

*Full Length Research Paper*

# Modified atmosphere packaging to improve the microbial stability of Ricotta

Del Nobile, M. A.<sup>1,2\*</sup>, Conte, A.<sup>1</sup>, Incoronato, A. L.<sup>1</sup> and Panza, O.<sup>1</sup>

<sup>1</sup>Department of Food Science, University of Foggia, Via Napoli, 25 – 71100 Foggia, Italy.

<sup>2</sup>Istituto per la Ricerca e le Applicazioni Biotecnologiche per la Sicurezza e la Valorizzazione dei Prodotti Tipici e di Qualità - BIOAGROMED, Università degli Studi di Foggia, Via Napoli, 52 - 71100 Foggia, Italy.

Accepted 27 February, 2012

**In this work, modified atmosphere packaging (MAP) was used to prolong the Ricotta shelf life. In particular, three different MAP were tested. The quality loss of the product was assessed by monitoring microbial and physico-chemical parameters over an 8-day period. Results suggest that MAP, and in particular that richer in carbon dioxide, inhibits microbial growth without a significant effect on lactic acid bacteria, probably due to their facultative anaerobic nature. The longest shelf life was obtained with MAP containing 95% of carbon dioxide. With regard to the visual quality, a substantial difference in the yellow index was also observed between Ricotta stored in air and that packaged under MAP.**

**Key words:** Dairy products, map, microbial stability, ricotta.

## INTRODUCTION

Ricotta is a popular whey cheese of the Mediterranean area, consumed as both table cheese or combined with other ingredients for typical dishes. It is prepared traditionally by heating whey, or combinations of whey and milk, in open kettles. The coagulated curd muss floats to the surface and is scooped off and placed in perforated trays for drainage. Ricotta is made mainly from sheep or goat milk, but also from cow and buffalo milk (Pizzillo et al., 2005). Its shelf life is generally limited to a few days due to the exposure of the product to the atmosphere prior to packaging. Moreover, due to the high moisture level, the initial pH above 6 and the low salt content, ricotta is very susceptible to spoilage by molds, yeasts and bacteria, substantially represented by Enterobacteriaceae (Fleet and Mian, 1987; Pintado et al., 2001). Ricotta characteristics possibly act selectively on survival and permit the growth of salt-tolerant microorganisms (Lioliou et al., 2001). Additionally, as other soft and whey cheeses, it could constitute a major food safety concern showing a high incidence and potential for survival / growth of pathogens (Papaioannou et al., 2007). Very little information is available in the literature regarding with

shelf life of whey cheeses. For cottage cheese, similar in composition to Ricotta, Labuza et al. (1982) reported a shelf life of 14 days at 2°C. Low storage temperatures can obviously influence microbial growth of fresh whey cheese. Hough et al. (1999) reported shelf life values of 33, 13.5 and 5.5 days for commercial Ricotta cheese packaged in polyethylene bags and kept at 6, 17 and 25°C, respectively.

Among the preservation methods to ensure safety of whey cheeses are irradiation combined with vacuum packaging (Tsiotsias et al., 2002) and using of antimicrobial compounds (Samelis et al., 2003 and Content et al., 2007), both of which had been applied to typical Greek whey cheese. A useful technique to overcome the problems associated with oxygen-sensitive foods is represented by packaging under modified atmosphere conditions (MAP) (Kerry and Papkovsky, 2002). In the literature the application of MAP to dairy products is abundant (Colchin et al., 2001; Gonzalez-Fandos et al., 2000; Piergiovanni et al., 1993; Juric et al., 2003); however, no data are available on packaging of Ricotta.

It is well known that CO<sub>2</sub> has a bacteriostatic effect, especially on Gram-negative aerobic bacteria, and a fungistatic effect on molds, whereas the N<sub>2</sub> acts as an inert gas preventing package collapse (Daniels et al., 1985). Interesting shelf life prolongation was obtained by packaging fresh creamed-style cottage cheeses at middle

\*Corresponding author. E-mail: [ma.delnobile@unifg.it](mailto:ma.delnobile@unifg.it). Tel.: (+39) 881 589 242. Fax: (+39) 881 589 242.

levels, ranging from 25 to 40% (Moir et al., 1993; Mannheim and Soffer, 1996; Sarais et al., 1996). Also, Gonzalez-Fandos et al. (2000) studied five different: N<sub>2</sub> mixtures (from 20 - 100%), compared to vacuum, to preserve the quality of a typical Spanish fresh cheese stored at 4°C. The authors demonstrated that the most effective gas combinations to extend the shelf life and retain good sensory characteristics are based on CO<sub>2</sub> content ranging between 50 and 60%. With regard to MAP applied to fresh whey cheeses, a high CO<sub>2</sub> concentration was found very effective. Pintado and Malcata (2000) demonstrated that 100% CO<sub>2</sub> to package a Portuguese whey cheese ensured more consistent product and better overall visual appearance. Papaioannou et al. (2007) proved that the use of MAP (70:30 CO<sub>2</sub>:N<sub>2</sub>), at refrigerated temperature extended the shelf life of a typical Greek whey cheese by approximately 20 days, compared to another gas mixture with a lower CO<sub>2</sub> concentration.

Even though the use of MAP to preserve different kinds of cheeses seems to be widely studied, no data have been reported on Ricotta packaging. Due to this lack of knowledge and considering that the optimal gas composition for cheese preservation is strictly dependent on each cheese nature, the current study make a new contribution to this field by investigating the effects of different MAP on Ricotta during storage at 4°C.

## MATERIALS AND METHODS

### Ricotta packaging

Ricotta obtained from sheep milk was purchased from a local dairy market in Lucera (Foggia, Italy) and immediately transported to the laboratory, where it was portioned in samples of 100 g and packaged in Nylon-based high-barrier multi-layer plastic bags (250 x 350 mm and thickness 200 µm), provided by Valco (Bergamo, Italy), having an Oxygen Transmission Rate of 30 cm<sup>3</sup> m<sup>-2</sup> 24 h<sup>-1</sup> at 1 atm (measured at 23°C and 75% Relative Humidity), and a Water Vapor Transmission Rate of 1 g m<sup>-2</sup> 24 h<sup>-1</sup> (measured at 23°C and 85% Relative Humidity). Three gas mixtures were used: 50:50 (CO<sub>2</sub>:N<sub>2</sub>) (MAP50), 70:30 (CO<sub>2</sub>:N<sub>2</sub>) (MAP70) and 95:5 (CO<sub>2</sub>:N<sub>2</sub>) (MAP95). Gas mixtures were prepared using a gas mixer (PBI-Dansensor Model 9000, Ringsted, Denmark). Pouches were heat-sealed by means of a packaging machine (R 200, Reepack, Italy) connected to the gas mixer. Ricotta samples were also packed under ordinary atmosphere as controls. All packaged Ricotta bags were stored at 4°C for 8 days.

### Microbiological analyses

For microbiological analyses, 10 g of each sample were diluted with 90 ml of Ringer's solution in a Stomacher bag (Interscience, France) and homogenized for 1 min in a Stomacher Lab Blender 400 (Interscience, France). Serial dilutions of homogenates were plated onto the surface of the appropriate media in Petri dishes. The media and the conditions used, were: Plate Count Agar (PCA) incubated at 5°C for a week or at 32°C for 48 h for psychrotrophic and mesophilic bacteria, respectively; Pseudomonas Agar Base (PAB), modified by adding Pseudomonas CFC selective supplement after autoclaving at 121°C for 15 min, incubated at 30°C for 48 h for Pseudomonas spp.; violet red bile glucose agar (VRBGA, Oxoid) incubated at 37°C for 18 - 24 h for Enterobacteriaceae; MRS

agar incubated at 30°C for 48 h under anaerobic conditions for lactic acid bacteria; Sabouraud Dextrose Agar, supplemented with chloramphenicol (0.1 g/l), incubated at 25°C for 48 h or 5 days for yeasts and molds, respectively. All media were from Oxoid (Milan, Italy). The analyses were carried out twice, on two different batches.

### pH measurements

The pH was determined for each Ricotta sample with a pH meter (Crison Instrument, Barcelona, Spain).

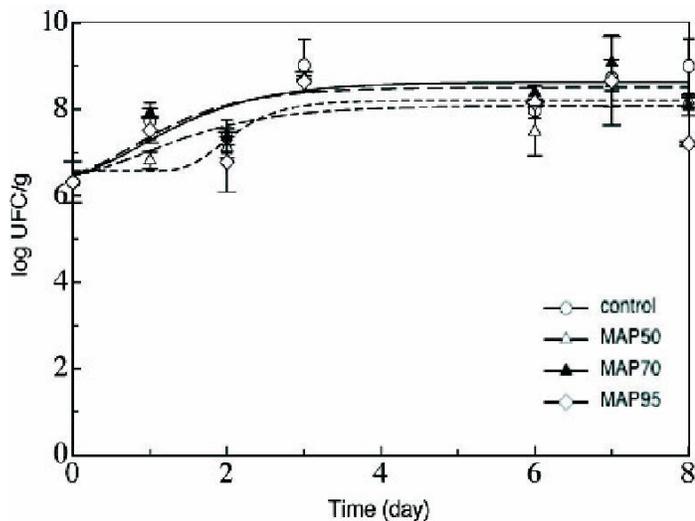
### Colour measurement

Colour measurements were performed with a Chroma Meter CR-400 (Konica Minolta, Osaka, Japan), according to the standard conditions of the Commission International d'Eclairage (CIE). The values were measured three times on the surface of packaged Ricotta slices after 0, 1, 2, 3, 6, 7 and 8 days of storage at 4°C. Results were expressed as L\* (lightness), a\* (redness) and b\* (yellowness). Before each series of measurements, the instrument was calibrated using a white ceramic tile having the following values: Y = 93.0, x = 0.3134, y = 0.3193. To determine if the colour differences among samples were statistically significant, one-way variance analysis (ANOVA) and Duncan's test (p < 0.05) was performed. The statistical package Statistica for Windows (Statsoft, Tulsa, USA) was used for this purpose.

## RESULTS AND DISCUSSION

The initial microbial counts in packaged Ricotta were very high, in the region of 10<sup>6</sup>-10<sup>7</sup> cfu g<sup>-1</sup>, possibly reflecting milk quality, survival of heat-sensitive microorganisms during the cheese-making process and post-processing microbial contamination. During cold storage, different microbial evolutions were recorded, depending on the selected modified atmospheres.

Figure 1 show the growth of lactic acid bacteria in Ricotta stored at 4°C under ordinary atmosphere and MAP conditions. As expected, for all monitored samples, except MAP95, a steady increase in the cell counts was observed at the early stage of storage. Afterwards, cell counts remained practically constant, suggesting that the stationary phase has been attained. In the case of the MAP95 sample, a lag phase of about 2 days was observed. After an initial delay, the lactic acid bacteria population reached stationary phase with a cell count similar to that of the control. Indeed, the moisture content and pH of whey cheeses are usually high, thus favouring lactic acid bacteria growth (Pintado et al., 2001). As can be seen in the figure, the tested MAP conditions did not seem to influence, to a great extent the growth of lactic acid bacteria, probably due to their facultative anaerobic nature (Papaioannou et al., 2007). As reported in the literature for a commercial French-style goat milk cheese (Park et al., 2004), the correlation between the viable cell count of a specific microbial group (lactic acid bacteria) and pH was established. Figure 2 (a, b, c and d) shows the microbial counts plotted as a function of pH. As can be seen, the bacterial counts decreased as the pH incre-

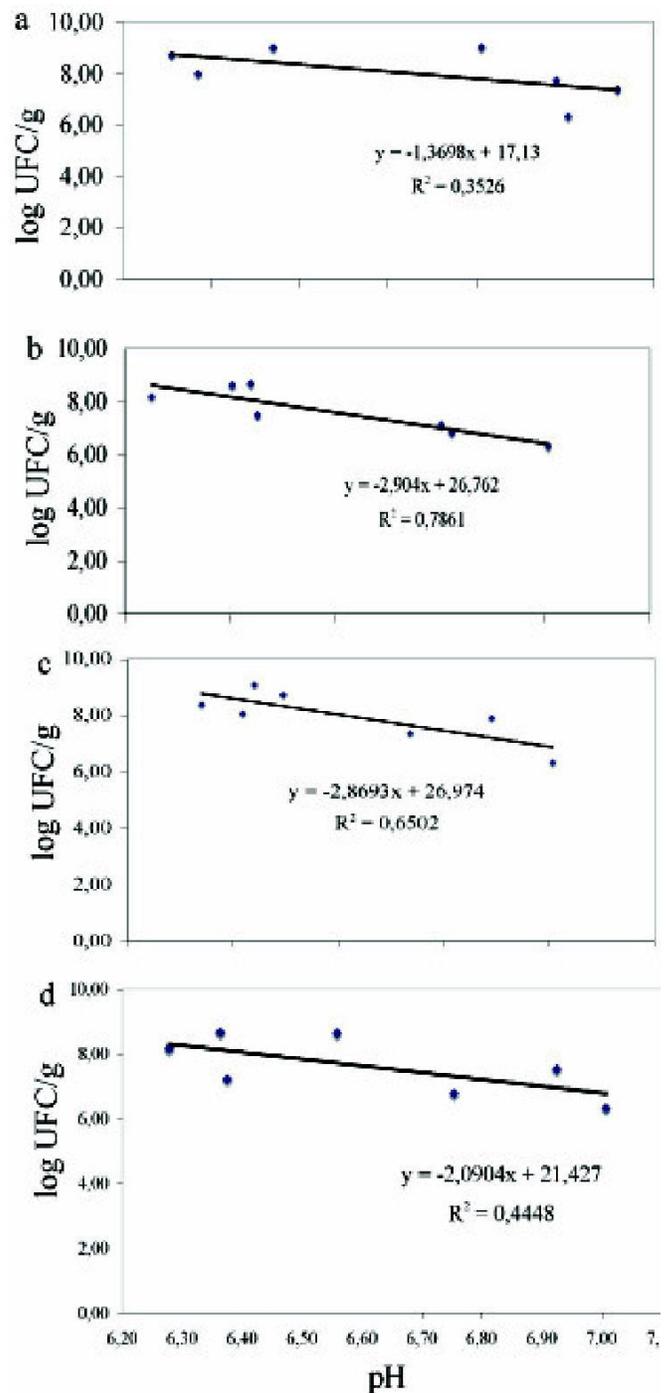


**Figure 1.** Growth of lactic acid bacteria in Ricotta stored at 4°C.

increased under all tested packaging conditions. However, the degree of correlation is acceptable only for MAP50 ( $R^2 = 0.7861$ ); for the control and the other two investigated MAP conditions the degree of correlation is too low. Unfortunately, due to the poor level of correlation no definitive conclusion can be drawn from the above data.

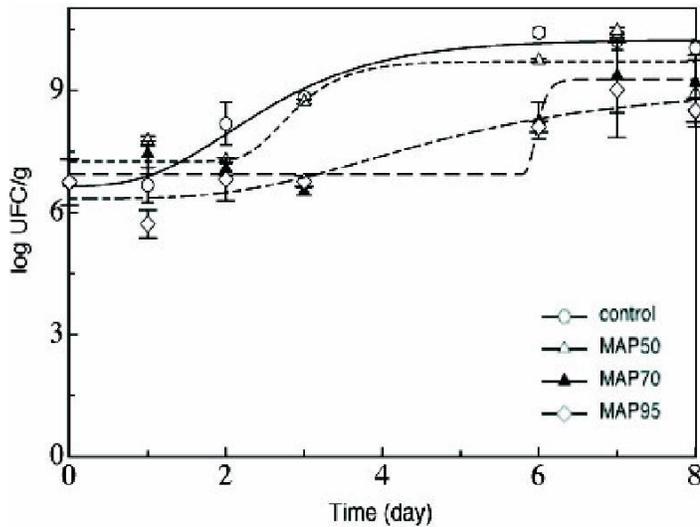
Contrary to what was observed for lactic acid bacteria, a substantial difference between samples packaged under ordinary atmosphere and those packaged under MAP70 and MAP95 was observed in the case of total mesophilic and psychrotrophic viable cell counts. Indeed, high concentrations of  $CO_2$  are reported to produce similar effects in other types of refrigerated fresh food matrices, probably due to the increased solubility of  $CO_2$  at lower temperatures (Farber, 1991). Total mesophilic bacteria were selected to calculate the Ricotta shelf life. For this, a modified version of Gompertz equation (Zwietering et al., 1990) was used (Figure 3). Considering that spoilage generally starts to occur at  $10^7$  CFU/g of total viable count (ICMSF, 1984), this level was set as the microbial threshold. The fitting procedure allowed us to calculate the following shelf life values:  $1.14 \pm 0.34$ ,  $0.55 \pm 0.5$ ,  $0.36 \pm 0.6$  and  $3.37 \pm 0.7$ , respectively, for the control, MAP50, MAP70 and MAP95 samples. Ricotta is supposed to be consumed within a short time after manufacture; however, the shelf life, among other factors, is strictly related to the initial microbial load. As can be seen, MAP with high concentrations of  $CO_2$  could affect microbial proliferation, thus prolonging shelf life.

Figure 4 shows the fungal population plotted as a function of storage time recorded in Ricotta. The fungal population only comprises yeasts, because no molds were recovered in the investigated samples. The yeast cell load in the control sample steadily increased and it did not seem to reach stationary phase over the entire

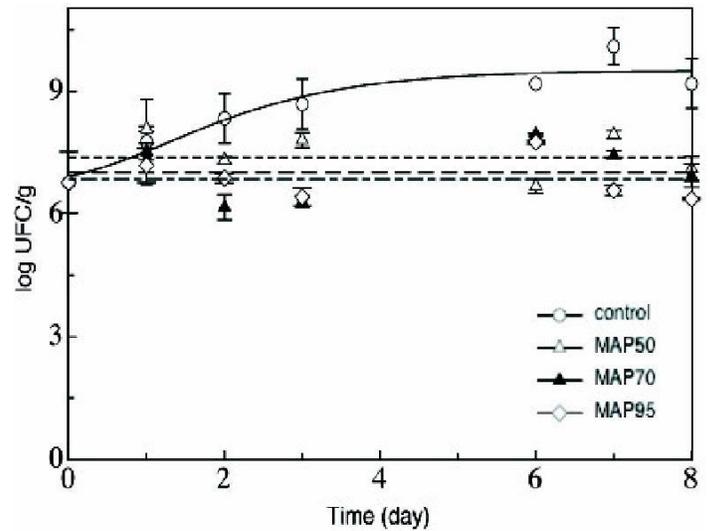


**Figure 2.** Correlation between pH and lactic acid bacteria. (a) Control, (b) MAP50, (c) MAP70, (d) MAP95

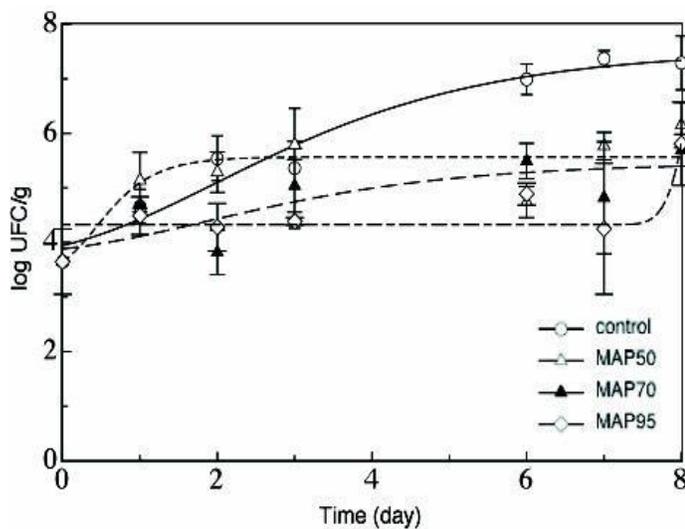
observation period. On the other hand, the yeast concentration in Ricotta packaged under MAP50 first increased earlier than in the control and afterwards it reached a population lower than that recovered for the control at the stationary phase. It is worth noting that the yeast counts in Ricotta packaged under the two higher  $CO_2$  concentrations did not reach stationary phase over the 8 days of



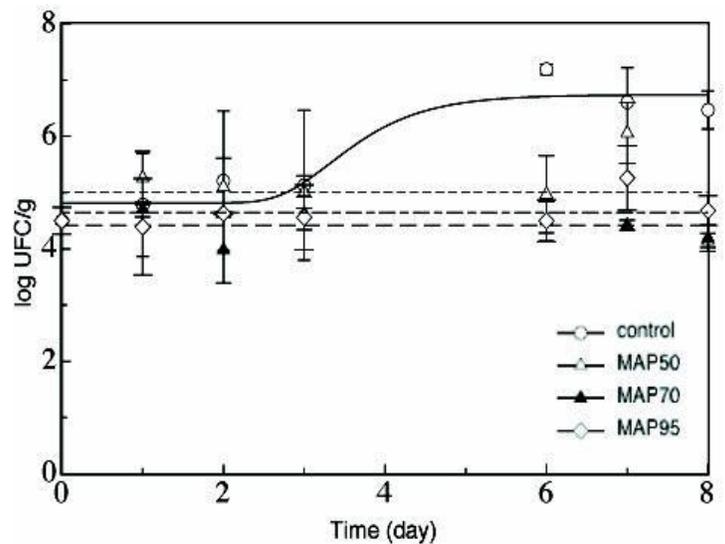
**Figure 3.** Growth of total mesophilic bacteria in Ricotta stored at 4°C.



**Figure 5.** Growth of *Pseudomonas* spp. in Ricotta stored at 4°C.



**Figure 4.** Growth of yeasts in Ricotta stored at 4°C.



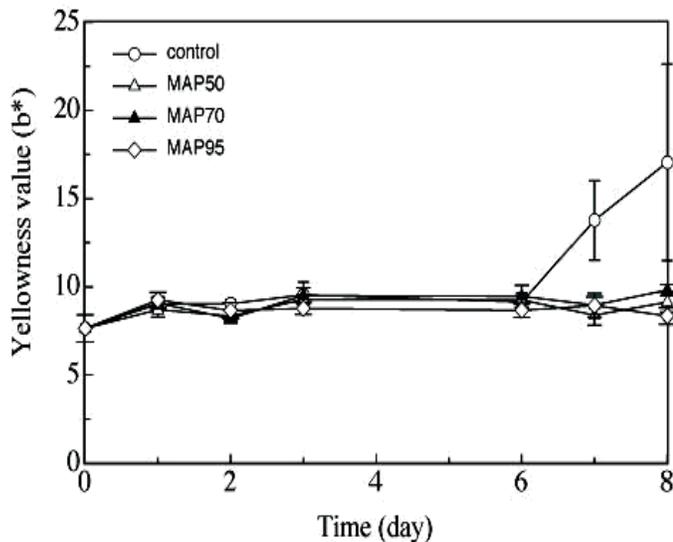
**Figure 6.** Growth of Enterobacteriaceae in Ricotta stored at 4°C.

monitoring. Moreover, for both MAP conditions the yeast counts at the end of the observation period was lower than that measured in Ricotta packaged under ordinary atmosphere. The above data are in agreement with what has been reported in the literature, where it was found that MAP tested on a typical Greek whey cheese is effective in reducing the yeast/mold populations during storage, thus representing an enhanced preservation method compared to atmospheric air or vacuum packaging (Gonzalez-Fondos et al., 2000; Papaioannou et al., 2007).

Figure 5 shows the growth of *Pseudomonas* spp. in the packaged dairy product. As can be inferred from the figure, the control showed a typical growth cycle: an initial lag - phase, followed by an exponential increase in the

viable cell count, up to a plateau. On the other hand, the samples packaged under each MAP do not show any significant growth of *Pseudomonas* spp., suggesting that CO<sub>2</sub> is effective in inhibiting their proliferation. Other authors also reported similar MAP effects for various types of cheese (Papaioannou et al., 2007; Pintado and Malcata, 2000).

Results analogous to those obtained for the other spoilage microbial groups were also recorded for Enterobacteriaceae (Figure 6). A substantial difference was observed between samples packed under ordinary atmosphere and those packed under MAP. In fact, the control showed a lag phase of about 3 days, followed by a steady increase until the attainment of the stationary phase. On the con-



**Figure 7.** Evolution of  $b^*$  colour parameter of each packaged Ricotta sample.

trary, for samples packed under MAP, a longer lag phase was observed compared to the control. Moreover, the viable cell count reached at the stationary phase was lower than that recorded on Ricotta packaged in air. The number of Enterobacteriaceae in other similar cheeses, such as Greek whey cheese or Manouri cheese or fresh Cameros cheese, was lower than that recorded in this study, thus indicating worse manufacturing practices and conditions during production (Gonzalez-Fondos et al., 2000; Lioliou et al., 2001; Papaioannou et al., 2007).

With regard to the visual quality of the tested dairy product, colour evolution was monitored and  $L^*$ ,  $a^*$  and  $b^*$  values were compared statistically. No differences were recorded by comparing  $L^*$  (Lightness) values, as well as  $a^*$  values (redness) (data not shown). On the contrary, substantial differences between samples stored under ordinary atmosphere and MAP were recorded in the yellow index ( $b^*$ ) (Figure 7). As shown, similar values of  $b^*$  were measured for all samples until the sixth day of storage. After this time the  $b^*$  value recorded for Ricotta packaged in air increased rapidly. On the contrary,  $b^*$  values remained fairly constant for samples stored under MAP. Moreover, the difference between MAP samples and the control was found to be statistically significant. The observed changes in the yellowness index are in accordance with microbiological data. In fact, after 6 days of storage the spoilage bacteria counts increased substantially in Ricotta stored in air, suggesting that the changes in colour of the investigated product is a consequence of the detrimental phenomena provoked by the high microbial proliferation.

## Conclusion

The MAP conditions tested in this work seem to be effec-

tive in slowing down the growth of spoilage microorganisms. Moreover, MAP packaging could be further optimized to become a technological strategy that assures Ricotta microbial stability. Results also suggest that the modified packaging headspace conditions could also improve the product aspect. However, further work is necessary to establish the optimum  $\text{CO}_2$  concentrations to package the product and then correlate the microbial, physical and chemical aspects to the sensorial attributes that play a major role in consumer acceptability.

## ACKNOWLEDGEMENTS

This work was financially supported by Ministero dell'Economia e delle Finanze, Ministero dell'Istruzione, dell'Università e delle Ricerca Scientifica e Tecnologica e l'Assessorato Bilancio e Programmazione Regione Puglia by the programme "Accordo di Programma Quadro in Materia di Ricerca Scientifica della Regione Puglia - Progetto Strategico - Title: "Improvement of nutritional properties and safety of traditional dairy products of Capitanata".

## REFERENCES

- Colchin LM, Owens SL, Lyubachevskaya G, Boyle-Roden E, Russek-Cohen E, Rankin SA (2001). Modified atmosphere packaged cheddar cheese shreds: influence of fluorescent light exposure and gas type on color and production of volatile compounds. *J. Agric. Food Chem.* 49: 2277-2282.
- Conte A, Speranza B, Sinigaglia M, Del Nobile MA (2007). Effect of lemon extract on food borne microorganisms. *J. Food Prot.* 70:1896-1900.
- Daniels JA, Krishnamurthi R, Rizvi SSH (1985). A review of effect of carbon dioxide on microbial growth and food quality. *J. Food Prot.* 48:532-537.
- Farber JM (1991). Microbiological aspects of modified atmosphere packaging technology-A review. *J. Food Prot.* 54:58-70.
- Fleet GH, Mian MA (1987). The occurrence and growth of yeasts in dairy products. *Int. J. Food Microbiol.* 4:145-155.
- Gonzalez-Fondos E, Sanz S, Olarte C (2000). Microbiological physicochemical and sensory characteristics of Cameros cheese packaged under modified atmospheres. *Food Microbiol.* 17: 407-414.
- Hough G, Puglieso ML, Sanchez R, Mendez da Silva O (1999). Sensory and microbiological shelf life of a commercial Ricotta. *J. Dairy Sci.* 82: 454-459.
- ICMSF (International Commission on Microbiological Specifications for Foods) (1984). *Microorganismos de los alimentos. 1 Tecnicas de analisis microbiologica.* Ed. Acribia, Zaragoza.
- Juric M, Bertelsen G, Mortensen G, Petersen MA (2003). Light-induced color and aroma changes in sliced modified atmosphere packaged Kerry JP, Papkovsky DB (2002). Development and use of non-destructive continuous assessment chemical oxygen sensors in packs containing sensitive foodstuffs. *Res. Advan. Food Sci.* 3:121-140.
- Labuza TP (1982). *Shelf life dating of foods.* Food and Nutrition Press Inc., Westport, Connecticut. pp. 54-58.
- Lioliou K, Litopoulou-Tzanetaki E, Tzanetakis N, Robinson RK (2001). Changes in the microflora of hanouri, A Transitional Greek whey cheese, Uring Storage. *Int. J. Dairy Technol.* 54: 100-106.
- Mannheim CH, Soffer T (1996). Shelf life extension of cottage cheese by modified atmosphere packaging. *LWT.* 29: 767-771.
- Papaioannou G, Chouliara I, Karatapanis AE, Kontominas MG, Savvaidis IN (2007). Shelf life of a Greek whey cheese under modified atmosphere packaging. *Int. Dairy J.* 17: 358-364.

- Park WY, Kalantari A, Frank JF (2004). Changes in the microflora of commercial soft goat milk cheese during refrigerated and frozen-storage. *Small Rum. Res.* 53: 61-66.
- Piergiiovanni L, Fava P, Moro M (1993). Shelf life extension of Taleggio cheese by modified atmosphere packaging. *Int. J. Food Sci.* 5:115-119.
- Pintado ME, Macedo AC, Malcata FX (2001). Review: Technology, chemistry and microbiology of whey cheeses. *Food Sci. Tech. Int.* 7:105-116.
- Pintado ME, Malcata FX (2000). The effect of modified atmosphere packaging on the microbial ecology in Requeijao a Portuguese whey cheese. *J. Food Process. & Pres.* 24 :07-124.
- Pizzillo M, Claps S, Cifuni GF, Fedele V, Rubino R (2005). Effect of goat breed on the sensory chemical and nutritional characteristics of Ricotta. *Lives Prod. Sci.* 94: 33-40.
- Samelis J, Kakouri A, Rogga KJ, Savvaidis IN, Kontominas MG (2003). Nisin treatments to control *Listeria monocytogenes* post-processing contamination of Anthotyros a traditional Greek whey cheese stored at 4°C in vacuum packages. *Food Microbiol.* 20: 661-669.
- Sarais I, Piuksi D, Aquili V, Stecchini ML (1996). The behavior of yeast populations in Stracchino cheese packaged under various conditions. *J. Food Prot.* 59: 541-544.
- semi-hard cheeses. *Int. Dairy J.* 13:239-249.
- Tsiotsias A, Savvaidis I, Vassila A, Kontominas MG, Kotzekidou P (2002). Control of *Listeria monocytogenes* by low-dose irradiation in combination with refrigeration in the soft whey cheese Anthotyros. *Food Microbiol.* 19:117-126.
- Zwietering MH, De Koos JT, Hasenack BE, De Wit JC, van't Riet K (1990). Modeling of bacterial growth as a function of temperature. *Appl. Env. Microbiol.* 57:1875-1881.