

Shelf Life Determination of Fresh Cheese Subjected to Different Modified Atmospheres Packaging

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ABSTRACT

The aim of this study was to determine the physicochemical, microbiological, and sensory properties as well as shelf life of fresh mozzarella cheese samples. Fresh mozzarella cheese samples were packaged under five different Modified Atmospheres (MAP): Vacuum (Atm 2), 40% CO₂/60% N₂ (Atm 3), 60% CO₂/40% N₂ (Atm 4), 100% CO₂ (Atm 5) and 100% N₂ (Atm 6). Identical cheese samples were packaged in air (Atm 1), taken as the control. All cheese samples were kept under refrigeration (4±1°C) for 6 weeks. Atm 5 gas mixture was the most effective for inhibiting aerobic microflora growth in cheese samples stored at 4°C during 6 weeks. Lactic acid bacteria were not affected by CO₂ presence even in high concentrations. Yeasts and moulds were totally inhibited by Atm 5 gas mixture throughout the entire storage period. Sensory evaluation showed that cheese packaged under Atm 3 retained good sensory characteristics for 6 weeks of storage while control samples were appreciated the least. Atm 5 provided the best shelf life extension at 4°C by 81 days, compared to the control.

Keywords: Aerobic microflora, Mozzarella cheese, Sensory evaluation, Yeasts.

INTRODUCTION

Several preservation methods are useful to extend the shelf-life of various food products, among which Modified Atmosphere Packaging (MAP) technique is the most promising. The packaging technology under modified atmospheres is a multidisciplinary technology based on the fundamental principles of chemistry, physics, microbiology, food science, engineering and polymer chemistry (Lioutas, 1988). This technique undertakes many basic roles such as improving the product image, preventing microbial growth and chemical deterioration, protecting sensorial properties and extending the shelf-life of many food products (Bal, 2016), including dairy products, mainly cheeses (Khoshgozaran *et al.*, 2012). The cheese preservation implies to ensure its

protection against dehydration, which can be done easily using packaging with low permeability to water vapour, and to reduce the excessive microorganisms' growth. These microorganisms are responsible for cheese lipolysis and proteolysis causing changes in flavour and odour, while contaminants such as yeasts or molds modify the texture and the appearance (Fedio *et al.*, 1994). Generally, the microorganisms' growth in the cheeses depends on the availability of nutrients, water activity, pH, ionic strength, temperature and the composition of the atmosphere in the headspace (Pintado *et al.*, 2001).

Several studies have been conducted on the effect of MAP on the shelf-life as well as the properties of hard and semi-hard cheeses (Juric *et al.*, 2003), fresh cheeses (Olarte *et al.*, 2002) and whey cheese such as Requejase (Pintado and Malcata, 2000). These authors summarized that the cheese packaging

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depends on several parameters such as the nature of ferments for cheese production, the type of cheese, the initial microbial contamination and the storage conditions. Gonzalez-Fandos *et al.* (2000) have studied the effect of different gas mixtures on a typical Spanish fresh cheese quality preservation at a storage temperature of 4 °C. These authors have shown that the most effective gas combination to extend the shelf life and retain good sensory characteristics of the studied cheeses corresponds to that with a CO₂ content ranging between 50 and 60%. Estimating the shelf-life of foods is essential to protect the consumer health and successful marketing (Schmidt and Bouma, 1992). Accelerated Shelf-Life Testing (ASLT) is a method which evaluates product stability, based on data that is obtained in a significantly shorter period than the actual shelf-life of the product (Labuza, 1994). In this context, the aim of this work was to determine the physicochemical, microbiological, and sensory changes in prepared Mozzarella cheese during storage under vacuum and different modified atmospheres at 4°C and to determine the shelf life of these cheeses under the same packing conditions using ASLT method.

MATERIALS AND METHODS

Sample Preparation

Fresh cow milk was purchased from a local breeding located in Tunis region (Tunis governorate, Tunisia). Once arrived to the laboratory at 4°C, a pH (Metrohm pH meter) determination was realized. Then, cow milk was skimmed by centrifugation at 3,500×g during 15 minutes at 20°C (Gyrozen 1580MGR, Multi-purpose Centrifuge, Daejeon, Korea), heat treated to 72±0.1°C for 3 seconds and then cooled to 35±0.5°C and liquid calf rennet was added (*M. miehei*, strength 1:10 000, Laboratories ARRAZI, PARACHIMIC, Sfax, Tunisia). The curd formation was achieved after approximately 30 minutes and then the curd was cut, collected and drained into

polyvinylchloride moulds, kept at room temperature (20±2°C) for 2 hours, and then placed in a cooling chamber (5±0.5°C, 80–90% RH) for 24 hours. Cheeses were shredded to be immediately used for physico-chemical, microbiological and sensory analyses as well as shelf-life determination. The shredded form is particularly suitable for the production of such a study since it increases very significantly the gas contact with the surface flora.

Cheese samples of approximately 90 g were packaged in plastic bags (150×150 mm and thickness 200 µm). These bags were composed of 99.35% Low Density PolyEthylene (LDPE) layer (114.120°C, 27.97 mW) and 99.58% PolyAmide 6 (PA6) layer (219.108°C, 22.79 mW), and had an oxygen permeation transmission rate of 6.63±0.06 (cm³ m⁻² d⁻¹) (OX-TRAN, model 2/60, 7500 Mendelssohn Ave N, Minneapolis, MN 55428, USA) and water vapour permeation transmission rate of 1.29 ± 0.19 (g m⁻² d⁻¹) (MOCON PERMATRAN-W, model 3/60, 7500 Boone Ave N, Minneapolis, MN 55428, USA). The different mixtures of carbon dioxide / nitrogen were injected into these bags using a gas mixer (Dansensor, Model Mix 9000, PBI, Ringsted, Denmark). Pouches were heat-sealed by means of a packaging machine (R 200, Reepack, Italy) connected to the gas mixer. Cheese samples were separated into 5 lots, each of which constituted 30 samples, and were packaged as follows: Atm 1 (control samples, packaged under air), Atm 2 (packaged in vacuum), Atm 3 (packaged in 40% CO₂/60% N₂), Atm 4 (packaged in 60% CO₂/40% N₂), Atm 5 (packaged in 100% CO₂) and Atm 6 (packaged in 100% N₂). All samples were stored at 4°C for 6 weeks. The determinations of headspace gas composition, physicochemical attributes, and microbial count were carried out before packaging and after 1, 2, 3, 4, 5 and 6 weeks of storage and repeated at least 3 times.

Headspace Gas Composition

The headspace gas composition was determined using an analyzer (Dansensor model checkmate 9000, PBI, Denmark). About 10 cm³ was taken from each package headspace for gas analysis with a gastight syringe. The composition of the headspace gas was determined using a gas chromatograph (HP 5890 Series II, Toronto, Ontario) with a thermal conductivity detector. Each package was used only for one single determination.

Microbiological Analyses

Ten grams of the shredded cheese samples were diluted with 90 mL of a buffered peptone water (BPW, Merck, Darmstadt, Germany) of 25.5 g L⁻¹ using Stomacher bags (PBI, International Milan, Italy) for 60 seconds. Then, decimal dilutions were prepared with the same diluents and plated onto the surface of the appropriate Petri dishes. Analyses were carried out using the following procedures: Total microbial counts were enumerated on Plate Count Agar (PCA, Merck) and incubated at 30°C for 48 hours (APHA, 1985); Lactic Acid Bacteria (LAB) on Man Rogosa Sharpe Agar (MRS, pH 6.2) and incubated at 30°C for 48 hours (Gerhardt *et al.*, 1994), and yeast and moulds on Sabouraud Dextrose Agar, supplemented with chloramphenicol (0.1 g L⁻¹) and incubated at 25°C for 5 days (APHA, 1992). The results were expressed as common logarithm colony forming units per gram of cheese. Enumeration of microorganisms in each experiment was conducted in triplicate and the level of detection was 1 log CFU g⁻¹.

Physico-Chemical Analyses

The pH values of the cheese samples were determined using pH meter (Hanna Instruments, Portugal) connected to an electrode 406 M 6 (Mettler Toledo, France).

The moisture (%) of the samples was determined by dehydration at 103°C for 7 hours using a drying oven (WTC binder, 78532 Tuttlingen, Germany) according to AOAC (1995) procedures. The weight loss (%) of the cheese samples was calculated by deducing the difference between the initial weight of the cheese sample at $t = 0$ and its weight at t_i . Three packages per treatment were randomly selected on each sampling day and every 3 days during the storage life.

Sensorial Analyses

Shredded cheese samples (4±0.5°C, 80–90% RH) were evaluated by a 100-member panel recruited among staff and students of both the Technical Center of Food Packaging and the High School of Food Analysis (Tunis, State of Tunisia) who stated that they were cheese lovers and users. Each of the cheese samples studied in this paper was coded with three-digit random numbers, and randomly presented to the panel. Panel members evaluated cheeses for colour, texture, and flavour (odour and taste) using a 0–5 point scale, with 0 being the worst and 5 the best quality. Importance was given predominantly to the attributes of flavour, and texture over the appearance of the cheeses, as advised by the IDF (1995). Panellists were also asked to score the overall quality as an average of the above-mentioned sensorial attribute values as weighted by the panellists. Cheese samples were evaluated before packaging and after 1, 3, and 6 weeks of storage.

Shelf-Life Determination

The shelf-life of the cheese samples was determined. The technique consisted of an Accelerated Shelf-Life Testing (ASLT) of the cheese samples. The end of the shelf-life of foods has often been related to the microbiological counts and/or values of physicochemical parameters in different products categories (Calligaris *et al.*, 2007).



Therefore, in this study, yeast and moulds count was chosen as the alteration factor determining the shelf life of the cheese samples. For this purpose, cheese samples were stored for 2 weeks at 3 different storage temperatures (5, 10, and 20°C). The determination of yeast and mould counts of each sample were determined every 3 days during 2 weeks for each of the indicated storage temperature. For the determination of the cheese samples shelf-life at the indicated temperatures, Q_{10} values, which defines the change in shelf-life of foods at storage temperatures differing by 10°C, and the activation Energy (E_a), which gives a measure of the temperature dependence of the sensory quality change upon storage, were used. These values are of practical significance in predicting the changes in the shelf-life with variations in temperatures during the distribution and storage of foods (Torri *et al.*, 2010). These quantities are related through the following equation:

$$\log Q_{10} = \frac{2.189E_a}{(T+10)T} = \log \left[\frac{\text{Shelf life at } T+10}{\text{Shelf life at } T} \right]$$

Where, E_a is an activation Energy in cal mol⁻¹, and T is temperature in °C

Statistical Analyses

The physico-chemical, microbiological, and sensorial values of all the investigated samples were compared by one-way variance analysis (ANOVA) using the software SPSS statistics 19. Significant differences ($P < 0.05$) among treatments were detected using Duncan's multiple range tests. Values expressed are means±standard deviation of triplicate measurements.

RESULTS AND DISCUSSION

Headspace Gas Composition

Figure 1 shows the dynamics of N₂, CO₂ and O₂ concentrations during 6 weeks of

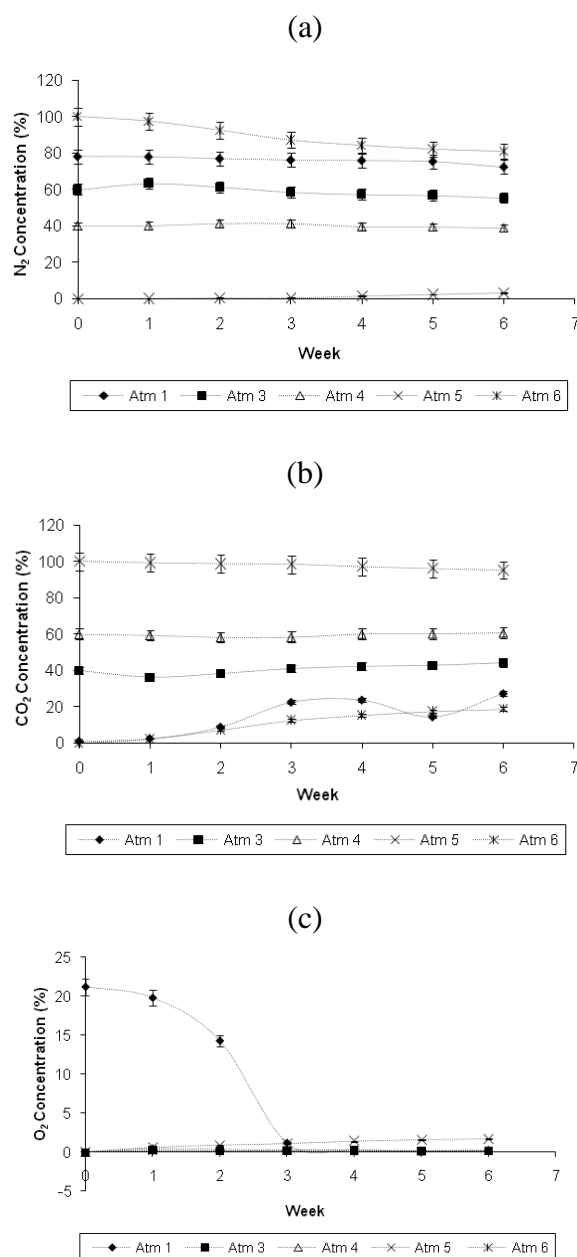


Figure 1. Evolution of N₂ (a), CO₂ (b) and O₂ (c) concentrations in the packages : Atm 1: Air; Atm 3: 40% CO₂/60% N₂; Atm 4: 60 % CO₂/40% N₂; Atm 5: 100% CO₂, Atm 6: 100% N₂.

storage. In samples of atmosphere 6 (100% N₂), a slight decrease in N₂ concentrations during the cheese storage was observed (Figure 1-a). This decrease was followed by stabilization after 30 days of storage. For samples of atmospheres 3 and 4 (60% and 40% N₂, respectively), the

decrease was much less pronounced and N₂ concentrations remained constant with time. As for the samples of atmosphere 5 which were devoid of nitrogen, N₂ proportion increased and reached 1.5% on week 6 (Figure 1-a).

It is noteworthy that in cheese samples of atmosphere 3 (initial CO₂ concentration equal to 40%), an increase in CO₂ occurred simultaneously with the decrease in N₂ (Figure 1-b). For samples stored under atmosphere 1 (air), a remarkable increase in CO₂ concentration was observed. In fact, it started with 0.03% at the beginning of the experiment to more than 8% on week 2 to ultimately achieve 27.3 % on week 6 (Figure 1-b). This accumulation of CO₂ inside the packages is explained by the carbon dioxide production due to the growth of the micro-organisms in the cheeses. This increase in CO₂ concentrations could explain the apparent decrease in N₂ concentrations reported as a percentage of gas content. Eliot *et al.* (1998) reported that the detection sensitivity of gas composition evolution in the package during storage was lower at higher concentrations of CO₂ or N₂, thus, the variations observed may not be dependably reliable. In samples of atmosphere 4, the CO₂ concentration remained relatively stable at around 60%. This result is probably due to the equilibrium established between the CO₂ produced by the microorganisms in the cheese and the CO₂ released through the package. In the samples of atmosphere 5 (100% CO₂), CO₂ concentration decreased with time to reach 95.2% on week 6, in parallel, the nitrogen concentration increased (Figure 1-b). An increase in CO₂ concentration was obtained for atmosphere 6 (100% N₂), which reached 18.7% on week 6.

Oxygen is the second main component of the ambient air and its concentration is 21%. A tremendous drop in O₂ concentrations after the increase of the CO₂ concentration in the samples packaged with the atmosphere 1 was observed (Figure 1-c). Indeed, it decreased from 19.8% on week 1 to 1.22% on week 3 and then remained constant at around 0.2%. The majority of

oxygen disappeared after 3 weeks. This result is probably due to micro-organisms oxygen consumption phenomenon that occurred inside the package (Figure 1-c). In the case of the other samples, the O₂ concentrations, initially equal to zero, increased slightly on the first week of storage. There is a diffusion of oxygen from the outside to the inside of the package. These contents have then been stabilized around relatively low values, between 0.2% for Atm 3 (40% CO₂/60% N₂), Atm 4 (60% CO₂/40% N₂), and Atm 6 (100% N₂) and 1.7% for Atm 5 (100% CO₂). Under these conditions, it can be concluded that the oxygen is consumed gradually as it penetrates through the packaging. The same behaviour was observed by Maniar *et al.* (1994) for cottage cheese. In samples of Atm 5, however, it was noted that the residual oxygen concentration in the packages (1.7%) was higher than in the samples kept under atmospheres 1 to 4 (0.2 to 0.3%). This might be related to the development of microorganisms. It is thus likely that an atmosphere consisting solely of CO₂ (Atm 5) could slow the microbial growth resulting in a decrease in O₂ consumption. The storage of cheese samples under air condition (Atm 1) results in lower concentrations of O₂ and higher CO₂ concentrations (Figure 1-c). Indeed, it declined from 19.8% on week 1 to 1.2% on week 3 and stabilized at around 0.2%. The majority of the oxygen was consumed after 3 weeks of storage.

The gas composition evolution inside the package highlighted the existence of two major factors: (1) Oxygen consumption, and (2) Production of carbon dioxide. Similar events have been reported in the literature for different types of cheese. In fact, Fedio *et al.* (1994) noted a decrease in oxygen concentrations simultaneously with CO₂ concentrations increase in cottage cheese packaged under air. They assigned this result to the respiration of the residual microflora. Alves *et al.* (1996) observed the same two phenomena with Mozzarella slices packaged under pure nitrogen (100% N₂) and they



associated the oxygen consumption (from the residual air initially present between the cheese slices) to the growth as well as the metabolism of the aerobic microorganisms while the CO₂ production was rather related to the growth of aerobic and anaerobic microorganisms. Meanwhile, Piergiovanni *et al.* (1993) demonstrated the endogenous production of CO₂ in modified atmosphere packaging and they connected this to the microbial metabolism and to the enzymatic reactions in the cheese, but these reactions are independent of the consumption of oxygen. Eliot (1997) has implemented the same phenomena with shredded Mozzarella packaged under different conditions of modified atmospheres: regular oxygen consumption by aerobic bacteria and the production of carbon dioxide due to metabolism of the microbial flora. However, the close relationship between these phenomena with the microbial metabolism is shrinking with high CO₂ concentrations (> 75%). In this study, the oxygen consumption (present initially or diffused from outside) was observed in all the packages (Figure 1-c), and the concentrations were rapidly stabilized at relatively high levels: about 0.2% for the CO₂ concentrations ≤ 60%, and about 1.7% for the 100% CO₂. This stabilization suggests that there is an establishment of an equilibrium between the input of the O₂ by diffusion and its consumption. The differences between the residual oxygen concentrations (higher when CO₂ concentrations are important) lead to the fact that the oxygen consumption is slowed in the presence of high CO₂ concentrations. Regarding CO₂ production, it was remarkable in all samples where the CO₂ concentration was ≤ 60%. But for samples of 60% CO₂, stabilization of this concentration throughout the 6 weeks was observed.

Physicochemical Analyses

Table 1 shows the physicochemical characteristics of the cheese samples packaged

under different modified atmospheres during 6 weeks of storage. The pH of the stored cheese samples varied depending on the storage time and the packing conditions (Table 1). The changes of the pH of the investigated cheese samples were of a different nature. The initial pH drop was attributed to the degradation of lactose to lactic acid and was associated with the formation of carbonic acid resulting from the dissolution of CO₂ in water as well as the decrease in moisture in the cheese samples. From week 4 of cheese storage, pH increased to reach 5.35-5.65 at the end of the experimentation. This result could be explained by an intensive proteolysis during cheese samples storage. The highest drop of the pH values was noted in cheeses packaged under carbon dioxide atmospheres (Atms 3, 4, and 5) while the lowest fall was observed in cheeses of atmospheres 1 and 6 (air and 100% N₂, respectively) (Table 1). The obtained results confirmed those reported in the literature. Dermiki *et al.* (2008) have shown that the presence of CO₂ results in a pH drop which is associated with the formation of carbonic acid, acidic amino acids, and free fatty acid production during proteolysis and lipolysis, respectively. The obtained results showed that the presence of CO₂ results in a reduction in pH values during cheese samples stored at 4°C due to the formation of carbonic acid. The dissolution of CO₂ in water reduces partial pressure of the gas in the mixture leading, in extreme cases, to “shrinking” the packaging around the product (Pluta *et al.*, 2005). The moisture measurements carried out during 6 weeks showed a notable variation throughout the experiment (Table 1). For Atm 1, the moisture content was constant during cheese storage. As for the cheeses packaged under the different gas mixtures, the average moisture contents decreased during storage in a similar way for all cheese samples. There were no significant differences between cheeses packaged in different modified atmospheres. The moisture content fell from the mean initial value of 40.8% to the final value of 34.40-38.80%. It is well known that grated cheeses possess an unprotected surface, which facilitates free diffusion of water

Table 1. Physicochemical parameters of MAP cheeses stored at 4°C for 0-6 weeks.^a

	Samples	Weeks						
		0	1	2	3	4	5	6
pH	Atm 1	5.41 ± 0.01 ^{aA}	5.36 ± 0.01 ^{aA}	5.34 ± 0.01 ^{bA}	5.26 ± 0.01 ^{bA}	5.35 ± 0.01 ^{aA}	5.41 ± 0.01 ^{bA}	5.65 ± 0.01 ^{cB}
	Atm 2	5.41 ± 0.01 ^{aB}	5.34 ± 0.01 ^{aA}	5.32 ± 0.01 ^{bA}	5.26 ± 0.01 ^{bA}	5.37 ± 0.01 ^{aA}	5.40 ± 0.01 ^{bB}	5.55 ± 0.01 ^{bC}
	Atm 3	5.41 ± 0.01 ^{aC}	5.38 ± 0.01 ^{aB}	5.24 ± 0.01 ^{aA}	5.25 ± 0.01 ^{abA}	5.34 ± 0.01 ^{aB}	5.36 ± 0.01 ^{aB}	5.46 ± 0.01 ^{aC}
	Atm 4	5.41 ± 0.01 ^{aC}	5.38 ± 0.01 ^{aB}	5.24 ± 0.01 ^{aA}	5.24 ± 0.01 ^{aA}	5.32 ± 0.01 ^{aB}	5.33 ± 0.01 ^{aB}	5.42 ± 0.01 ^{aC}
	Atm 5	5.41 ± 0.01 ^{aB}	5.38 ± 0.01 ^{aB}	5.22 ± 0.01 ^{aA}	5.22 ± 0.01 ^{aA}	5.29 ± 0.01 ^{aA}	5.31 ± 0.01 ^{aA}	5.35 ± 0.01 ^{aB}
	Atm 6	5.41 ± 0.01 ^{aA}	5.35 ± 0.01 ^{aA}	5.32 ± 0.01 ^{bA}	5.29 ± 0.01 ^{aA}	5.35 ± 0.01 ^{aA}	5.39 ± 0.01 ^{bA}	5.49 ± 0.01 ^{bb}
Moisture (%)	Atm 1	40.80 ± 0.14 ^{aA}	39.20 ± 0.14 ^{aA}	40.00 ± 0.00 ^{bA}	40.00 ± 0.00 ^{aA}	38.00 ± 1.41 ^{bA}	39.00 ± 0.71 ^{bA}	40.80 ± 0.28 ^{bA}
	Atm 2	40.80 ± 0.07 ^{aC}	40.60 ± 0.14 ^{bc}	38.00 ± 0.00 ^{ab}	40.40 ± 0.14 ^{aC}	28.00 ± 0.71 ^{aA}	40.00 ± 0.00 ^{bc}	36.60 ± 0.14 ^{ab}
	Atm 3	40.80 ± 0.07 ^{aC}	41.00 ± 1.41 ^{bc}	36.00 ± 1.41 ^{ab}	39.20 ± 0.42 ^{aC}	42.00 ± 2.83 ^{cd}	32.00 ± 1.41 ^{aA}	37.00 ± 2.83 ^{ab}
	Atm 4	40.80 ± 0.07 ^{ab}	40.20 ± 0.14 ^{ab}	42.00 ± 0.00 ^{cC}	40.00 ± 1.41 ^{ab}	42.00 ± 0.00 ^{cC}	34.00 ± 1.41 ^{aA}	36.20 ± 0.28 ^{aA}
	Atm 5	40.80 ± 0.07 ^{ad}	40.60 ± 0.14 ^{bd}	38.00 ± 0.00 ^{aC}	40.00 ± 0.71 ^{ad}	36.00 ± 1.41 ^{bc}	38.00 ± 1.41 ^{bc}	34.40 ± 0.28 ^{aA}
	Atm 6	40.80 ± 0.07 ^{aC}	40.80 ± 0.14 ^{bc}	40.00 ± 0.00 ^{bc}	40.60 ± 0.14 ^{aC}	40.00 ± 0.00 ^{cC}	36.00 ± 0.71 ^{bA}	38.80 ± 0.42 ^{bb}
Weight loss (%)	Atm 1	0.00 ± 0.00 ^{aA}	1.74 ± 0.07 ^{cd}	1.75 ± 0.14 ^{ad}	1.84 ± 0.01 ^{aE}	1.63 ± 0.00 ^{aC}	1.54 ± 0.01 ^{aB}	1.50 ± 0.01 ^{aB}
	Atm 2	0.00 ± 0.00 ^{aA}	1.74 ± 0.07 ^{ca}	3.62 ± 0.01 ^{cd}	1.96 ± 0.28 ^{ab}	2.99 ± 0.07 ^{cC}	6.14 ± 0.07 ^{dE}	1.78 ± 0.03 ^{aA}
	Atm 3	0.00 ± 0.00 ^{aA}	1.73 ± 0.02 ^{cb}	2.17 ± 0.15 ^{bc}	3.03 ± 0.09 ^{bcd}	3.13 ± 0.23 ^{cd}	3.15 ± 0.17 ^{cd}	3.22 ± 0.02 ^{cd}
	Atm 4	0.00 ± 0.00 ^{aA}	1.67 ± 0.01 ^{bb}	3.09 ± 0.01 ^{cG}	1.82 ± 0.01 ^{aC}	2.76 ± 0.06 ^{bE}	2.13 ± 0.08 ^{bd}	2.78 ± 0.01 ^{bF}
	Atm 5	0.00 ± 0.00 ^{aA}	1.32 ± 0.01 ^{ab}	6.09 ± 0.14 ^{cG}	2.91 ± 0.04 ^{bc}	3.80 ± 0.71 ^{dF}	3.13 ± 0.03 ^{cd}	3.37 ± 0.14 ^{cE}
	Atm 6	0.00 ± 0.00 ^{aA}	2.08 ± 0.01 ^{dc}	5.98 ± 0.28 ^{dE}	2.16 ± 0.06 ^{bd}	2.23 ± 0.07 ^{bd}	1.80 ± 0.03 ^{ab}	2.04 ± 0.04 ^{bC}

^a Means±Standard Deviation (SD) of three separate determinations. Atm 1: Air; Atm 2: Vacuum; Atm 3: 40% CO₂/60% N₂; Atm 4: 60 % CO₂/40% N₂; Atm 5: 100% CO₂; Atm 6: 100% N₂. ^{ab,c,d,e} Values sharing the same lowercase letter within a column are not significantly different by Duncan's multiple-range test (P< 0.05). ^{A,B,C,D,E,F,G} Values sharing the same capital letter within a row are not significantly different by Duncan's multiple-range test (P< 0.05).



from the inside of the cheese towards its surface. These results agree with those reported by Sendra *et al.* (1994) for goat fresh cheese. On the other hand, Garabal *et al.* (2010) indicated that different gas mixtures did not significantly influence the moisture content or weight loss in the cheese when packaged under modified atmospheres. The highest weight losses were found in samples packaged under 100% CO₂. This result confirms that of Sendra *et al.* (1994) who studied the effect of modified atmospheres on goat fresh cheese.

Microbiological Analyses

Figure 2 (a) indicated a change in Lactic Acid Bacteria (LAB) counts for different samples during the storage period. This means that the CO₂ is not a factor inhibiting these microorganisms. Thus, its presence, even in high quantities, seemed to increase the growth of these microorganisms (Atm 5, 100% CO₂). These results are in accordance with those found earlier by other researchers (Dermiki *et al.*, 2008; Gammariello *et al.*, 2011). These authors have shown that LAB were slightly affected under the modified atmospheres and were able to grow well since LAB are facultative anaerobic Gram-positive in nature. However, Whitley *et al.* (2000) have observed a decrease in LAB rate for Stilton cheese, due to the presence of CO₂. The evolution of lactic flora seemed obviously referring to the results of pH measurements (Table 1), i.e. where there is an increase in pH, there is a decrease in the rate of lactic acid bacteria and vice versa.

Figure 2 clearly shows that the yeasts and moulds are particularly sensitive to modified atmospheres. The fastest and the strongest reduction in the yeast and moulds count was observed in cheese samples packaged under the atmosphere of 100% CO₂ (Figure 2 -b). Indeed, yeasts and moulds were not detected under this condition. For the other atmospheres, a significant reduction in yeasts and moulds count was noted, they have fluctuated around relatively low values

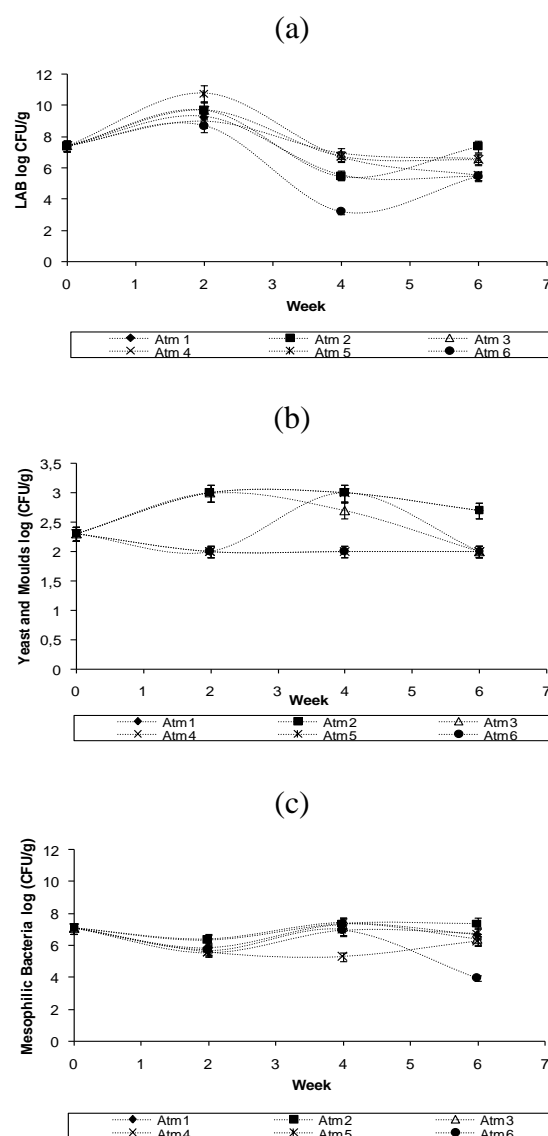


Figure 2. The growth of LAB (a), Yeast and molds (b) and Mesophilic bacteria (c) in MAP cheese stored at 4°C. Mean log value (n= 3). Atm 1: Air; Atm 2: Vacuum; Atm 3: 40% CO₂/60% N₂; Atm 4: 60% CO₂/ 40% N₂; Atm 5: 100% CO₂, Atm 6: 100% N₂.

(Figure 2-b). The obtained results are in accordance with previous investigations. Indeed, the inhibitory and killing effect that carbon dioxide had on yeasts and moulds, already mentioned by Gammariello *et al.* (2011), was clearly demonstrated in this study. Yeast and moulds growth was similar in both air and vacuum packaging. Their

high initial growth under Atm 1 was slowed following week 2 due to the oxygen depletion as well as the CO₂ accumulation. Nitrogen atmosphere packaging showed little effect on the reduction of yeast and moulds growth in comparison with air packaging. This result confirms that found by Alves *et al.* (1996) who investigated the effect of packing in the atmosphere of N₂ and a 1:1 mixture of CO₂ and N₂ on the development of yeast, and observed a smaller inhibitory effect for packing in the atmosphere of N₂ than in the mixture of these gases.

The trend of the total mesophilic flora count was similar in all samples (Figure 2-c). Indeed, it started with a slight decrease followed by an increase in week 4, then, remained relatively stable. The initial content of mesophilic flora before packaging was about 7.4 log CFU g⁻¹ as shown in Figure (2-c). The obtained results are in accordance with those of Piergiovanni *et al.* (1993) who have found no significant differences between different samples packaged under modified atmospheres. Papaioannou *et al.* (2006) have shown that the gas mixture (70%/30%) (CO₂/N₂) is the most effective for inhibition of the total flora. Eliot *et al.* (1998) showed that in high concentration, the CO₂ was more effective than the vacuum in reducing the growth of mesophilic flora.

Sensory Evaluation

The panel's scores for the cheese samples are presented in Table 2. A significant difference ($P < 0.05$) was observed between various packaged cheeses, reflecting the generally recognized negative effect of CO₂ on appearance scores of cheeses. The lowest note was attributed to the cheese sample packed under 100% CO₂. However, for CO₂ concentrations equal to 40 and 60% (Atms 3 and 4), the appearance of the cheeses retained its brilliance and clarity during the storage period. However, for the other samples, the color was fairly clear. At the

end of the experimentation, Atms 3 and 4 received the highest scores, i.e. 3.23 and 3.40, respectively. This result disagrees with those found by Scott and Smith (1971) who have reported that CO₂ has some negative effects on milk products in general, with respect to the color and aroma. However, Maniar *et al.* (1994) found that CO₂ did not affect the sensorial characteristics of cottage cheese. These different results can be explained in terms of CO₂ concentrations used and types of products studied. Furthermore, the packaging under different modified atmospheres decreased the intensity of the odour of all the samples compared to the control cheese sample. The modified atmosphere packaging increased the salty taste. The more the concentration of CO₂, the more the saltiness increased. The scores given to the cheese samples packaged under 100% CO₂ (Atm 5) was the least important, i.e. this package increased the acidity of the sample throughout the experimental period. This result confirmed those of Alves *et al.* (1996). For all cheese samples, an increase in bitterness was noticed compared to the control sample. The effectiveness of the modified atmospheres on improving the taste and the odour of the cheeses was studied by Dermiki *et al.* (2008). An improvement in hardness attributes for cheese samples packaged under different CO₂ concentrations (Atms 3, 4 and 5) was noted (Table 2). These results confirmed those found by Mannheim and Soffer (1996) who studied the effect of modified atmospheres on cottage cheeses. The samples of Atm 6 (100% N₂) maintained also a fairly uniform texture. The cheese sample packaged under air (Atm 1) had a fairly homogeneous texture. For the vacuum packed sample, the structure deteriorated since the gas injection allowed pasting different fragments of the cheese, this was the reason that this sample had the lowest score.

The results of the sensorial evaluation (overall acceptability) of all systems are presented in Table 2. It should be noted that the highest score was awarded to the



Table 2. Sensory attribute ratings of MAP cheeses before and after storage at 4°C for 1, 3 and 6 weeks (scores from 100 naïve panellists).^a

Weeks	Samples	Appearance	Odour	Saltiness	Acidity	Bitterness	Hardness	Overall impression
0	Atm 1	3.85 ± 1.42 ^{de}	3.51 ± 1.11 ^c	2.57 ± 1.00 ^{cA}	3.54 ± 1.20 ^{ad}	4.35 ± 1.19 ^{bf}	2.95 ± 1.32 ^{ab}	3.55 ± 1.25 ^{aC}
	Atm 2	3.95 ± 1.01 ^{de}	3.07 ± 1.12 ^{bb}	2.35 ± 0.91 ^{aA}	3.50 ± 1.20 ^{cd}	4.33 ± 1.05 ^{bf}	3.37 ± 1.13 ^{bc}	3.65 ± 1.15 ^{bd}
	Atm 3	3.89 ± 1.50 ^{cd}	2.84 ± 1.14 ^{ab}	2.43 ± 0.76 ^{aA}	3.25 ± 1.17 ^{aC}	4.35 ± 1.09 ^{be}	3.37 ± 1.14 ^{bc}	3.85 ± 0.99 ^{cd}
	Atm 4	3.57 ± 1.05 ^{ad}	3.27 ± 1.10 ^{cb}	2.54 ± 1.07 ^{bA}	3.44 ± 1.26 ^{bc}	4.07 ± 1.32 ^{aF}	3.81 ± 0.88 ^{ce}	3.87 ± 1.22 ^{ce}
	Atm 5	3.86 ± 1.15 ^c	2.77 ± 0.95 ^{aA}	2.63 ± 1.14 ^{cA}	3.35 ± 1.20 ^{bb}	4.33 ± 1.00 ^{bd}	3.29 ± 1.09 ^{bb}	3.38 ± 1.12 ^{ab}
	Atm 6	3.75 ± 1.22	2.88 ± 1.10 ^{aA}	2.70 ± 1.11 ^{dA}	3.35 ± 1.25 ^{bc}	4.35 ± 1.31 ^{bf}	3.12 ± 1.04 ^{ab}	3.90 ± 1.19 ^{ce}
1	Atm 1	3.77 ± 1.25 ^{de}	3.40 ± 1.10 ^c	2.67 ± 0.99 ^{cA}	3.50 ± 1.22 ^{bd}	4.27 ± 1.17 ^{bf}	2.87 ± 1.41 ^{ab}	3.40 ± 1.28 ^{aC}
	Atm 2	3.90 ± 1.12 ^{de}	2.97 ± 1.10 ^{bb}	2.40 ± 0.89 ^{aA}	3.40 ± 1.22 ^{cd}	4.30 ± 0.95 ^{bf}	3.27 ± 1.23 ^{bc}	3.50 ± 1.11 ^{ad}
	Atm 3	3.80 ± 1.10 ^{bd}	2.73 ± 1.17 ^{ab}	2.33 ± 0.66 ^{aA}	3.23 ± 1.07 ^{aC}	4.30 ± 0.99 ^{be}	3.27 ± 1.26 ^{bc}	3.80 ± 0.97 ^{cd}
	Atm 4	3.53 ± 1.25 ^{ad}	3.17 ± 1.02 ^{bb}	2.43 ± 1.04 ^{aA}	3.27 ± 1.28 ^{aC}	3.97 ± 1.30 ^{aF}	3.73 ± 0.98 ^{ce}	3.70 ± 1.26 ^{be}
	Atm 5	3.63 ± 1.35 ^{bd}	2.67 ± 0.92 ^{ab}	2.43 ± 1.04 ^{aA}	3.33 ± 1.27 ^{bc}	4.27 ± 1.08 ^{be}	3.27 ± 1.11 ^{bc}	3.30 ± 0.99 ^{aC}
	Atm 6	3.70 ± 1.26 ^{cd}	2.80 ± 1.13 ^{ab}	2.60 ± 1.04 ^{cA}	3.33 ± 1.27 ^{bc}	4.20 ± 1.21 ^{be}	3.20 ± 1.24 ^{bc}	3.70 ± 1.09 ^{bd}
3	Atm 1	3.33 ± 1.27 ^{bd}	3.23 ± 0.97 ^{dc}	2.70 ± 1.06 ^{aA}	3.10 ± 1.30 ^{ab}	3.14 ± 1.16 ^{bb}	3.33 ± 1.03 ^{ad}	3.10 ± 0.93 ^{bb}
	Atm 2	3.50 ± 1.22 ^{de}	3.03 ± 1.19 ^{cb}	2.77 ± 0.94 ^{bA}	3.37 ± 1.07 ^{bd}	3.23 ± 1.16 ^c	3.50 ± 1.22 ^{be}	3.20 ± 1.14 ^c
	Atm 3	3.50 ± 1.17 ^{cd}	2.90 ± 1.21 ^{bb}	2.60 ± 1.04 ^{aA}	3.17 ± 1.28 ^{bc}	3.13 ± 1.13 ^{bc}	3.50 ± 0.87 ^{bd}	3.10 ± 1.07 ^{bc}
	Atm 4	3.30 ± 1.26 ^{bc}	2.87 ± 1.04 ^{ba}	2.80 ± 1.10 ^{bA}	3.47 ± 1.28 ^{cd}	3.15 ± 1.33 ^{bb}	3.30 ± 1.07 ^{aC}	3.10 ± 0.97 ^{bb}
	Atm 5	3.20 ± 1.10 ^{bc}	2.50 ± 0.82 ^{aA}	2.90 ± 1.06 ^{bb}	3.27 ± 1.20 ^c	3.01 ± 1.36 ^{ab}	3.20 ± 0.94 ^{aC}	3.00 ± 0.98 ^{ab}
	Atm 6	3.50 ± 1.20 ^{cd}	3.07 ± 0.94 ^{cb}	2.87 ± 0.86 ^{ba}	3.30 ± 1.26 ^c	3.25 ± 1.09 ^c	3.50 ± 0.92 ^{bd}	3.20 ± 0.96 ^c
6	Atm 1	3.03 ± 1.38 ^{bc}	2.87 ± 1.31 ^{db}	2.07 ± 1.28 ^{aA}	3.07 ± 1.36 ^{dc}	2.81 ± 1.88 ^{bb}	2.87 ± 1.20 ^{db}	2.80 ± 1.45 ^{bb}
	Atm 2	2.43 ± 1.52 ^{aA}	2.40 ± 1.28 ^{aA}	2.87 ± 1.33 ^{dc}	2.83 ± 1.09 ^c	2.59 ± 1.52 ^{ab}	2.40 ± 1.31 ^{aA}	2.60 ± 1.59 ^{ab}
	Atm 3	3.23 ± 1.45 ^{cd}	2.70 ± 1.15 ^{cb}	2.40 ± 1.13 ^{bA}	2.97 ± 1.25 ^{dc}	2.91 ± 1.57 ^c	2.70 ± 1.19 ^{cb}	2.90 ± 1.33 ^c
	Atm 4	3.40 ± 1.43 ^{de}	2.63 ± 1.27 ^{ba}	2.67 ± 1.15 ^{ca}	2.77 ± 1.55 ^{bb}	2.97 ± 1.63 ^c	2.63 ± 1.07 ^{ba}	3.00 ± 1.27 ^{bd}
	Atm 5	3.03 ± 1.43 ^{be}	2.47 ± 1.20 ^{aA}	2.70 ± 1.24 ^{cc}	2.60 ± 1.33 ^{ab}	2.77 ± 1.74 ^{bd}	2.47 ± 1.25 ^{aA}	2.80 ± 1.47 ^{bd}
	Atm 6	2.97 ± 1.40 ^{bc}	2.70 ± 1.12 ^{cA}	2.67 ± 0.96 ^{cA}	2.80 ± 1.37 ^{cb}	2.82 ± 1.49 ^{bb}	2.70 ± 1.29 ^{cA}	2.80 ± 1.11 ^{bb}

^a Means±Standard Deviation (SD) of three separate determinations. Atm treatments as define in the text and Table 1. ^{a,b,c,d,e} Values sharing the same lowercase letter within a column are not significantly different by Duncan's multiple-range test (P< 0.05). ^{A,B,C,D,E,F} Values sharing the same capital letter within a row are not significantly different by Duncan's multiple-range test (P< 0.05).

samples packaged under modified atmosphere. These samples were the most appreciated among all the other samples. This result confirmed those found in the literature. From Table 2, it is observed that for the first week of storage, the cheese samples packaged under Atm 3 (40% CO₂/60% N₂) was appreciated the most. The Atms 4 and 6 were appreciated just after Atm 3. During week 3 of storage, the Atm 3 remained the most appreciated sample with the same average scores. As for the Atm 4, the overall impression decreased during the same week. Regarding the 6th week, the global appreciation of all samples decreased remarkably. However, the tasting panel appreciated the Atm 3 but less than during the other weeks. Alves *et al.* (1996) and Maniar *et al.* (1994) reported that plain CO₂ was the best at maintaining the sensorial characteristics of Mozzarella and Cottage cheeses, respectively. Mannheim and Soffer (1996), as well, showed that Cottage cheese samples do not suffer from packaging with 100% CO₂ with regard to the sensorial characteristics, and no changes in taste or even texture were recorded. However, Esmer *et al.* (2009) noted that the highest score was given to the Crottin de Chavignol cheeses with a 20% CO₂ gas composition.

Shelf-Life Determination

In this section, our approach to calculate the shelf-life of the investigated cheese

samples is based on the Arrhenius model and the Q_{10} values as reported by Labuza (1994). At storage temperature of 4°C, the investigated packaged cheese samples are expected to have yeasts and moulds of 1.5-2 log₁₀ CFU g⁻¹ of cheese. From the curves of $\ln(\text{yeasts and moulds}) = f(\text{storage period})$, the shelf-life of each packaged cheese sample was determined. Table 3 shows the different values of shelf-life values of the investigated cheese samples packaged under different modified atmospheres. As illustrated in Table 3, the most appropriate storage temperature of the cheese samples corresponds to 4 °C for Atms 3 (40% CO₂/60% N₂), 4 (60% CO₂/40% N₂), and 5 (100% CO₂). These results confirmed those found earlier by many researchers. Indeed, Papaioannou *et al.* (2006) have shown that the shelf-life of the goat cheese type "Anthotryros" packaged under modified atmosphere of 30%/70% (CO₂/N₂) and stored at 4°C was extended by 10 days compared to that packaged under vacuum, while it was extended by 20 days when stored at 4°C for cheese packaged under 70% CO₂/30% N₂) and by 4 days at 12°C under the same modified atmosphere conditions. Furthermore, for atmospheres 1 (air), 2 (vacuum), and 6 (100% N₂), the temperature of 10 °C appeared to be the best since it guaranteed a shelf life of 93 days for Atm 1, 100 days for Atm 2, and 126 days for Atm 6. Kamleh *et al.* (2012) showed that for a storage temperature of 5°C, the cheese's shelf-life determined was about 79.6 days

Table 3. Shelf life values (days) of MAP cheeses basing on Q_{10} values using ASLT method.^a

Samples	Shelf life (days)		
	T= 4°C	T= 10°C	T= 20°C
Atm 1	85.05 ± 0.01 ^a	92.97 ± 0.06 ^b	89.28 ± 0.03 ^c
Atm 2	97.53 ± 0.05 ^b	100.14 ± 0.11 ^c	95.88 ± 0.08 ^a
Atm 3	143.59 ± 0.12 ^c	136.63 ± 0.09 ^b	118.93 ± 0.11 ^a
Atm 4	154.53 ± 0.17 ^a	118.67 ± 0.15 ^b	118.68 ± 0.21 ^b
Atm 5	166.14 ± 0.09 ^b	118.06 ± 0.01 ^a	118.68 ± 0.10 ^a
Atm 6	122.39 ± 0.10 ^b	125.72 ± 0.13 ^c	107.99 ± 0.05 ^a

^a Means±Standard Deviation (SD) of three separate determinations. Atm treatments as define in the text and Table1. ^{a,b,c} Values sharing the same lowercase letter within a column are not significantly different by Duncan's multiple-range test (P< 0.05).



while at 15°C, it was about 37.8 days and for a storage temperature of 25°C, the cheese's shelf-life was about 2.6 days. Atmosphere 5, i.e., 100% CO₂, promoted the best shelf-life (166 days) among all the investigated packaged cheese samples, i.e., extended by 81 days compared to the control sample. But, for sensory reasons, this atmosphere was neglected in favour of the Atm 3 representing 40% CO₂/60% N₂, because on one hand, it improved the microbiological, physico-chemical and sensorial attributes and, on the other hand, it extended the cheese shelf-life of 46 days compared to vacuum.

CONCLUSIONS

The evolution of gas composition inside the package indicated the existence of both oxygen consumption and carbon dioxide production phenomena. The physico-chemical results showed that the presence of CO₂ results in a reduction in pH values during cheese samples stored at 4°C from 5.41 to 5.35 (Atm 5) while it had no effect on moisture content during the storage period. The highest weight losses were found in samples packaged under 100% CO₂. Indeed, 6.09% of weight loss was recorded for fresh Mozzarella cheese stored at 4 °C for 2 weeks. The lactic acid bacteria were not inhibited even for the atmospheres of high CO₂ concentrations (Atm 5). Atm 3, representing 40% CO₂/60% N₂, exhibited the best sensory characteristics of the investigated cheese samples during the storage period. The same atmosphere (Atm 3) allowed a shelf-life extension of 46 days at 4°C compared to vacuum.

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تعیین عمر انباری پنیر تازه در معرض اتمسفرهای اصلاح شده مختلف بسته بندی

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چکیده

هدف این پژوهش تعیین خواص فیزیکوشیمیایی، میکروبیولوژیکی و حسی و نیز عمر انباری نمونه های پنیر موزارلا (mozzarella) بود. نمونه های این پنیر در بسته بندی هایی با 5 اتمسفر اصلاح شده شامل خلاء (Atm 2)، $40\% \text{ CO}_2/60\% \text{ N}_2$ (Atm 3)، $60\% \text{ CO}_2/40\% \text{ N}_2$ (Atm 4)، $100\% \text{ CO}_2$ (Atm 5) و $100\% \text{ N}_2$ (Atm 6) بود مطالعه شد و تیمار شاهد مطالعه هم نمونه های یکسانی از پنیر مزبور در هوای معمولی بود. همه نمونه ها به مدت 6 هفته در یخچال ($4 \pm 1^\circ\text{C}$) نگهداری شد. در طی این شش هفته و در درجه حرارت 4°C ، مخلوط گازی تیمار 5 از نظر ممانعت از رشد میکروفلور هوازی از همه تیمارهای دیگر موثرتر بود. گاز CO_2 حتی در غلظت های زیاد هم روی باکتری های لاکتیک اسید تاثیری نداشت. در تمام مدت آزمایش، مخلوط گازی Atm 5 از رشد مخمر و کپک ها کاملاً جلوگیری کرد. ارزیابی حسی نشان داد که پنیر بسته بندی شده در تیمار 3 ویژگی های حسی را به خوبی در شش هفته انبار داری حفظ کرد در حالیکه نمونه های شاهد کمترین پسندیدگی را داشتند. در مقایسه با تیمار شاهد، تیمار 5 Atm در 4°C موجب بیشترین افزایش عمر انبارداری به مدت 81 روز شد.