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A combination of chitosan, coating and modified atmosphere packaging for prolonging Fior di latte cheese shelf life

Matteo A. Del Nobile [a,b,*], Daniela Gammariello [a], Amalia Conte [a], Massimiliano Attanasio [a]

[a] Istituto per la Ricerca e le Applicazioni Biotecnologiche per la Sicurezza e la Valorizzazione dei Prodotti Tipici e di Qualità, Università degli Studi di Foggia, Via Napoli, 25, 71100 Foggia, Italy
[b] Department of Food Science, University of Foggia, Via Napoli, 25, 71100 Foggia, Italy

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ABSTRACT

In this work, the addition of chitosan into cheese making, combined with either coating or active coating (lysozyme and ethylenediamine tetraacetic acid, disodium salt) and MAP (modified atmosphere packaging) was used to prolong the shelf life of "Fior di latte" cheese. On the packaged cheese stored at 4 °C microbiological, pH, gas composition and sensory changes were monitored over an 8-day period. Results showed that the combination of chitosan, active coating and MAP improved "Fior di latte" cheese preservation by increasing the shelf life in comparison with the traditional packaging. In fact, the latter showed a very short shelf life limited to more or less 1 day, whereas the integrated approach developed in this study allowed us to obtain a significant shelf life prolongation to 5 days, most probably due to the synergic effect between the active compounds and the atmospheric conditions in the package headspace.

1. Introduction

"Fior di latte" cheese is a widely known fresh dairy product and has a rather short shelf life mainly due to the variety of microorganisms, including spoilage bacteria (Cantoni, Iacumin, & Comi, 2003; Parisi, 2003). Numerous studies have characterized this cheese from a microbiological point of view (Altieri, Manganelli, & Giudici, 1994; Massa, Gardini, Sinigaglia, & Guerzoni, 1992; Ottogalli, Rondinini, & Conti, 1979); in contrast, there is little on methods to prolong its shelf life.

Currently, the packaging of "Fior di latte" cheese consists of rigid or flexible films of multilayer material, trays made of polyethylene/paper laminated films and tetrapack-type packages (Robertson, 1993). Today, many researchers focus their attention on active packaging defined as "a mode of packaging in which the package, the product, and the environment interact to prolong shelf life or enhance safety or sensory properties, while maintaining the quality of the product" (Suppakul, Miltz, Sonneveld, & Bigger, 2003). However, in the scientific literature only a few applications of active packaging systems or modified atmosphere packaging (MAP) for to mozzarella cheese are reported. Conte, Scrocco, Sinigaglia, and Del Nobile (2007) successfully investigated a release system based on lemon extract to prolong shelf life of mozzarella cheese; Sinigaglia, Bevilacqua, Corbo, Pati, and Del Nobile (2008) demonstrated the antimicrobial effectiveness of lysozyme and Na_2–EDTA, dissolved in brine, in prolonging the mozzarella storability. The antimicrobial effects of lysozyme and EDTA are well known (Bester & Lombard, 1990; Branen & Davidson, 2004; Cunningham, Proctor, & Goetsch, 1991; Davidson, Post, Branen, & McCurdy, 1993; Gill & Holley, 2000; Ibrahim et al., 1996; Razavi-Rohani & Griffiths, 1994; Stevens, Sheldon, Klapes, & Klaenhammer, 1991), whilst the antimicrobial activity of essential oils were recognized long ago, but their application as natural antimicrobials has recently received increased attention in the food industry (Davidson, 2001b; Draughon, 2004). In fact, Gammariello, Di Giulio, Conte, and Del Nobile (2008) demonstrated that some essential oils, dissolved in brine, exerted an inhibitory effect on the microorganisms responsible for spoilage of "Fior di latte" cheese. Furthermore, as regards the potential application of MAP, Eliot, Vuillemard, and Emond (1998) reported that shredded mozzarella cheese packaged under MAP containing levels of 75% CO_2 was well preserved from undesirable microorganisms and gas formation. Alves, Sarantopoulos, Van Dender, and Faria (1996) also found that the microbial growth in sliced mozzarella cheese, packaged in MAP and stored at 7 °C, was delayed with high concentrations of CO_2. Finally, the combination of coating as a carrier of natural antimicrobials (lysozyme and EDTA) to MAP conditions in a sealed packaging system represented a strategic solution to prolong the shelf life of "Fior di latte" cheese (Conte, Gammariello, Di Giulio, Attanasio, & Del Nobile, 2008).

The shelf life of this product is also closely linked to the type of raw material and processing technology. Chitosan has gained significant attention: it has been evaluated for numerous applications

* Corresponding author. Address: Department of Food Science, University of Foggia, Via Napoli, 25, 71100 Foggia, Italy. Tel./fax: +39 881 589 242.
E-mail address: ma.delnobile@unifg.it (M.A. Del Nobile).

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in the medical, food, agricultural, and chemical industries, mainly due to its high biodegradability and antimicrobial properties (Arvanitoyannis, 1999; Arvanitoyannis, Nakayama, & Aiba, 1998; Hague et al., 2005; Kim, Chen, Wang, & Rajapakse, 2005; Yamada et al., 2005). Altieri, Scrocco, Sinigaglia, and Del Nobile (2005) successfully tested the use of chitosan as an antimicrobial agent in the process of mozzarella cheese. The biological activity of chitosan depends on its molecular weight, deacetylation degree, chitosan derivatization, degree of substitution, length and position of a substituent in glucosamine units of chitosan, pH of chitosan solution and, of course, the target organism (Chirkov, 2002; Devlieghere, Vermeulen, & Debevere, 2004; Hirano & Nagao, 1989; Kendra & Hadwiser, 1984; Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003; Uchida, Izume, & Ohtakara, 1989; Ueno, Yamaguchi, Sakairi, Nishi, & Tokura, 1997; Zheng & Zhu, 2003).

Due to the above considerations, the goal of this research was to study an integrated approach to prolong the shelf life of "Fior di latte" cheese. The investigated strategy was based on the combination of chitosan in the manufacture, either coating or active coating, combined with MAP. To this aim, the microbiological, physico-chemical and sensory changes of packaged "Fior di latte" cheese were monitored over an 8-day period at 4 °C.

2. Materials and methods

2.1. "Fior di latte" manufacturing procedure

"Fior di latte" cheese samples were manufactured in the cheese-making factory "Posta la via" (Foggia, Italy), according to the following procedure: 50 kg of pasteurized cow's milk was acidified with 0.28% of lactic acid (80%, Henan Jir, Tecno Milk, Bari) and 0.025% liquid rennet (strength 1:10,000) was added. Curd formation was achieved after about 15–20 min. When the curd pH reached a value of about 5.80, the whey was removed. The curd was cut, stretched and shaped, after tempering in cold water. Simultaneously, a modified "Fior di latte" cheese was manufactured, adding a high-molecular-weight chitosan (Sigma–Aldrich, Milan, Italy). Briefly, a chitosan solution containing lactic acid (1%) was put into the working milk, in order to get a final concentration of 0.012% chitosan. The "Fior di latte" manufacture was carried out as described above. Samples were transported to the laboratory in polystyrene boxes containing ice and were used within 3 h after production.

2.2. Coating procedure and packaging

"Fior di latte" cheese was dipped into sodium alginate solution prepared by dissolving sodium alginic acid (8% w/v) both in distilled water (COAT) and in a solution of 0.25 mg mL^{-1} lysozyme and 50 mM of ethylenediamine tetraacetic acid, disodium salt (Na_2–EDTA) (COAT-ACT). The coated samples were immersed into a 5% (w/v) calcium chloride ($CaCl_2$) solution for 1 min, to physically crosslink the polymeric matrix. Sodium alginic acid and calcium chloride were provided by Sigma–Aldrich (Milan, Italy). All samples were dried at room temperature for 2 min. Each coated sample was packaged in commercially available bags with thickness of 95 μm, provided by Valco (Bergamo, Italy). These were obtained by laminating a nylon layer and a polyolefin layer, and have an Oxygen Transmission Rate (OTR) of 50 mL m^{-2} 24 h^{-1} at 1 atm, measured at 23 °C and 75% relative humidity and a Water Vapour Transmission Rate (WVTR) of 1.64 g m^{-2} 24 h^{-1} at 1 atm, measured at 23 °C and 85% relative humidity. During packaging, modified atmosphere conditions (30:5:65 CO_2:O_2:N_2) were used. As controls, chitosan-free and chitosan samples without coating were also packaged in trays with the traditional brine. The brine consisted of 2% NaCl solution.

Determinations of microbial count, pH, headspace gas composition and sensory evaluation were carried out before packaging and after 1, 4, 5, 6, 7 and 8 days of storage at 4 °C on different cheese samples.

Samples will be named as follow: CNTL (chitosan-free "Fior di latte", stored in brine), CNTL-COAT-MAP (chitosan-free "Fior di latte", coated with alginate, stored under MAP), CNTL-ACT-COAT-MAP (chitosan-free "Fior di latte", coated with alginate loaded with lysozyme and Na_2–EDTA, stored under MAP), CHT (chitosan-loaded "Fior di latte", stored in brine), CHT-COAT-MAP (chitosan-loaded "Fior di latte", coated with alginate, stored under MAP), CHT-ACT-COAT-MAP (chitosan-loaded "Fior di latte", coated with alginate loaded with lysozyme and Na_2–EDTA, stored under MAP).

2.3. Microbiological analysis

Twenty grams of "Fior di latte" cheese were diluted in 180 mL of Ringer's solution in a Stomacher bag and blended with a Stomacher Lab Blender mod. 4153-50 (PBI, International Milan, Italy). Serial dilutions of homogenates were plated on the appropriate media in Petri dishes. The media and conditions used were PCA (Oxoid), incubated at 30 °C for 48 h for total microbial count, MRS agar (Oxoid), supplemented with cycloheximide (0.1 g L^{-1}, Sigma), incubated under anaerobiosis (Anaerogen Gas Pack, Oxoid) at 37 °C for 48 h for lactic acid bacteria; M17 agar (Oxoid), incubated at 37 °C for 48 h for coccus-shaped lactic acid bacteria; yeast peptone dextrose agar (YPD, Oxoid), supplemented with chloranphenicol (0.1 g L^{-1}, Oxoid) incubated at 30 °C for 48 h for yeasts; VRBLA (Oxoid) incubated at 37 °C for 24 h for total coliforms; Pseudomonas Agar Base (Oxoid), added with SR103 E selective supplement (Oxoid) and incubated at 25 °C for 48 h for *Pseudomonas* spp. All analyses were performed in duplicate.

In order to determine the microbial acceptability limit (i.e., the storage time at which the viable cell concentration reaches its threshold value), the Gompertz equation, as re-parameterized by Corbo, Del Nobile, and Sinigaglia (2006), was fitted to the experimental data:

$$\log(N(t)) = \log(N_{max}) - A \cdot \exp\left\{-\exp\left\{\left[(\mu_{max} \cdot 2.71) \cdot \frac{\lambda - MAL}{A}\right] + 1\right\}\right\} + A \cdot \exp\left\{-\exp\left\{\left[(\mu_{max} \cdot 2.71) \cdot \frac{\lambda - t}{A}\right] + 1\right\}\right\} \quad (1)$$

where $N(t)$ is the viable cell concentration at time t, A is related to the difference between the decimal logarithm of maximum bacterial growth attained at the stationary phase and decimal logarithm of the initial value of cell concentration, μ_{max} is the maximal specific growth rate, λ is the lag time, N_{max} is the microbial threshold value, MAL is the microbiological acceptability limit (i.e., the time at which $N(t)$ is equal to N_{max}), and t is the storage time. The value of N_{max} was set to 10^6 CFU/g for *Pseudomonas* spp. ($MAL^{Pseudomonas}$) and 10^5 CFU/g for coliforms ($MAL^{coliforms}$). The latter is imposed by the DPR 54/97 (European Union, 1997), whereas the former value is the contamination level at which the alterations of the product start to appear (Bishop & White, 1986).

2.4. Headspace gas composition

Prior to opening the cheese bags, headspace gas composition was determined, by using a Checkmate 9900 gas analyzer (PBI Dansensor, Ringsted, Denmark). The volume taken from the package headspace for gas analysis was about 10 cm^3. To avoid modifications in the headspace gas composition due to gas sampling, each package was used only for a single determination of the headspace gas composition. Two samples were used for each test.

2.5. pH determination

The pH values on each sample were determined by direct reading whit pH-metre (Crison, Barcelona, Spain). Each value was the average of measures recorded on sample from two different batches.

2.6. Sensory analysis

A panel of six trained participants evaluated the sensory attributes of consistency, colour and odour. They were asked to describe differences between samples by using a scale from 0 to 7 (Corradini & Innocente, 2002), where 4 indicated the attribute threshold for acceptability. On the basis of the above-mentioned attributes, panelists were also asked to score the *overall quality* of the product using the same 0–7 scale. Before evaluating, each coated "Fior di latte" cheese was deprived of the coating and immersed in water at room temperature for few minutes, in order to tie these samples to wet uncoated cheese.

In order to determine the sensory acceptability limit (i.e., the storage time at which the sensory attribute reaches its threshold value), the Gompertz equation as re-parameterized by Corbo et al. (2006) was fitted to the sensory data:

$$\mathrm{SA}(t) = \mathrm{SA}_{\min} + A^{\mathrm{SA}} \cdot \exp\left\{-\exp\left\{\left[\left(\mu^{\mathrm{SA}}_{\max} \cdot 2.71\right) \cdot \frac{\lambda^{\mathrm{SA}} - \mathrm{SAL}}{A^{\mathrm{SA}}}\right] + 1\right\}\right\} + A^{\mathrm{SA}} \cdot \exp\left\{-\exp\left\{\left[\left(\mu^{\mathrm{SA}}_{\max} \cdot 2.71\right) \cdot \frac{\lambda^{\mathrm{SA}} - t}{A^{\mathrm{SA}}}\right] + 1\right\}\right\} \quad (2)$$

where $\mathrm{SA}(t)$ is the sensory attribute at time t, A^{SA} is related to the difference between the sensory attribute attained at the stationary phase and the initial value of sensory attribute, $\mu^{\mathrm{SA}}_{\max}$ is the maximal rate at which $\mathrm{SA}(t)$ decreases, λ^{SA} is the lag time, $\mathrm{SA}_{\min}$ is the sensory attribute threshold value, SAL is the sensory acceptability limit (i.e., the time at which $\mathrm{SA}(t)$ is equal to $\mathrm{SA}_{\min}$), and t is the storage time. As reported above, the value of $\mathrm{SA}_{\min}$ is equal to 4.

3. Results and discussion

As reported above, an integrated approach was proposed in this study to prolong the shelf life of "Fior di latte" cheese. In particular, modifications to both manufacturing and packaging processes were put into practice to slow down the quality loss kinetic of the selected dairy product during a refrigerated storage period. The main quality sub-indices of Fior di latte (microbial and sensory) were monitored for 8 days to determine the effectiveness of the proposed approach in prolonging the shelf life. In the following, results obtained for the above-mentioned quality sub-indices were reported and discussed separately.

3.1. Microbial growth

Fig. 1 shows the viable cell concentration of *Pseudomonas* spp. and coliforms plotted as a function of storage time for all the investigated samples. *Pseudomonas* spp. and coliforms are acknowledged as the main spoilage microorganisms for "Fior di latte" cheese. The curves were obtained by fitting Eq. (1) to the experimental data, whereas the horizontal solid line is the viable cell concentration threshold value. The $\mathrm{MAL}^{Pseudomonas}$ and $\mathrm{MAL}^{\mathrm{coliforms}}$ values, obtained according to the procedure reported in Section 2, were listed in Table 1. Data highlight that the proposed integrated approach (see the $\mathrm{MAL}^{Pseudomonas}$ and $\mathrm{MAL}^{\mathrm{coliforms}}$ values of CHT-ACT-COAT-MAP sample, resulting from the combination of chitosan, active coating and MAP) was very effective in inhibiting

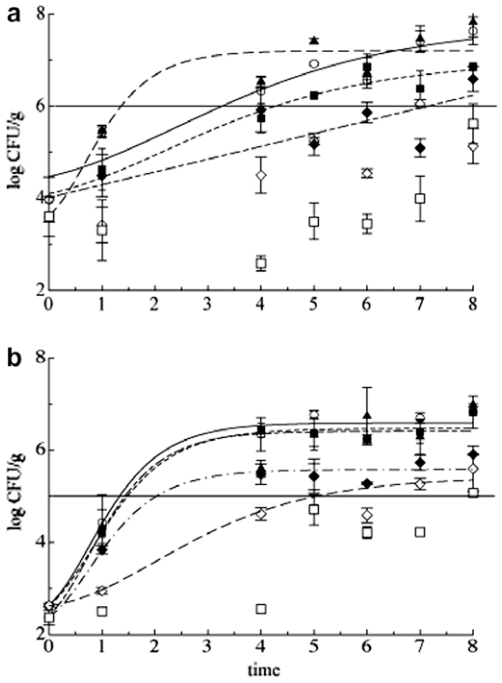

Fig. 1. (a) Evolution of *Pseudomonas* spp. count in "Fior di latte" cheese during the storage period. (b) Evolution of coliform count in "Fior di latte" cheese during the storage period. The curves are the best fit of the Eq. (1) to the experimental data. (○ CNTL, chitosan-free Fior di latte in brine; ■ CNTL-COAT-MAP, chitosan-free Fior di latte, coated with alginate, stored under MAP; ◇ CNTL-ACT-COAT-MAP, chitosan-free Fior di latte, coated with alginate loaded with lysozyme and Na_2–EDTA, stored under MAP; ▲ CHT, chitosan-loaded Fior di latte in brine; CHT-COAT-MAP chitosan-loaded Fior di latte, coated with alginate, stored under MAP; □ CHT-ACT-COAT-MAP, chitosan-loaded Fior di latte, coated with alginate loaded with lysozyme and Na_2–EDTA, stored under MAP).

the growth of monitored spoilage microorganisms. In particular, all tested strategies act synergistically to inhibit the growth of both *Pseudomonas* spp. and coliforms. In fact, among the tested samples, CHT-ACT-COAT-MAP was the sole one whose *Pseudomonas* spp. and coliforms viable cell concentration did not overcome the threshold value for the entire period of observation (i.e., the difference between the viable cell concentration and its threshold value, wherever it was positive, was never statistically significant).

Concerning coliform growth, data suggest that chitosan, coating and MAP alone did not seem to affect, to a great extent, the cell growth cycle, whereas the use of active coating seemed to inhibit the growth of these microorganisms. It can also be noted that the presence of chitosan enhances the antimicrobial efficacy of the active coating. The hurdle theory in literature stated that the preservative action of antimicrobials depends on the type, genus, specie and strain of microorganism tested. Efficiency of an antimicrobial also depends greatly on environmental factors such as pH, water activity, temperature, atmosphere, initial microbial load and acidity of the food substrate (Davidson, 2001a; Gould, 1989; Wiley, 1994). Many of these environmental factors can be considered individually as preservation methods when they are used at high doses, whereas the combined use of some of these treatments has been the basis of the hurdle concept which consists in the use of more than one treatment in a logical sequence to provide fresh-like quality food products (Wiley, 1994). It is worth noting that microbiological activity of chitosan has been detected for many bacteria, filamentous fungi and yeasts (Badawy et al., 2004; Chirkov, 2002; Hirano & Nagao, 1989; Kendra & Hadwiser, 1984; Muzzarelli et al., 1990, 2001; Papineau, Hoover, Knorr, & Farkas, 1991; Rabea et al., 2003; Rhoades & Roller, 2000; Sudarshan,

Table 1
Shelf life (days) of "Fior di latte" samples, assumed as the lowest value among $MAL^{Pseudomonas}$, $MAL^{coliforms}$ and $SAL^{O.Q.}$.

Sample	Microbial parameters		Sensorial parameters				Shelf life
	$MAL^{coliforms}$	$MAL^{Pseudomonas}$	$SAL^{COL.}$	$SAL^{OD.}$	$SAL^{CONS.}$	$SAL^{O.Q.}$	
CNTL	1.33 ± 0.17 a	3.26 ± 0.67 b	>8	>8	6.65 ± 0.16 b	6.75 ± 0.19 b	1.33 ± 0.17 a
CNTL-COAT-MAP	1.48 ± 0.18 a	4.27 ± ± 0.45 b	4.52 ± 3.61 a	>8	5.50 ± 0.69 a	5.16 ± 1.03 a	1.48 ± 0.18 a
CNTL-ACT-COAT-MAP	5.03 ± 1.05 b	>8	4.72 ± 0.77 a	>8	4.93 ± 0.81 a	4.77 ± 0.92 a	4.77 ± 0.92 b
CHT	1.43 ± 0.37 a	1.36 ± 0.39 a	>8	7.82 ± 0.11 b	7.23 ± 0.00 b	7.69 ± 0.23 b	1.36 ± 0.39 a
CHT-COAT-MAP	2.02 ± 0.32 a	7.15 ± 1.97 c	3.87 ± 1.53 a	6.97 ± 0.25 a	6.58 ± 0.33 b	4.88 ± 0.25 a	2.02 ± 0.32 a
CHT-ACT-COAT-MAP	>8	>8	4.09 ± 0.85 a	7.88 ± 0.20 b	6.35 ± 0.36 b	5.05 ± 0.30 a	5.05 ± 0.30 b

Data are presented ±standard deviation.
Data in column with different small letters are significantly different ($p < 0.05$).
(CNTL, chitosan-free Fior di latte in brine; CNTL-COAT-MAP, chitosan-free Fior di latte, coated with alginate, stored under MAP; CNTL-ACT-COAT-MAP, chitosan-free Fior di latte, coated with alginate loaded with lysozyme and Na_2–EDTA, stored under MAP; CHT, chitosan-loaded Fior di latte in brine; CHT-COAT-MAP chitosan-loaded Fior di latte, coated with alginate, stored under MAP; CHT-ACT-COAT-MAP, chitosan-loaded Fior di latte, coated with alginate loaded with lysozyme and Na_2–EDTA, stored under MAP).
$MAL^{coliforms}$ Microbiological Acceptability Limit for Coliforms. $MAL^{Pseudomonas}$ Microbiological Acceptability Limit for *Pseudomonas* spp.
$SAL^{COL.}$ Sensorial Acceptability Limit for colour. $SAL^{OD.}$ Sensorial Acceptability Limit for odour. $SAL^{CONS.}$ Sensorial Acceptability Limit for consistency. $SAL^{O.Q.}$ Sensorial Acceptability Limit for overall quality.

Hoover, & Knorr, 1992; Uchida et al., 1989; Ueno et al., 1997; Wang, 1992) and the main factors affecting its antibacterial activity are molecular weight and concentration (Gerasimenko, Avdienko, Bannikova, Zueva, & Varlamov, 2004; Tikhonov, Radigina, & Yamskov, 1996; Zheng & Zhu, 2003). In mozzarella cheese chitosan, at a low molecular weight, had more antimicrobial effect (Altieri et al., 2005) in comparison with chitosan, at a higher molecular weight, used in this work. This effect can be also due to different concentrations (i.e., in the former case was 0.075% and in the latter was 0.012%), dissolving medium (whey in contrast to lactic acid) and of course, to different types of chitosan. However, some residual antimicrobial activity was still present as the $MAL^{coliforms}$ of CNTL-ACT-COAT-MAP was lower than that of CHT-ACT-COAT-MAP.

Data listed in Table 1 highlight that $MAL^{Pseudomonas}$ was affected by coating and MAP as well as by the active coating. The presence of chitosan alone did not affect significantly the $MAL^{Pseudomonas}$, as observed for coliforms. Data also suggest that chitosan works in synergy with the other hurdles tested in this study (i.e., coating, MAP, and active coating) to inhibit the growth of *Pseudomonas* spp.

Fig. 2 shows the viable counts of lactic acid bacteria for all the investigated sample. As can be inferred from the figure the cell load of lactic acid bacteria of CNTL-ACT-COAT-MAP and CHT-ACT-

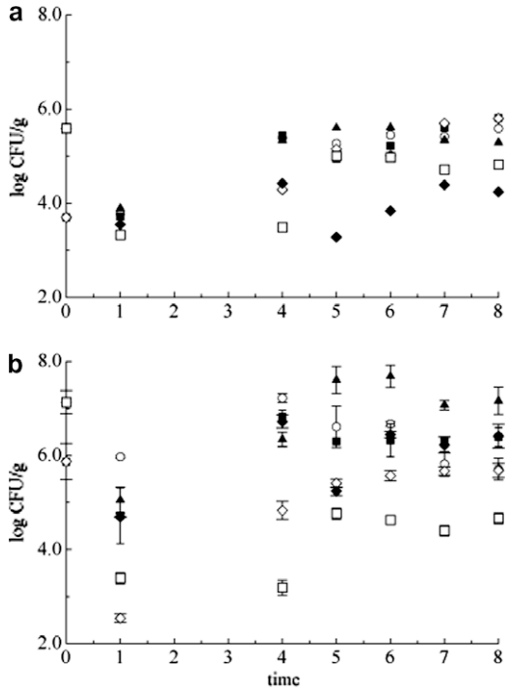

Fig. 2. (a) Evolution of rod lactic acid bacteria count in "Fior di latte" cheese during the storage period. (b) Evolution of coccus-shaped lactic acid bacteria count in "Fior di latte" cheese during the storage period. (○ CNTL, chitosan-free Fior di latte in brine; ■ CNTL-COAT-MAP, chitosan-free Fior di latte, coated with alginate, stored under MAP; ◇ CNTL-ACT-COAT-MAP, chitosan-free Fior di latte, coated with alginate loaded with lysozyme and Na_2–EDTA, stored under MAP; ▲ CHT, chitosan-loaded Fior di latte in brine; ♦ CHT-COAT-MAP chitosan-loaded Fior di latte, coated with alginate, stored under MAP; □ CHT-ACT-COAT-MAP, chitosan-loaded Fior di latte, coated with alginate loaded with lysozyme and Na_2–EDTA, stored under MAP).

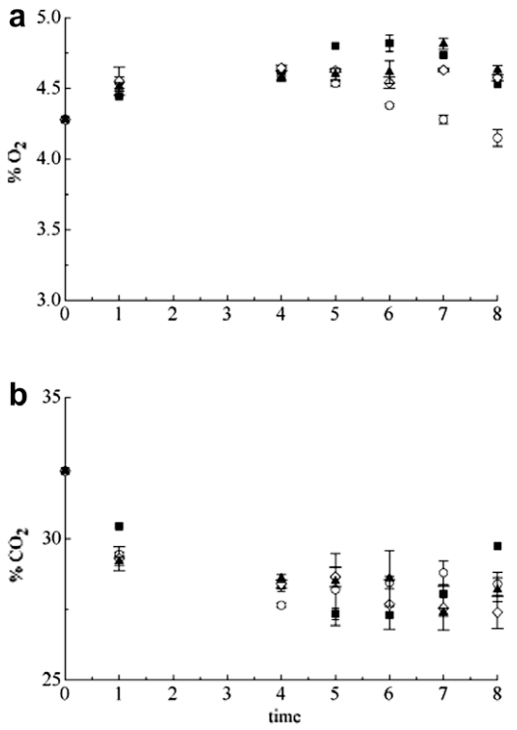

Fig. 3. (a) Oxygen evolution in the hermetically sealed bags of "Fior di latte" cheese. (b) Carbon dioxide evolution in the hermetically sealed bags of "Fior di latte" cheese. (■ CNTL-COAT-MAP, chitosan-free Fior di latte, coated with alginate, stored under MAP; ◇ CNTL-ACT-COAT-MAP, chitosan-free Fior di latte, coated with alginate loaded with lysozyme and Na_2–EDTA, stored under MAP; ♦ CHT-COAT-MAP chitosan-loaded Fior di latte, coated with alginate, stored under MAP; □ CHT-ACT-COAT-MAP, chitosan-loaded Fior di latte, coated with alginate loaded with lysozyme and Na_2–EDTA, stored under MAP).

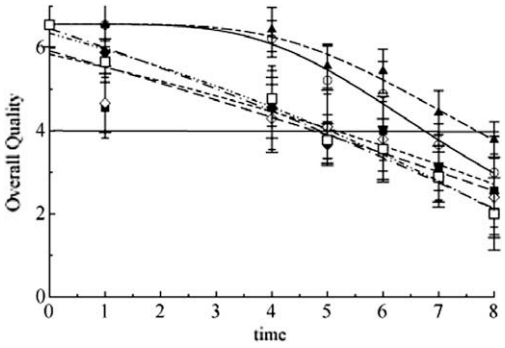

Fig. 4. "Fior di latte" cheese Overall Quality during the storage period. The curves are the best fit of Eq. (2) to the experimental sensorial data. (○ CNTL, chitosan-free Fior di latte in brine; ■ CNTL-COAT-MAP, chitosan-free Fior di latte, coated with alginate, stored under MAP; ◇ CNTL-ACT-COAT-MAP, chitosan-free Fior di latte, coated with alginate loaded with lysozyme and Na_2–EDTA, stored under MAP; ▲ CHT, chitosan-loaded Fior di latte in brine; ♦ CHT-COAT-MAP chitosan-loaded Fior di latte, coated with alginate, stored under MAP; □ CHT-ACT-COAT-MAP, chitosan-loaded Fior di latte, coated with alginate loaded with lysozyme and Na_2–EDTA, stored under MAP).

COAT-MAP sample decrease, especially for coccus-shaped. In contrast, chitosan alone did not seem to affect the growth of lactic bacteria; this concurs with Altieri et al. (2005); whereas Papaioannou, Chouliara, Karatapanis, Kontominas, and Savvaidis (2007) and Eliot et al. (1998) indicated that modified atmosphere slightly affected growth of these bacteria in whey and mozzarella cheese, respectively.

The yeasts (data not shown) were found in similar amounts in all samples, and the following trend was observed: there was a period of stability in the counts followed by a considerable rise, which was more evident in the CNTL sample.

3.2. Physico-chemical analysis

Fig. 3 shows the headspace oxygen and carbon dioxide concentration evolution during the storage period for the samples packaged under MAP. As expected, a decrease in the headspace oxygen concentration was observed along with an increase in headspace carbon dioxide concentration. However, this change in gas composition was relatively small, suggesting that the initial MAP conditions were protracted for the entire observation period. Most probably, this can be ascribed to high barrier properties of the packaging film (see OTR and WVTR), which limits the mass exchange rate between package headspace and environment.

The pH of samples tested in this study slightly increases during the storage period (data not shown). In particular, it ranges from about 5.3 to about 5.9, suggesting that the observed antimicrobial activity of some of tested packaging strategies cannot be ascribed to the change in the pH. The increase in pH observed could be the consequence of the mass exchange between the product and the "covering" liquid, which takes place during storage.

3.3. Sensory analysis

Fig. 4 shows the evolution during the storage period of the "Fior di latte" *overall quality*. The curves were obtained by fitting Eq. (2) to the experimental data, whereas the horizontal dashed line is the sensory threshold value. As expected, the quality of the tested cheese steadily decreased, regardless of the packaging strategies adopted. The SAL values of the monitored sensory attributes as well as that of the "Fior di latte" *overall quality* are also listed in Table 1.

As can be inferred from data, CNTL and CHT samples have the highest $SAL^{O.Q.}$ values among those of the tested samples, suggesting that all the investigated packaging strategies slightly speed up the quality loss of "Fior di latte" during the storage. Consistency (see $SAL^{Cons.}$) limits the $SAL^{O.Q.}$ of CNTL and CHT samples, this was most probably due to the mass exchange between "Fior di latte" and brine; whilst, the colour was the limiting attribute for all the other samples ($SAL^{Col.}$). Most probably the colour modification could be ascribed to the absence of brine, which in turn induced a dehydration of "Fior di latte" surface with consequent colour variation. To date, there are no data reported in the literature on the effect of brine on mozzarella cheese colour. However, MAP did not affect the colour of the packaged cheese as stated by Olarte, Gonzales-Fandos, and Sanz (2001). Regarding the influence of the active compounds (i.e., lysozyme and EDTA) on $SAL^{Col.}$, it seems that the effect, if present, was negligible. The presence of chitosan improved the $SAL^{Cons.}$ of all the tested samples, as stated by Altieri et al. (2005), however the differences were not significant.

3.4. Shelf life

The "Fior di latte" shelf life is listed in Table 1 for each of the samples tested in this study. It was calculated as the lowest value among $MAL^{Pseudomonas}$, $MAL^{coliforms}$, and $SAL^{O.Q.}$ (Conte et al., 2008). It can be emphasized from data that microbial quality limits the shelf life of lysozyme-free samples, whereas the sensory quality controls the shelf life of the samples coated with the active alginate solution (i.e., 4.77 ± 0.92 for CNTL-ACT-COAT-MAP and 5.05 ± 0.30 for CHT-ACT-COAT-MAP). Chitosan affects only slightly the shelf life of packaged cheese, the differences in shelf life between CNTL and CHT samples being not statistically significant.

To sum up, the combination of chitosan, active coating and MAP increased the shelf life of the packaged "Fior di latte" to 5 days, thus representing a strategic solution to prolong the shelf life of this dairy product.

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