa^{-1}$ (23 °C, 0% RH) and a water vapour transmission rate of 7.5 $g\ day^{-1}\ m^{-2}$ (38 °C, 100% RH). Two packaging methods were evaluated: vacuum (V) and packaging under a mixture of equal parts of CO_2 and N_2 (G). Gas mixture was selected considering previous results reported in literature (Eliot et al., 1998; Trobetas, Badeka, & Kontominas, 2008). The gases were industrial mixtures provided by Praxair Argentina S.R.L. (Buenos Aires, Argentina). The packages were evacuated, flushed and sealed on a Turbovac Serie S packaging machine (Cerveny, 's-Hertogenbosch, Netherlands). For each packaging method, 60 bags containing 10 slices were obtained and stored at 4 °C for 8 weeks.

2.2. Physicochemical analysis

Samples obtained at 1, 8, 15, 29, 43 and 57 days of storage were used for physicochemical analysis. The pH was determined with an electrode for solid foods (pH spear, Oakton Instruments, Vernon Hills, IL, USA). Chloride content was determined as suggested by Fox (1963) with an automatic titrator model DL40 RC (Mettler Instrumente AG, Greifensee, Switzerland). Moisture content was determined in a microwave CEM AVC 80 (CEM, Matthews, NC, USA) (AOAC, 1990). Water-soluble fraction extraction at pH 4.6 was performed with a modified procedure developed by Kuchroo and Fox (1982) and modified by Sihufe, Zorrilla, and Rubiolo (2003). Total nitrogen (TN) and water-soluble nitrogen (WSN) were determined using the micro-Kjeldahl method with an automatic digestor model 430, a distillation unit model 322 and a control unit model 342 (Büchi, Flawil, Switzerland). Fat content was determined for initial composition (International Dairy Federation Standard 5A; IDF, 1969). Physicochemical analyses were carried out in duplicate.

Maturation index (MI) was calculated as a percentage of WSN of the cheese TN (WSN × 100/TN), and it was used to follow the proteolysis degree during ripening (Kuchroo & Fox, 1982). Proteolysis was also evaluated by urea-PAGE using the procedure described by Sihufe, Zorrilla, and Rubiolo (2010).

2.3. Microbiological analysis

Samples obtained at 1, 29 and 57 days of storage were used for microbiological analysis. Packages were aseptically opened and 20 g of cheese were dispersed in 180 mL of a 0.02 $g\ mL^{-1}$ sterile sodium citrate solution (15 min, 121 °C) using a lab paddle blender (Lab Cima, Buenos Aires, Argentina) for 2 min to obtain a 10^{-1} dilution. Serial decimal dilutions of this suspension were prepared with 0.001 $g\ mL^{-1}$ sterile peptone solution. Total aerobic mesophilic microorganisms were counted on Plate Count Agar (Britania, Buenos Aires, Argentina) with the addition of 0.1 $g\ mL^{-1}$ reconstituted and sterilized skim milk powder (Milkaut S.A., Franck, Argentina) (30 min, 112 °C) after incubation at 30 °C for 48 h (Frank, Christen, & Bullerman, 1993). Total presumptive coliforms were counted on Violet Red Bile Agar (Britania, Buenos Aires, Argentina) after incubation at 32 °C for 24 h (Christen, Davidson, McAllister, & Roth, 1993). Yeast and moulds were enumerated on Yeast and Mould agar (Britania, Buenos Aires, Argentina) after incubation at 25 °C for 5–7 days (IDF Standard 94A; IDF, 1985). All microbiological analyses were carried out in duplicate.

2.4. Rheological measurements

Samples obtained at 8, 15, 29, 43 and 57 days of storage were used for rheological measurements. A rheometer Haake RheoStress RS80 (Haake Instrument Inc., Paramus, NJ, USA) with parallel plates (35 mm diameter, 2 mm gap) at a frequency of 1 Hz was used for rheological measurements. Sand paper in the upper plate was used to eliminate slippage. The temperature of the lower plate of the measuring system was maintained by circulating water from water bath. The disk-shaped cheese sample was placed on the lower plate and then the upper plate was brought in contact with the sample to attain temperature equilibrium. A thin film of silicone oil (20 cP) covered the edge of samples to avoid evaporation during measurements.

The dynamic rheological data obtained included the 2 components of complex shear modulus (G^*): the storage modulus (G'), which is a measure of the energy stored and recovered per cycle of deformation (elastic component), and the loss modulus (G''), which is a measure of the energy dissipated or lost as heat per cycle of deformation (viscous component) (Gunasekaran & Ak, 2003). These parameters are related as follows:

$$\left|G^*\right|^2 = (G')^2 + (G'')^2 \quad (1)$$

Temperature sweep tests were carried out from 20 to 60 °C (1.33 $°C\ min^{-1}$) at a strain amplitude of $0.01 \pm 5 \times 10^{-4}$. The linear viscoelastic region was determined by performing strain sweep tests from 0.001 to 0.1 at 20 °C and 60 °C for all storage times studied. From temperature sweeps, the crossover temperature (T_c) of moduli ($G' = G''$) was determined, which may be used for identifying the transition to the melted state as cheese is heated. Moreover, the effect of temperature on complex viscosity ($|\eta^*| = |G^*|/\omega$, ω: frequency of oscillation) was studied by an Arrhenius-type equation:

$$\left|\eta^*\right| = A_{VISC} \exp(Ea/RT) \quad (2)$$

where A_{VISC} is the pre-exponential factor, Ea is the activation energy (cal mol^{-1}), R is the gas constant (1.9872 cal mol^{-1} K^{-1}), and T is the temperature (K) (Rao, 1999, chap. 2; Tunick, 2000, 2010).

2.5. Statistical analysis

Data were analysed by ANOVA using Statgraphics (Statgraphics Inc., Rockville, MD, USA). When differences between treatment effects were significant ($P < 0.05$), a multiple comparison of means was performed.

3. Results and discussion

3.1. Physicochemical characteristics

The initial fat content was $19.90 \leq 0.06$ g/100 g. Physicochemical composition of Mozzarella cheese for the different conditions studied is shown in Table 1. Expected values of moisture, total protein, and chloride contents and of pH were observed (McMahon, Fife, & Oberg, 1999; Olivares, Zorrilla, & Rubiolo, 2009; Subramanian & Gunasekaran, 1997).

Maturation index increased during the storage period studied, approximately from 3.12 g/100 g to 5.78 g/100 g in samples V and from 3.88 g/100 g to 6.24 g/100 g in samples G, possibly because the residual coagulant and plasmin cause the proteolysis of α_{s1} and β-casein (Farkye, Kiely, Allshouse, & Kindstedt, 1991; Fox & Guinee, 1987). The partial thermal inactivation of the coagulant during kneading and stretching process may be responsible of the low level of proteolysis (Feeney, Fox, & Guinee, 2001; Yun, Barbano, & Kindstedt, 1993). The storage method did not significantly affect any of the physicochemical parameters studied. Results obtained from the urea-PAGE patterns showed that α_{s1}-I-casein formation (and consequently, α_{s1}-casein degradation) was more noticeable than β-casein degradation during the storage period, but no difference due to storage method was detected (Table 2).

3.2. Microbiological characteristics

Microbiological characteristics of cheese for the different conditions studied are shown in Table 3. It was observed that coliform microorganisms were not detected for both storage methods, while counts of yeast and moulds remained controlled and in acceptable levels. Expected values of total aerobic mesophilic counts were obtained, which decreased with storage time without a significant effect due to the storage method. These results agree with those reported in the literature (Eliot et al., 1998).

Table 1
Physicochemical composition of Mozzarella cheese samples studied.[a]

Storage method	Storage time (days)	pH	Moisture (g/100 g)	Total protein (g/100 g)	Chloride (g/100 g)	MI (g/100 g)
V	1	5.44	49.64	22.34	0.77	3.12a
	8	5.40	49.45	23.57	0.76	4.04ab
	15	5.44	50.56	23.44	0.72	3.87ab
	29	5.47	48.79	22.68	0.68	5.13cd
	43	5.49	49.21	23.91	0.71	5.79de
	57	5.42	50.16	24.02	0.71	5.78de
G	1	5.48	49.41	24.13	0.74	3.88ab
	8	5.47	48.87	24.16	0.76	3.48ab
	15	5.40	49.49	23.94	0.63	4.29bc
	29	5.37	49.35	23.30	0.70	5.20cd
	43	5.47	50.02	23.50	0.71	5.35de
	57	5.42	49.95	24.43	0.78	6.24e

V: cheese slices packaged under vacuum.
G: cheese slices packaged using a gas mixture of equal parts of CO_2 and N_2.
MI: maturation index.
[a] Mean values with different letters in a column indicate significant differences ($P < 0.05$).

Table 2
Mean values of integrated optical density (IOD g^{-1} of cheese) of the fractions determined by urea-PAGE in the Mozzarella cheese samples studied.[a]

Storage method	Storage time (days)	β-CN	α_{s1}-CN	α_{s1}-I-CN
V	1	61.7	75.6cde	2.4a
	8	72.4	83.2e	3.8a
	15	65.2	72.7bc	5.1ab
	29	63.4	69.3abc	9.1c
	43	70.2	69.4abc	14.1d
	57	78.4	71.6bc	21.1f
G	1	54.2	70.3abc	1.7a
	8	69.1	81.2de	3.1a
	15	68.3	74.3cd	3.6a
	29	59.2	66.3ab	8.5bc
	43	79.7	76.1cde	16.0de
	57	69.8	63.4a	18.4ef

V: cheese slices packaged under vacuum.
G: cheese slices packaged using a gas mixture of equal parts of CO_2 and N_2.
[a] Mean values with different letters in a column indicate significant differences ($P < 0.05$).

3.3. Rheological characteristics

Using the temperature sweep tests, the influence of temperature on rheological properties of Mozzarella cheese was analysed. During a temperature sweep, the crossover temperature T_c is considered as the beginning of the cheese melting (Gunasekaran & Ak, 2003, chap. 5). In addition, this rheological assay allows studying the viscoelastic behaviour of the material at the temperature range analysed.

The changes of G' and G'' with temperature is shown in Fig. 1 where the transition from solid to melted state is clearly observed. Below 40 °C, the material behaves predominantly as an elastic solid (indicated by G' values greater than G'' ones). As temperature exceeds 40 °C, the cheese begins melting, undergoing structural changes. These changes include the fat transition from solid to liquid state and the increase of mobility of the protein phase. Above T_c, G'' values are greater than G' ones and the material behaves mainly as a viscous liquid. These results agree with literature data for this type of material (Gunasekaran & Ak, 2003, chap. 5; Muliawan & Hatzikiriakos, 2007). In addition, water acts as plasticizer and modifies some properties of this type of food polymer (Gunasekaran & Ak, 2003, chap. 5). As proteolysis takes place, a more hydrated protein structure allows the protein to slip

Table 3
Mean values of log CFU g^{-1} of cheese corresponding to the microbial groups in Mozzarella cheese samples studied.[a]

Storage method	Storage time (days)	Total aerobic mesophilics	Coliforms	Yeast and moulds
V	1	9.40a	ND	2.23
	29	8.90c	ND	4.57
	57	8.97bc	ND	3.99
G	1	9.24ab	ND	3.61
	29	8.91c	ND	3.92
	57	8.95bc	ND	2.62

ND: not detected.
V: cheese slices packaged under vacuum.
G: cheese slices packaged using a gas mixture of equal parts of CO_2 and N_2.
[a] Mean values with different letters in a column indicate significant differences ($P < 0.05$).

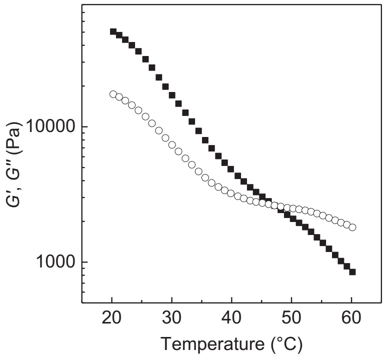

Fig. 1. Typical temperature sweep of a Mozzarella cheese sample stored under vacuum during 29 days. (■) G', (○) G''.

past one another more easily and, when combined with the lubricating properties of the fat, it results in improved meltability and in a decrease of T_c value (McMahon et al., 1999; Ribero, Rubiolo, & Zorrilla, 2007).

Crossover temperatures of cheese for the different conditions studied are listed in Table 4. ANOVA indicated that main factors, storage time and storage method, had significant effect, while the interaction between them had not significant effect. It was observed that T_c decreased as storage time increased, while greater values of T_c were obtained in samples G (Fig. 2). Differences between samples V and G were observed from the very beginning of the storage period.

Microstructural information was obtained from temperature sweeps by analysing the activation energy of flow (*Ea*) estimated by Eq. (2). It is worth mentioning that in the case of cheese, *Ea* has not the typical significance of an energy barrier required to overcome resistance to flow. The value of *Ea* quantifies how quickly the structure degrades with heating (Tunick, 2010). Therefore, the effect of storage time and storage method on *Ea* values should provide more extensive information of cheese matrix changes during heating. Moreover, as it was illustrated above, cheese can be classified as a multiphase system that exhibits a solid-like behaviour, and that gradually changes to a liquid-like behaviour as temperature is increased. As reported by Muliawan and Hatzikiriakos (2007), this implies that the data obtained at temperatures above 40 °C correspond to a structurally different material. For this reason, temperature sweep data were divided in two temperature ranges and *Ea* values were obtained from a solid-like region (20–40 °C) and a liquid-like region (40–60 °C). Fig. 3 clearly shows the different rheological behaviour of cheese at the two temperature ranges.

Table 4
Mean values of T_c corresponding to Mozzarella cheese samples studied.

Storage method	Storage time (days)	T_c (°C)
V	8	48.46
	15	45.05
	29	44.35
	43	42.42
	57	41.59
G	8	50.68
	15	46.13
	29	45.00
	43	44.23
	57	42.76

V: cheese slices packaged under vacuum.
G: cheese slices packaged using a gas mixture of equal parts of CO_2 and N_2.
T_c: crossover temperature of moduli G' and G''.

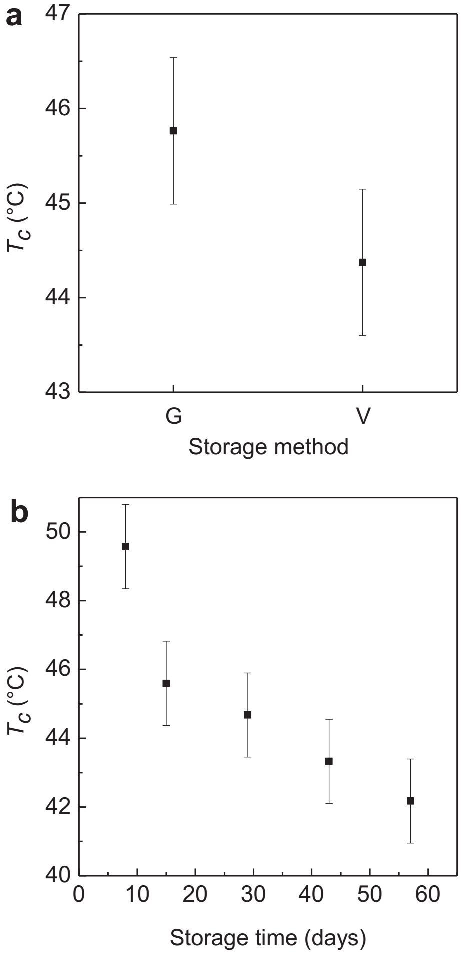

Fig. 2. Mean values of T_c (crossover temperature of moduli G' and G'') of Mozzarella cheese samples. (a) Storage method effect, (b) storage time effect. Bars indicate confidence interval.

Values of *Ea* for the different studied conditions are listed in Table 5. It can be observed that *Ea* values are higher in the solid-like region than those in the liquid-like region. That indicates a more rapid change in viscosity with temperature (Steffe, 1996, chap. 1) at the lowest temperature range evaluated (20–40 °C). ANOVA indicated that main factors, storage time and storage method, had

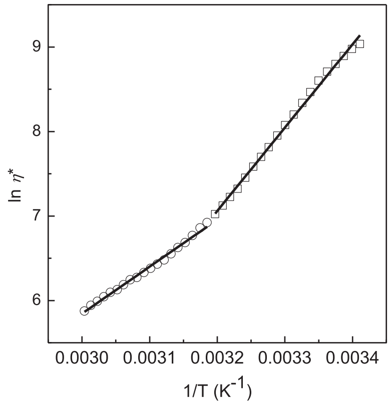

Fig. 3. Typical complex viscosity values versus reciprocal absolute temperature of a Mozzarella cheese sample stored under vacuum during 8 days. (□) range 20–40 °C, (○) range 40–60 °C.

Table 5
Mean values of activation energy corresponding to Mozzarella cheese samples studied.[a]

Storage method	Storage time (days)	*Ea* (kcal mol^{-1}) range: 20–40 °C	*Ea* (kcal mol^{-1}) range: 40–60 °C
V	8	18.9a	11.6c
	15	20.7bc	10.4b
	29	21.7cde	9.9ab
	43	21.9de	13.5d
	57	22.5e	13.7d
G	8	18.6a	11.6c
	15	20.0b	10.9bc
	29	20.2b	9.0a
	43	20.9bcd	10.0ab
	57	21.9de	11.5c

V: cheese slices packaged under vacuum.
G: cheese slices packaged using a gas mixture of equal parts of CO_2 and N_2.
[a] Mean values with different letters in a column indicate significant differences ($P < 0.05$).

significant effect on *Ea* values obtained from both temperature ranges. In the 20–40 °C range, *Ea* increased as storage time increased, while greater values of *Ea* were obtained for samples V. In the 40–60 °C range, *Ea* increased as storage time increased but that behaviour was not clearly observed in samples G. In addition, *Ea* values in samples V were greater than in samples G.

Studying *Ea*, it can be observed that cheese matrix was degraded more quickly with heating as storage time increased and when storage method V was applied. These results are in agreement with the lower values of T_c obtained for samples V. However, the cheese matrix degradation was less perceptible in the liquid-like range. Therefore, the temperature range for *Ea* evaluation is critical for determining cheese microstructural changes.

The differences observed in T_c and *Ea* values between the storage methods studied may not be ascribed to changes in proteolysis degree of samples because no differences were found in results obtained by MI and urea-PAGE. Therefore, it may be inferred that the compression induced by the storage method V modifies other cheese microstructural characteristics, such as, the arrangement of protein matrix, size and shape of fat globules or water distribution. The greater T_c values obtained in samples G indicate that in spite of cheese maturation continues for both storage systems studied, samples stored with method G maintain the rheological characteristics of the original material for a longer period of time.

4. Conclusions

It is concluded that both storage methods are appropriate to preserve Mozzarella cheese during the studied storage period, although a different rheological behaviour was observed in samples packaged with the storage methods analysed. These differences may be associated with microstructural characteristics of the material. It may be inferred that the compression induced by the storage method V modifies other cheese microstructural characteristics, such as the arrangement of protein matrix, size and shape of fat globules or the water distribution. These results also show that rheological assays are capable to detect microstructural changes that may affect some functional properties of Mozzarella cheese.

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