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Active coating to prolong the shelf life of Fior di latte cheese

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This study explains how active coating can serve to prolong the shelf life of Fior di latte cheese. The active coating was prepared by dissolving, in two sodium alginic acid solutions (5 and 8% w/v), different concentrations of lysozyme (0·25, 0·50 and 1·00 mg ml^{-1})+50 mM of Ethylene-Diamine Tetraacetic Acid (EDTA). Samples of Fior di latte cheese packaged in brine and active brine (lysozyme+EDTA, at the above concentrations) were also used as controls. The quality decay of the Fior di latte cheese stored at 10 °C was assessed by monitoring the viable cell concentration of the main spoilage microorganism, as well as its sensory quality (i.e., external appearance, consistency, colour and flavour). The concentration of rod-or coccus-shaped Lactic Acid Bacteria (LAB) was also monitored to assess the effect of the proposed packaging strategies on the flora type of Fior di latte cheese. The results show that an increase in the shelf life equal to 104% was recorded for the coated samples, compared with controls packaged in brine without active compounds. This shelf life increase is slightly lower than that recorded with samples packaged in the active brine (151%), as a result of a more pronounced microbial proliferation; however, the coating could be a better packaging solution for the reduced weight of tray.

Keywords: Fior di latte cheese, coating, lysozyme, shelf life.

Traditional dairy products of the Mediterranean area are increasing in popularity among consumers because of their unique taste. Fior di latte cheese is the most popular member of the pasta filata cheeses, with high moisture (55 to 60%) and high fat (45% dry matter basis) content (Salvadori del Prato, 2001), characterized by a pH 4·9 to 5·6, a soft body and a juicy appearance, and by a fresh and slightly sour flavour (UNI 10979:2002). The manufacture of this cheese has been described in detail in the literature (Parente & Moschetti, 1997; Salvadori del Prato, 2001). It is produced either according to traditional procedures (raw milk inoculated with natural whey or milk cultures, raw milk ripened under special conditions, without starter addition) or by using pasteurized milk and commercial starter cultures of LAB (Salvadori del Prato, 2001). Although mozzarella cheese receives a heat treatment during curd stretching, post-processing contamination by microorganisms may occur, causing cheese spoilage, health risks for consumers, and shelf life reduction (Spano et al. 2003). Few reports are available dealing with methods to prolong the shelf life of fresh cheeses.

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). These systems do not represent a strategic solution to prolong the shelf life of the selected dairy product.

Today, many researchers focus their attention on the development of new minimal processing technologies and innovative materials to increase food shelf life. One of the most interesting strategies is the active packaging intended 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 et al. 2003). The most diffuse active packaging systems are based on oxygen scavenger, moisture absorbers, carbon dioxide or ethanol generator, and antimicrobial systems. Among the active packaging solutions, the antimicrobial release systems are receiving considerable attention as a means of prolonging the bacterial lag phase, or to delay the growth rate of spoilage microorganisms (Vermeiren et al. 1999; Han, 2000; Cutter, 2002; Quintavalla & Vicini, 2002;

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Mauriello et al. 2005; Rojas-Grau et al. 2007). Several natural substances, such as chitosan, bacteriocins essential oils and lysozyme, are suitable to develop active films or edible coatings. Lysozyme is a lytic enzyme found in many natural systems, and has been used in cheese manufacture to prevent the growth of lactate-fermenting, gas-forming *Clostridia* spp. with a dosage of 25 mg l^{-1} milk (Crapisi et al. 1993). The antimicrobial spectrum of lysozyme could be enhanced when it is used with other substances, such as EDTA (Branen & Davidson, 2004; Sinigaglia et al. 2008), disodium pyrophosphate, pentasodium tripolyphosphate (Boland et al. 2003), caffeic acid and cinnamic acid (Masschalck & Michiels, 2003).

In the scientific literature, a few applications of active packaging systems to fresh dairy products are reported. In particular, a release system based on lemon extract was successfully investigated by Conte et al. (2007). Sinigaglia et al. (2008) demonstrated the antimicrobial effectiveness of lysozyme+Na_2-EDTA, dissolved in brine, in prolonging mozzarella storability. Furthermore, Pantaleao et al. (2007) studied an active packaging able to extend the shelf life of a fresh cheese, by using sachets impregnated with sodium propionate.

Another important factor that limits the distribution of fresh dairy products beyond the market borders is the weight of the packaging. Laurienzo et al. (2006) showed that the use of a gel packaging, based on natural polysaccharides, could represent a strategic solution to both reduce the packaging weight and increase the shelf life of mozzarella cheese.

Due to the above considerations, the objective of this study is to determine the effectiveness of a new active packaging system. To this aim, two alginate-based coatings (5 and 8% w/v) were loaded with different concentrations of lysozyme and Na_2-EDTA and applied to Fior di latte cheeses. The effectiveness of the same active compound directly dissolved into brine was also tested for sole comparative purposes.

Materials and Methods

Samples preparation

Samples of Fior di latte cheese (weight 50 g, diameter 5–7 cm, pH 6·00) produced through chemical acidification were purchased from a cheese factory located in Puglia (Southern Italy) and brought to our laboratory under refrigeration (4 °C). Fior di latte cheese was removed from its package and dipped into two active sodium alginate-based solutions (Sigma-Aldrich, Gallarate, Italy). The solutions were prepared by dissolving sodium alginic acid (5 and 8% w/v) in distilled water and by adding lysozyme (Sigma-Aldrich), at three different concentrations (0·25, 0·50 and 1·00 mg/ml), and 50 mM of Na_2–EDTA (J.T. Baker, Milan, Italy). All the coated samples were immersed into a 5% (w/v) calcium chloride ($CaCl_2$, Sigma-Aldrich) solution for 1 min to crosslink the polymeric matrix. All samples were dried at room temperature for 2 min. Each sample was packed in polypropylene tubs and stored at 10 °C for 8 d.

As controls, samples of Fior di latte were also packaged in tubs with 170 ml brine (2% NaCl, pH 6·2–6·5), and with the same brine added with three different concentrations of lysozyme (0·25, 0·50 and 1·00 mg/ml) and 50 mM of Na_2–EDTA. These controls are referred to as CNTR and CNTR-ACT, respectively. To prevent the decrease of pH due to the addition of Na_2–EDTA, 100 mM-phosphate buffer (K_2HPO_4/KH_2PO_4, J.T. Baker) was previously added to the active brine; in this way, pH ranged between 5·5–6·0 avoiding effects of brine acidification on spoiling microorganisms (Sinigaglia et al. 2008).

Microbiological analyses

Twenty grams Fior di latte cheese were diluted in 180 ml 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, Milan, Italy), incubated at 30 °C for 48 h for total microbial count, MRS agar (Oxoid), supplemented with cycloheximide (100 mg l^{-1}, Sigma-Aldrich, Gallarate, Italy), incubated under anaerobiosis (Anaerogen Gas Pack, Oxoid) at 37 °C for 48 h for lactic acid bacilli; M17 agar (Oxoid), incubated at 37 °C for 48 h for coccus-shaped LAB; yeast peptone dextrose agar (YPD, Oxoid), supplemented with chloranphenicol (0,1 g l^{-1}, Oxoid) incubated at 30 °C for 48 h for yeasts and moulds; 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. Microbiological analyses were performed twice, before packaging and after 1, 2, 3, 4, 7, and 8 d storage.

In order to quantitatively determine the efficiency of the packaging strategy proposed in this work, the Gompertz equation (1) as re-parameterized by Corbo et al. (2006), was fitted to the *Pseudomonas'* data:

$$\log(N(t)) = \log(N_{max}) - A \cdot \exp\left\{-\exp\left\{\left[(\mu_{max} \cdot 2{\cdot}71) \cdot \frac{\lambda - MAL}{A}\right] + 1\right\}\right\} + A \cdot \exp\left\{-\exp\left\{\left[(\mu_{max} \cdot 2{\cdot}71) \cdot \frac{\lambda - t}{A}\right] + 1\right\}\right\}$$

where: N(t) is the viable cell concentration at time t, A is related to the difference between the decimal logarithm of maximum bacteria 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 time.

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pH determination

The measurement of pH was performed twice on all Fior di latte cheese samples during storage, with a pH meter (Crison, Barcelona, Spain).

Sensory analysis

To detect the effect of packaging on cheese sensory quality decay during storage, samples were examined at the same time intervals of microbiological analysis by a panel of seven-laboratory staff members, well experienced and familiar with cheese. They graded Fior di latte cheese for *exterior appearance, consistency, colour*, and *odour*. All these attributes were graded on a 0–7 scale (0=lowest quality, 7=best quality) (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 quantitatively determine the efficiency of the packaging system proposed in this work in terms of sensory quality preservation, the Gompertz equation (2) as re-parameterized by Corbo et al. (2006) was fitted to data relative to *overall quality*:

$$OSQ(t) = OSQ_{min} - A^{Q} \cdot \exp\left\{-\exp\left\{\left[(\mu^{Q}_{max} \cdot 2{\cdot}71) \cdot \frac{\lambda^{Q} - SAL}{A^{Q}}\right] + 1\right\}\right\} + A^{Q} \cdot \exp\left\{-\exp\left\{\left[(\mu^{Q}_{max} \cdot 2{\cdot}71) \cdot \frac{\lambda^{Q} - t}{A^{Q}}\right] + 1\right\}\right\}$$

where OSQ(t) is the Fior di latte overall sensory quality at time t, A^{Q} is related to the difference between the Fior di latte overall sensory quality attained at the stationary phase and the initial value of Fior di latte'' overall sensory quality, μ^{Q}_{max} the maximal rate at which OSQ(t) change, λ^{Q} is the lag time, OSQ_{min} is the Fior di latte overall sensory quality threshold value, SAL is the sensory acceptability limit (i.e., the time at which OSQ(t) is equal to OSQ_{min}), and t is the storage time. The value of the sensory quality threshold limit (OSQ_{min}) is equal to 4.

Shelf life calculation

Wherever the global quality of a given product depends on several quality sub-indices, the shelf life of the packed product is, by definition, the time at which one of the product quality sub-indices reaches its threshold value (Corbo et al. 2008). In the case under investigation, the shelf life of each tested sample was calculated as the lowest value between the MAL and the SAL values.

Statistical analysis

The MAL and SAL values were compared, respectively, by one-way variance analysis (ANOVA). A Duncan's multiple range test, with the option of homogeneous groups ($P<0{\cdot}05$), was used to determine significance between the different treatments. STATISTICA 7.1 for Windows (StatSoft, Inc, Tulsa, OK, USA) was used for this purpose.

Results and Discussion

The Fior di latte cheese quality during storage was evaluated by monitoring microbial load, pH and sensory properties. The *Pseudomonas* count, as main spoilage microbial group, together with the *overall sensory quality* was used to determine the shelf life of this dairy product packaged in different systems, as reported in the following.

Figure 1a illustrates the evolution during storage of *Pseudomonas* spp. viable cell concentration for all Fior di latte cheese samples. The curves shown in fig 1 were obtained by fitting the Eq. (1) to the experimental data. The value of N_{max} for *Pseudomonas* spp. was set to 10^6 CFU/g, because at this contamination level the alterations of the product start to appear (Bishop & White, 1986). The values of MAL reported in Table 1 show that both types of active packaged samples (i.e., CNTR-ACT and coated samples) have MAL values higher than that of Fior di latte packaged in the traditional brine, due to the effectiveness of the selected antimicrobial compounds. Duan et al. (2007) and Sinigaglia et al. (2008) also assessed the efficacy of lysozyme on *Pseudomonas* spp., the enzyme being in mozzarella packaging. In the case under study, the CNTR-ACT samples did not reach the *Pseudomonas* spp. threshold value during the entire observation period, suggesting that MAL value is higher than 8 d. In contrast, the coated samples have a MAL ranging between 2 and 3 d, which correspond to an increase of 104%, compared with the MAL of CNTR sample. Lysozyme concentration did not greatly affect the MAL value of coated samples, whereas 8% coating seems to be more efficient in inhibiting the growth of *Pseudomonas*, compared with the 5% coating. As we can seen in Fig. 1a, an initial decrease, followed by an increase of cell number of *Pseudomonas* spp. after 3 d storage was observed in CNTR-ACT samples, whereas CNTR sample showed a gradual increase followed by a period of stability. The addition of lysozyme and Na_2-EDTA in the brine (CNTR-ACT) inhibited their growth, probably due to a reversible stress on the cells, as reported by Johnston & Brown (2002) and Richards & Cavill (1976). The use of Na_2-EDTA could cause reversible damage to the outer membrane, inhibiting the ability to make colonies on plates. Within a prolonged stress condition, the cell would repair the damages to the membrane acquiring the culturable ability, as stated by Sinigaglia et al. 2008. In fact, lysozyme is well known for its antimicrobial properties (Bester & Lombard, 1990; Cunningham et al. 1991; Davidson et al. 1993) and EDTA is known to amplify its

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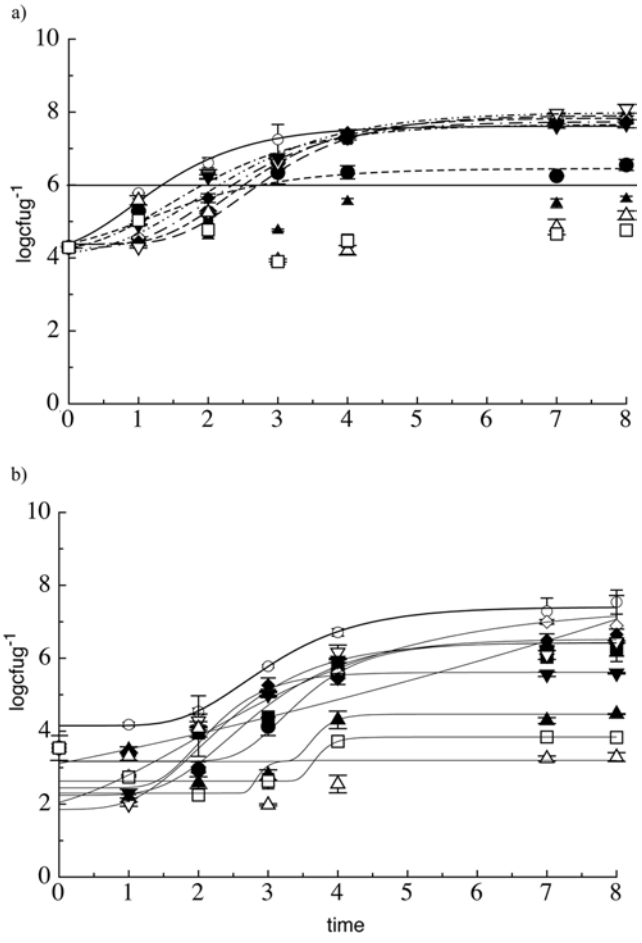

Fig. 1. a) Evolution of *Pseudomonas* spp. count in Fior di latte cheese during storage period. The curves are the best fit of Eq. (1) to the experimental data. b) Evolution of total coliform count in Fior di latte cheese during storage period. The curves are drawn with the sole aim to highlight the trend of data. (○ CNTR; ▲ CNTR-ACT 0·25 mg lysozyme/ml 50 mM-Na_2-EDTA; ● CNTR-ACT 0·50 mg lysozyme/ml 50 mM-Na_2-EDTA; □ CNTR-ACT 1·00 mg lysozyme/ml 50 mM-Na_2-EDTA; ● 8% sodium alginic acid 0·25 mg lysozyme/ml 50 mM-Na_2-EDTA; ■ 8% sodium alginic acid 0·50 mg lysozyme/ml 50 mM-Na_2-EDTA; ◇ 8% sodium alginic acid 1·00 mg lysozyme/ml 50 mM-Na_2-EDTA; ◆ 5% sodium alginic acid 0·25 mg lysozyme/ml 50 mM-Na_2-EDTA; ▽ 5% sodium alginic acid 0·50 mg lysozyme/ml 50 mM-Na_2-EDTA; ▼ 5% sodium alginic acid 1·00 mg lysozyme/ml 50 mM-Na_2-EDTA).

antimicrobial activities, especially against Gram-negative microorganisms (Stevens et al. 1991; Razavi-Rohani & Griffiths, 1994; Gill & Holley, 2000; Branen & Davidson, 2004). In contrast, for the coated samples there is a moderate decrease in *Pseudomonas* cell numbers, followed by an increase at the end of storage, except for coated sample (8%) with 0·25 mg lysozyme, which showed a marked decrease until the end of the storage time. The lower antimicrobial activity of coated samples could be ascribed to both lower macromolecular mobility and lower amount of lysozyme and EDTA in the alginate matrix, if compared with the brine. Both these factors reduce the availability of active compounds on Fior di latte surface, affecting their effect on microbial growth. Concerning the influence of alginate concentration on active coating antimicrobial efficacy, the higher alginate concentration (8%) increases the viscosity of the coating forming solution, which in turn increases the amount of alginate that adheres to the Fior di latte (coating thickness). Therefore, most probably coated samples with 8% alginate have a higher amount of antimicrobial compounds compared with samples coated with 5% renewable material.

Figure 1b shows the evolution of total coliforms over storage time for coated and control samples. As expected, coliforms were able to proliferate in the control sample (CNTR). The addition of lysozyme and Na_2-EDTA in the brine (CNTR-ACT) inhibited their growth from the second day of storage. However, this effect was similar to that observed for *Pseudomonas* spp. Among the coated samples, those covered with the highest concentration of sodium arginate (8%) and lower concentrations of lysozyme highlight a more pronounced microbial decrease. In contrast, for 5% coated samples wild fluctuations in coliform growth were recorded even if the trend was downward.

Figure 2 shows the evolution of LAB in all Fior di latte cheese samples. As shown in figure, the cell load of typical flora is not affected by the packaging system, suggesting that the selected agents, at the concentration applied, do not affect the growth of typical dairy microorganisms. Sinigaglia et al. (2008) also verified that lysozyme dissolved in brine solution did not inhibit LAB.

No moulds were detected on samples during the storage period. With regard to yeasts, similar trends were recorded between the different samples (data not shown). The yeasts' load in all cheese samples started from a low level (10^2 CFU/g) to arrive at 10^3–10^4 CFU/g at the stationary phase. The above evidences are in agreement with what reported in the literature by Nunez et al. (1981) and Coppola et al. (1988).

The total microbial count was similar in all samples (data not shown); the counts slightly increased but then remained relatively stable.

According to the literature data (Salvadori del Prato 2001), the pH (data not shown) slightly decreases with storage time, ranging between 6·0 and 5·0 for all samples, regardless of the packaging system. This experimental evidence suggests that the detected antimicrobial activity has to be ascribed to the investigated natural compound.

Figure 3 gives the *overall quality* plotted as a function of storage time for all samples. The curves shown in figure were obtained by fitting Eq. (2) to the experimental data; the values of SAL parameter are also listed in Table 1. It should be noted that the highest score of *overall quality* was awarded to the coated samples (alginate 5%) with 0·50 mg lysozyme, becoming unacceptable after almost 5 d. On the contrary, all other samples were refused after 2–3 d. In particular, the CNTR-ACT samples were found

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Table 1. Shelf life of Fior di latte samples evaluated on the basis of MAL and SAL

Values are means ± Standard error for $n=2$

	MAL (day)		SAL (day)		Shelf Life (day)	
CNTR	1·33 ± 0·12	A	3·70 ± 0·12	A	1·33 ± 0·12	A
CNTR-ACT 0·25 mg ml^{-1} Lysozyme 50 mM Na_2–EDTA	>8		3·37 ± 0·14	A	3·37 ± 0·14	B
CNTR-ACT 0·50 mg ml^{-1} Lysozyme 50 mM Na_2–EDTA	>8		3·16 ± 0·14	A	3·16 ± 0·15	B
CNTR-ACT 1·00 mg ml^{-1} Lysozyme 50 mM Na_2–EDTA	>8		2·71 ± 0·29	A	2·71 ± 0·29	E
8% sodium alginic acid 0·25 mg ml^{-1} Lysozyme 50 mM Na_2–EDTA	2·74 ± 0·52	D	3·69 ± 0·50	A	2·74 ± 0·52	E
8% sodium alginic acid 0·50 mg ml^{-1} Lysozyme 50 mM Na_2–EDTA	2·72 ± 0·00	D	3·09 ± 0·30	A	2·72 ± 0·00	E
8% sodium alginic acid 1·00 mg ml^{-1} Lysozyme 50 mM Na_2–EDTA	2·51 ± 0·05	CD	3·66 ± 0·71	A	2·51 ± 0·05	DE
5% sodium alginic acid 0·25 mg ml^{-1} Lysozyme 50 mM Na_2–EDTA	2·35 ± 0·07	CD	3·58 ± 0·24	A	2·35 ± 0·07	DE
5% sodium alginic acid 0·50 mg ml^{-1} Lysozyme 50 mM Na_2–EDTA	2·16 ± 0·24	BC	4·97 ± 0·75	B	2·16 ± 0·24	CD
5% sodium alginic acid 1·00 mg ml^{-1} Lysozyme 50 mM Na_2–EDTA	1·91 ± 0·09	B	3·72 ± 0·88	AB	1·91 ± 0·09	C

Data in column with different capital letters are significantly different ($P<0·05$)

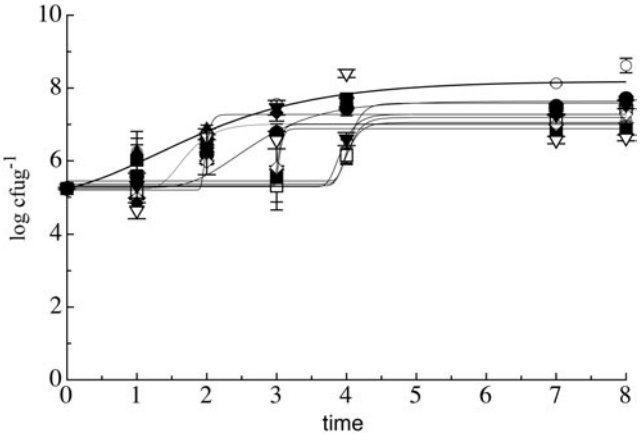

Fig. 2. Evolution of Lactic Acid Bacteria count in Fior di latte cheese during storage period. The curves are drawn with the sole aim to highlight the trend of data. For details of symbols see Fig. 1.

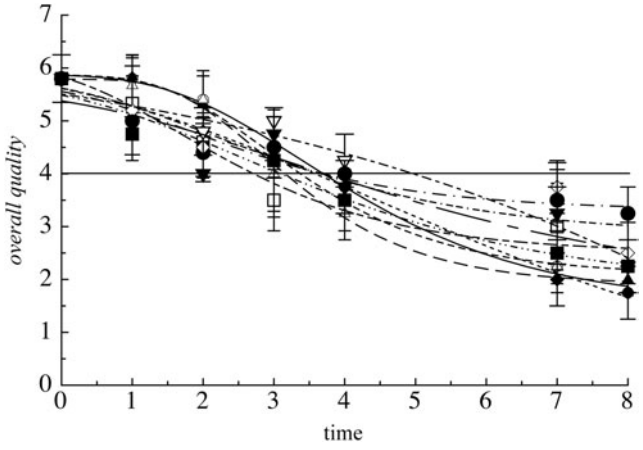

Fig. 3. Fior di latte cheese *overall quality* during storage period. The curves are the best fit of Eq. (2) to experimental data. For details of symbols see Fig. 1.

unacceptable for the aspect of the surface, probably compromised by the presence of Na_2–EDTA. Regards the coated cheese, they received slightly better scores of SAL, compared to the other samples packaged in active systems, regardless of coating concentration and lysozyme amount. This was probably due to a lower impact of the salt on the product, being it incorporated in the coating.

Shelf life values were also reported in Table 1. As can be inferred, a significant shelf life prolongation was recorded for the CNTR-ACT samples (i.e., an increase of about 151% compared with CNTR sample), the sensory quality being responsible for Fior di latte unacceptability. A slightly lower shelf life was obtained for active coated samples in comparison to products in active brine, the *Pseudomonas* count being the limiting factor. However, compared with the CNTR sample, an increase accounts for more than 100% was recorded for cheese in active coating, suggesting that this strategy could represent a valid packaging system because it is able to combine microbial proliferation prevention with reduced weight of packaging.

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