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Combined effect of active coating and modified atmosphere packaging on prolonging the shelf life of low-moisture Mozzarella cheese

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ABSTRACT

In this work, the effect of active coating on the shelf life of low-moisture Mozzarella cheese packaged in air and modified atmosphere (MAP) was studied. The active coating was based on sodium alginate (2%, wt/vol) and potassium sorbate (1%, wt/vol). The MAP was made up of 75% CO₂ and 25% N₂ (MAP1), 25% CO₂ and 75% N₂ (MAP2), or 50% CO₂ and 50% N₂ (MAP3). The product quality decay was assessed by monitoring microbiological and sensory changes during storage at 4, 8, and 14°C. Results showed that the combination of active coating and MAP was able to improve the preservation of low-moisture Mozzarella cheese. Specifically, the shelf life increased up to 160 d for samples stored at 4°C, and 40 and 11 d for those at 8 and 14°C, respectively. A faster quality decay for untreated samples packaged in air was observed. In particular, the *Pseudomonas* spp. growth and the appearance of molds were responsible for product unacceptability. The combination of active coating and MAP represents a strategic solution to prolong the shelf life of low-moisture Mozzarella cheese and to ensure the safety of the product under thermal abuse conditions.

Key words: low-moisture Mozzarella cheese, active coating, modified atmosphere packaging, shelf life

INTRODUCTION

Mozzarella cheese is one of several pasta filata cheeses originating in Italy. Two main types of Mozzarella are produced, based on moisture content (US FDA, 1989). In particular, low-moisture (LM) Mozzarella cheese (moisture content: 45–54%) is often used for pizza toppings or as an ingredient in other foods, whereas high-moisture Mozzarella cheese (moisture content: 56–65%), which is the principal Italian Mozzarella exported, is

usually consumed fresh as a table cheese (Gammariello et al., 2008; Conte et al., 2009; Del Nobile et al., 2009). Generally, LM Mozzarella cheese is packaged under vacuum in trays of polypropylene sealed with a plastic top film and stored at 8°C. In these conditions, the shelf life is less than 5 wk; as the product increases its firmness, the odor becomes unpleasant and develops a bitter taste.

Extending the shelf life of Mozzarella cheese is an important issue to the dairy industry, due to the high interest in extending the distribution of traditional products beyond market borders. The main ways of improving the food product quality and shelf life are based on using high-quality raw materials, developing process innovations, and adopting suitable storage conditions (Farkye et al., 1991; Kindstedt, 1993; Brody, 2001; Conte et al., 2009).

Edible films and coatings prepared from polysaccharides, proteins, and lipids have a variety of advantages, such as biodegradability, edibility, biocompatibility, aesthetic appearance, and barrier properties, against oxygen and physical stress. Edible films prepared from hydrocolloids, such as sodium alginate, form strong films and exhibit poor water resistance because of their hydrophilic nature (Guilbert, 1986).

The use of edible coatings for a wide range of food products has received increased interest, because coatings can serve as a carrier for a wide range of food additives, including antibrowning agents, colorants, flavors, nutrients, spices, and various antimicrobials that can extend product shelf life and reduce the risk of pathogen growth on the food surface (Cagri et al., 2004; Pranoto et al., 2005; Conte et al., 2009; Del Nobile et al., 2009).

The potential of modified atmosphere packaging (MAP) and active packaging to extend the shelf life of different dairy products has been demonstrated (Flores et al., 2000; Pantaleão et al., 2007; Papaioannou et al., 2007). Modified atmosphere packaging reduces physiological changes, respiration rates, oxidation reactions, and microbial growth by changing the level of gases that surround the product. To the best of our

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knowledge, very few papers have discussed Mozzarella cheese packaged in MAP. Eliot et al. (1998) reported that shredded Mozzarella cheese packaged in MAP containing levels of 75% CO₂ was well protected from undesirable organism and gas formation. Alves et al. (1996) also found that microbial growth in sliced Mozzarella cheese packaged in MAP was delayed with high concentrations of CO₂.

Considering the above, the aim of the current work was to evaluate how the combination of active coating and MAP can improve the shelf life of LM Mozzarella cheese. Specifically, the quality decay was assessed by monitoring the microbiological and sensorial subindices of the product and the effect of temperature was also investigated.

MATERIALS AND METHODS

Active Coating and Packaging of LM Mozzarella Cheese

Low-moisture Mozzarella cheese samples (~150–200 g) were kindly provided by a local cheese factory (Capurso Azienda Casearia SpA, Gioia del Colle, Bari, Italy) and brought to our laboratory in boxes containing 10 pieces each under refrigeration (4°C). According to information supplied by the manufacturer, all cheeses were produced by a common-milled curd procedure using starter culture that consisted exclusively of *Streptococcus thermophilus*, and with fermentation-produced chymosin as coagulant. Samples were removed from their packages and subjected to the active coating treatment. In particular, samples were dipped into a sodium alginate solution prepared by dissolving sodium alginate acid powder (2%, wt/vol) and potassium sorbate (1%, wt/vol) in sterile distilled water tempered to 50°C. Then, the coated samples were immersed into a crosslinking solution of calcium chloride (5%, wt/vol) for 1 min to promote the alginate gel-forming process. After treatment, the coated LM Mozzarella cheese samples (**COAT**) were air dried for 2 min. Then, 2 pieces per package were placed in commercially available bags (nylon/polyethylene) with a thickness of 95 µm (Valco S.r.l., Bergamo, Italy). The bags were 170 × 250 mm long, with an oxygen transmission rate of 49.39 cm³/(m²·d), carbon dioxide transmission rate of 162.83 cm³/(m²·d), and water vapor transmission rate of 1.64 g/(m²·d). In addition, LM Mozzarella cheese samples without active coating were used as control (**CTRL**). All samples were sealed in air and under modified atmosphere conditions. The gas combinations used were the following: **MAP1**: 75% CO₂ and 25% N₂, **MAP2**: 25% CO₂ and 75% N₂, and **MAP3**: 50% CO₂ and 50% N₂. All samples were stored at 4, 8, and

14°C. The sodium alginate, potassium sorbate, and calcium chloride were from Farmalabor S.r.l. (Canosa di Puglia, Italy).

Microbiological Analyses

Microbiological analyses for total viable count (**TVC**), *Pseudomonas* spp., *Enterobacteriaceae*, lactic acid bacteria (**LAB**), and lactococci were performed according to the International Organization for Standardization (ISO, 2001; ISO 8261:2001). Media and conditions used for the enumerations were as follows: plate count agar (Oxoid SpA, Milan, Italy) incubated at 30°C for 24 to 48 h for TVC; *Pseudomonas* agar base (Oxoid SpA) with 10 mg of cetrime/L, 10 mg of fucidine/L, and 50 mg of cephaloridine/L selective supplement (Oxoid SpA), incubated at 25°C for 48 h for *Pseudomonas* spp. count; for *Enterobacteriaceae*, violet red bile glucose agar (Oxoid SpA) incubated at 37°C for 18 to 24 h; de Man, Rogosa, and Sharpe (MRS) agar (Oxoid SpA) incubated anaerobically in HP 11 jars (Oxoid SpA) at 37°C for 2 to 4 d for LAB; and M17 agar (Oxoid SpA) incubated anaerobically in HP 11 jars (Oxoid SpA) at 37°C for 48 h for lactococci. The microbiological analyses were carried out twice on 2 different batches.

To determine the effectiveness of the packaging strategy, the microbiological acceptability limit (**MAL**), defined as the storage time at which microbial counts of the selected spoilage group reached the threshold value permitted, was calculated as reported by Del Nobile et al. (2009). The microbiological limit was fixed at 10⁶ cfu/g for *Pseudomonas* spp. (Conte et al., 2009).

Sensory Analysis

Low-moisture Mozzarella samples were subjected to sensory analysis by a panel consisting of 7 trained evaluators. The panel was selected and trained according to the International Organization for Standardization method 8586-1993 (ISO, 1993). The selection was made considering various aspects: interest and motivation, eating habits (consumption of dairy products), ability to communicate sensations, time available for analysis sessions, ability to concentrate, and performance training. Eight sessions of 1 h each were required to define the sensory profile and to familiarize the testers with the characteristics of LM Mozzarella cheese. After training, panelists were asked to evaluate color, odor, taste, and firmness attributes of LM Mozzarella cheese. Low-moisture Mozzarella samples were presented to panelists with and without active coating and they were asked to describe differences between samples using a scale from 1 to 7 (Corradini and Innocente,

2002). Panelists were asked to base their decision on the sample overall quality, only taking into account color, odor, taste, and firmness. Therefore, the samples' overall quality has to be considered as an average of the above-mentioned sensory attributes (i.e., color, odor, taste, and firmness), as weighted by the panelist (Conte et al., 2009). A score of 4 was the minimum threshold value for cheese acceptability. The sensorial acceptability limit (**SAL**; i.e., the storage time at which the sensory attribute reaches the threshold) was calculated as reported by Conte et al. (2009). During the panel test, the panelists were also asked to search for presence of visual molds and the visual molds time (**VMT**) was determined. The VMT is defined as the average time between the latest storage time at which molds were not visible and the earliest storage time at which molds were visible. The sensory evaluation of samples was stopped as soon as visible molds were detected on product surface.

Shelf-Life Calculation

The overall quality of a given product is related to the several quality subindices. Therefore, the shelf life of a packed product is, by definition, the time at which one of the product quality subindices reaches its threshold value. In this work, the shelf life of each tested sample was calculated as the lowest value among $MAL^{Pseudomonas}$, VMT, and the SAL in terms of overall quality (SAL^{OQ}).

Statistical Analysis

Experimental data of all tested samples were compared by one-way ANOVA. A Duncan's multiple range test, with the option of homogeneous groups ($P < 0.05$), was used to determine significant differences among treatments. To this aim, Statistica 7.1 for Windows software (StatSoft Inc., Tulsa, OK) was used.

RESULTS AND DISCUSSION

LM Mozzarella Cheese Stored at 4°C

Microbiological Analyses. Cheese microflora is dominated by LAB introduced with a cheese starter. The remaining microorganisms include thermoresistant bacteria originating from the raw material remaining after pasteurization of milk and microflora consisting of postpasteurization contamination (Molska, 1988).

The microbial counts of microorganisms investigated in this study is reported in Table 1. Microbial counts were monitored until the packaged LM Mozzarella cheese reached either its microbiological or sensorial

threshold value or until visible molds appeared on its surface. The observed changes of TVC depended on the type of packaging strategy used. A slight reduction in the TVC was observed for samples packaged in the atmosphere having high CO₂ concentration, with respect to the samples packaged in air and with low CO₂ levels. Specifically, TVC showed an initial cell load of about 4.3 log cfu/g and no significant differences between samples were observed up to 9 d. At 28 d of storage, control samples packaged with high CO₂ concentration (CTRL-MAP1 and CTRL-MAP3) showed a lower cell load (~4 log cfu/g) compared with samples in air (CTRL-air, 4.5 log cfu/g) and MAP with low CO₂ concentration (CTRL-MAP2, ~5.0 log cfu/g). A similar effect for the coated samples was also observed. This was probably due to the specific effect of CO₂ on TVC, as confirmed in previous studies (Eliot et al., 1998). At 50 d of storage, the control MAP1 samples showed the lowest cell load, whereas the highest value was observed for COAT-MAP3 samples (~6.0 log cfu/g). Then, the TVC progressively increased in all the cheese samples. The microbiological analyses for the control samples were interrupted at 78 d, as the samples were unacceptable from the sensory point of view, whereas for the coated samples, the TVC increased up to a value of about 7.0 log cfu/g at the end of storage. The analyses for coated samples in air and under low CO₂ levels (COAT-air and COAT-MAP2) were stopped at a shorter storage time than the other coated samples, due to unacceptable sensory characteristics.

Lactic acid bacteria counts varied little and significant differences between samples were observed only after 28 d of storage. In particular, control samples presented a lower cell load than the coated samples. Moreover, at 78 d of storage the cell load of LAB reached a value of about 5.0 log cfu/g for control samples and 6.0 log cfu/g for coated samples. Probably, the diffusion of the headspace oxygen to the surface of product was slowed down by the oxygen resistance of the coating. Therefore, oxygen sensitivity of anaerobic LAB would explain the lower cell load observed for the uncoated samples. The concentration of LAB increased up to a cell load of 7.0 log cfu/g at the end of storage.

For lactococci, significant differences at 5, 16, and 78 d of storage were observed. In particular, at 5 d of storage, CTRL-MAP3 samples (6.8 log cfu/g) were significantly different from CTRL-air and COAT-air samples (7.2 cfu/g). At 16 d of storage, the lowest cell load for coated and uncoated samples under low CO₂ levels (COAT-MAP2 and CTRL-MAP2) and coated samples under air (~7.0 log cfu/g) was observed compared with the COAT-MAP3 samples (7.4 log cfu/g). Then, the microbial counts did not show significant differences

Table 1. Microbial counts of microorganisms for low-moisture Mozzarella cheese stored at 4°C

Microorganism	Sample ¹	Cell load (log cfu/g)											
		d 0	d 5	d 9	d 16	d 28	d 50	d 78	d 98	d 145	d 249		
Total viable count	CTRL-air	4.29 ± 0.20 ^a	4.15 ± 0.21 ^a	4.24 ± 0.30 ^a	4.54 ± 0.18 ^{ab}	4.50 ± 0.28 ^b	4.76 ± 0.20 ^{ab}	6.18 ± 0.04 ^a	—	—	—	—	—
	CTRL-MAP1	4.29 ± 0.20 ^a	4.24 ± 0.34 ^a	4.44 ± 0.23 ^a	4.41 ± 0.15 ^{ab}	3.89 ± 0.16 ^a	4.26 ± 0.17 ^a	6.02 ± 0.09 ^a	—	—	—	—	—
	CTRL-MAP2	4.29 ± 0.20 ^a	4.21 ± 0.29 ^a	4.54 ± 0.23 ^a	4.19 ± 0.41 ^a	5.17 ± 0.18 ^{cd}	5.29 ± 0.08 ^{ab}	5.61 ± 0.22 ^a	—	—	—	—	—
	CTRL-MAP3	4.29 ± 0.20 ^a	4.25 ± 0.35 ^a	4.43 ± 0.09 ^a	4.38 ± 0.03 ^{ab}	3.92 ± 0.32 ^a	4.83 ± 0.10 ^{ab}	6.09 ± 0.33 ^a	—	—	—	—	—
	COAT-air	4.29 ± 0.20 ^a	4.69 ± 0.20 ^a	4.34 ± 0.23 ^a	4.45 ± 0.10 ^{ab}	5.40 ± 0.32 ^d	5.23 ± 0.26 ^{ab}	6.50 ± 0.14 ^a	6.63 ± 0.11 ^{ab}	6.60 ± 0.15 ^b	—	—	—
	COAT-MAP1	4.29 ± 0.20 ^a	4.25 ± 0.36 ^a	4.44 ± 0.17 ^a	4.63 ± 0.07 ^b	4.34 ± 0.37 ^{ab}	5.20 ± 1.64 ^{ab}	6.30 ± 0.18 ^a	6.30 ± 0.18 ^a	6.88 ± 0.12 ^a	7.10 ± 0.14 ^a	—	—
	COAT-MAP2	4.29 ± 0.20 ^a	4.31 ± 0.43 ^a	4.58 ± 0.24 ^a	4.41 ± 0.13 ^{ab}	5.15 ± 0.15 ^{cd}	5.06 ± 1.22 ^{ab}	6.42 ± 0.60 ^a	6.93 ± 0.11 ^b	6.85 ± 0.06 ^a	—	—	—
	COAT-MAP3	4.29 ± 0.20 ^a	4.31 ± 0.43 ^a	4.65 ± 0.12 ^a	4.33 ± 0.35 ^{ab}	4.74 ± 0.30 ^{bc}	6.14 ± 0.25 ^b	5.98 ± 1.73 ^a	6.55 ± 0.33 ^{ab}	6.89 ± 0.14 ^a	7.26 ± 0.20 ^a	—	—
	Lactic acid bacteria	CTRL-air	3.78 ± 0.02 ^a	3.47 ± 0.05 ^a	3.61 ± 0.72 ^a	3.49 ± 0.34 ^a	3.99 ± 0.12 ^{bc}	4.72 ± 0.26 ^{bc}	5.03 ± 0.19 ^{ab}	—	—	—	—
CTRL-MAP1		3.78 ± 0.02 ^a	3.41 ± 0.13 ^a	3.52 ± 0.15 ^a	3.37 ± 0.10 ^a	3.36 ± 0.03 ^{ab}	3.76 ± 0.06 ^a	5.22 ± 0.23 ^b	—	—	—	—	—
CTRL-MAP2		3.78 ± 0.02 ^a	3.52 ± 0.26 ^a	3.23 ± 0.12 ^a	3.44 ± 0.29 ^a	3.22 ± 0.25 ^a	3.52 ± 0.02 ^a	4.82 ± 0.13 ^{ab}	—	—	—	—	—
CTRL-MAP3		3.78 ± 0.02 ^a	3.75 ± 1.06 ^a	3.31 ± 0.01 ^a	3.26 ± 0.00 ^a	3.39 ± 0.55 ^{ab}	4.37 ± 0.19 ^{abc}	4.68 ± 0.60 ^a	—	—	—	—	—
COAT-air		3.78 ± 0.02 ^a	3.51 ± 0.24 ^a	3.35 ± 0.44 ^a	3.64 ± 0.37 ^a	4.45 ± 0.39 ^c	4.79 ± 0.41 ^{bc}	6.01 ± 0.14 ^c	6.23 ± 0.04 ^a	6.35 ± 0.01 ^a	—	—	—
COAT-MAP1		3.78 ± 0.02 ^a	3.22 ± 0.37 ^a	3.40 ± 0.28 ^a	3.48 ± 0.20 ^a	3.50 ± 0.28 ^{ab}	4.98 ± 1.14 ^c	6.25 ± 0.36 ^c	6.40 ± 0.46 ^{ab}	7.11 ± 0.30 ^b	7.13 ± 0.18 ^a	—	—
COAT-MAP2		3.78 ± 0.02 ^a	3.30 ± 0.06 ^a	3.15 ± 0.21 ^a	3.43 ± 0.07 ^a	4.21 ± 0.23 ^c	4.04 ± 0.19 ^{ab}	6.04 ± 0.06 ^c	6.82 ± 0.15 ^b	7.10 ± 0.02 ^b	—	—	—
COAT-MAP3		3.78 ± 0.02 ^a	3.28 ± 0.03 ^a	3.21 ± 0.13 ^a	3.37 ± 0.10 ^a	4.52 ± 0.54 ^c	5.13 ± 0.46 ^c	6.05 ± 0.21 ^c	6.20 ± 0.17 ^a	6.87 ± 0.08 ^b	7.35 ± 0.21 ^a	—	—
Lactococci		CTRL-air	5.32 ± 0.03 ^a	7.23 ± 0.18 ^b	7.10 ± 0.20 ^a	7.10 ± 0.20 ^{ab}	7.15 ± 0.10 ^a	6.57 ± 0.41 ^a	6.63 ± 0.32 ^a	—	—	—	—
	CTRL-MAP1	5.32 ± 0.03 ^a	7.01 ± 0.04 ^{ab}	7.34 ± 0.26 ^a	7.29 ± 0.24 ^{ab}	7.11 ± 0.05 ^a	6.76 ± 0.14 ^a	6.81 ± 0.07 ^{ab}	—	—	—	—	—
	CTRL-MAP2	5.32 ± 0.03 ^a	7.01 ± 0.32 ^{ab}	7.26 ± 0.05 ^a	7.06 ± 0.06 ^a	7.25 ± 0.30 ^a	6.67 ± 0.16 ^a	6.97 ± 0.12 ^b	—	—	—	—	—
	CTRL-MAP3	5.32 ± 0.03 ^a	6.83 ± 0.21 ^a	7.12 ± 0.22 ^a	7.11 ± 0.19 ^{ab}	7.02 ± 0.45 ^a	6.62 ± 0.14 ^a	7.05 ± 0.21 ^b	—	—	—	—	—
	COAT-air	5.32 ± 0.03 ^a	7.20 ± 0.18 ^b	7.33 ± 0.46 ^a	7.01 ± 0.01 ^a	7.15 ± 0.10 ^a	6.86 ± 0.15 ^a	6.87 ± 0.05 ^{ab}	7.35 ± 0.16 ^a	7.07 ± 0.16 ^a	—	—	—
	COAT-MAP1	5.32 ± 0.03 ^a	7.01 ± 0.21 ^{ab}	7.00 ± 0.20 ^a	7.19 ± 0.16 ^{ab}	6.84 ± 0.23 ^a	6.84 ± 0.26 ^a	6.78 ± 0.11 ^{ab}	6.98 ± 0.28 ^a	7.04 ± 0.19 ^a	7.20 ± 0.44 ^a	—	—
	COAT-MAP2	5.32 ± 0.03 ^a	6.98 ± 0.01 ^{ab}	6.90 ± 0.29 ^a	7.02 ± 0.16 ^a	7.02 ± 0.00 ^a	6.76 ± 0.34 ^a	6.99 ± 0.12 ^b	6.95 ± 0.08 ^a	7.05 ± 0.21 ^a	—	—	—
	COAT-MAP3	5.32 ± 0.03 ^a	6.88 ± 0.11 ^{ab}	6.89 ± 0.45 ^a	7.39 ± 0.06 ^b	6.96 ± 0.08 ^a	6.62 ± 0.40 ^a	6.99 ± 0.12 ^b	7.04 ± 0.11 ^a	7.02 ± 0.23 ^a	7.46 ± 0.64 ^a	—	—

^{a-d}Data within a column with different superscript letters are significantly different ($P < 0.05$). The statistical analyses were performed comparing means within each microbial group.

¹CTRL-air = control in ordinary atmosphere; CTRL-MAP = control in modified atmosphere packaging; COAT-air = coating in ordinary atmosphere; COAT-MAP = coating in modified atmosphere packaging; MAP1 = 75% CO₂ and 25% N₂; MAP2 = 25% CO₂ and 75% N₂; MAP3 = 50% CO₂ and 50% N₂.

Table 2. Shelf life of low-moisture Mozzarella cheese stored at 4°C¹

Sample ²	MAL ^{<i>Pseudomonas</i>} (d)	VMT (d)	SAL ^{OQ} (d)	Shelf life (d)
CTRL-air	>249	>249	52.52 ± 3.79 ^a	52.52 ± 3.79 ^a
CTRL-MAP1	>249	>249	57.78 ± 6.92 ^a	57.78 ± 6.92 ^a
CTRL-MAP2	>249	>249	58.40 ± 6.06 ^a	58.40 ± 6.06 ^a
CTRL-MAP3	>249	>249	59.62 ± 5.64 ^a	59.62 ± 5.64 ^a
COAT-air	>249	>249	107.85 ± 9.83 ^b	107.85 ± 9.83 ^b
COAT-MAP1	>249	>249	155.45 ± 19.06 ^c	155.45 ± 19.06 ^c
COAT-MAP2	>249	>249	102.05 ± 9.37 ^b	102.05 ± 9.37 ^b
COAT-MAP3	>249	>249	166.36 ± 38.01 ^c	166.36 ± 38.01 ^c

^{a-c}Data within a column with different superscript letters are significantly different ($P < 0.05$).

¹MAL = microbiological acceptability limit; VMT = visual molds time; SAL^{OQ} = sensorial acceptability limit in terms of overall quality.

²CTRL-air = control in ordinary atmosphere; CTRL-MAP = control in modified atmosphere packaging; COAT-air = coating in ordinary atmosphere; COAT-MAP = coating in modified atmosphere packaging. MAP1 = 75% CO₂ and 25% N₂; MAP2 = 25% CO₂ and 75% N₂; MAP3 = 50% CO₂ and 50% N₂.

until the end of storage and a cell load of about 7.0 log cfu/g for all samples was assessed.

Enterobacteriaceae and *Pseudomonas* spp. were not detected in any samples during the entire storage period. Therefore, for all samples, the MAL^{*Pseudomonas*} value reported in Table 2 was higher than the monitored period. The relatively high microbiological quality of LM Mozzarella cheese used in this study combined with storage at low temperature made it impossible to show the effect of different kinds of packaging on its microbiological quality during storage.

Sensory Analysis. The evolution of overall quality of LM Mozzarella cheese is shown in Figure 1a. The overall quality threshold value is highlighted in figure by the solid horizontal line. All the control samples reached the overall quality threshold value first compared with the coated samples. In particular, all the sensorial attributes such as color, odor, and texture compromised the overall quality of the control samples both in air and MAP (Figure 1b, c, and d), whereas the quality loss for the coated samples was mainly due to the texture. This was probably due to the weight loss that compromises the appearance of the product and, consequently, its acceptability. In fact, firmness was the parameter that affected the acceptability of control samples, whereas the other sensorial attributes such as color, odor, and taste did not influence the overall quality. Coated samples packaged in air and under MAP with low CO₂ concentration (MAP2) reached the threshold value before the coated samples under MAP1 and MAP3. In particular, an SAL^{OQ} value of about 57 d for all control samples was obtained and no significant difference was observed among them (Table 2). An SAL^{OQ} value of about 100 d was recorded for COAT-air and COAT-MAP2, whereas COAT-MAP1 and COAT-MAP3 samples showed a value of about 160 d. Most probably, edible coatings improved the aesthetic appearance and barrier properties against

oxygen and physical stress. Moreover, the use of active compounds loaded with coating combined with MAP reduced physiological changes, oxidation reactions, and microbial growth.

Shelf Life. The LM Mozzarella cheese shelf life is reported in Table 2. On the basis of the recorded results, shelf life of product seemed to be strongly dependent on sensory quality. In fact, no microbiological threshold was reached and no visual molds appeared on the surface to determine product unacceptability. In particular, a shelf-life value of about 57 d for all control samples was reached. Furthermore, a significant shelf-life prolongation was recorded for samples packaged according to the proposed techniques. Specifically, the combination of active coating and MAP was able to improve the shelf life of LM Mozzarella cheese, reaching a value of about 100 d for COAT-air and COAT-MAP2 and about 160 d for COAT-MAP1 and COAT-MAP3 samples.

LM Mozzarella Cheese Stored at 8°C

The evolution of TVC, LAB, and lactococci in LM Mozzarella cheese samples did not show differences between samples during the entire storage time (data not shown). In particular, the initial counts for TVC and lactococci were about 7.0 log cfu/g and increased until reaching a final value of about 8.0 log cfu/g. For LAB, the microbial counts ranged from a value of 4.0 to 7.0 log cfu/g at the end of storage.

The evolution of *Pseudomonas* spp. cell load for LM Mozzarella cheese stored at 8°C is shown in Figure 2. The microbiological analyses for control samples were stopped at 8 d because of the appearance of the visual molds on the product surface. The COAT-air samples showed a cell load that increased with time and exceeded the threshold value at about 13 d of storage. The combination of active coating and MAP was able

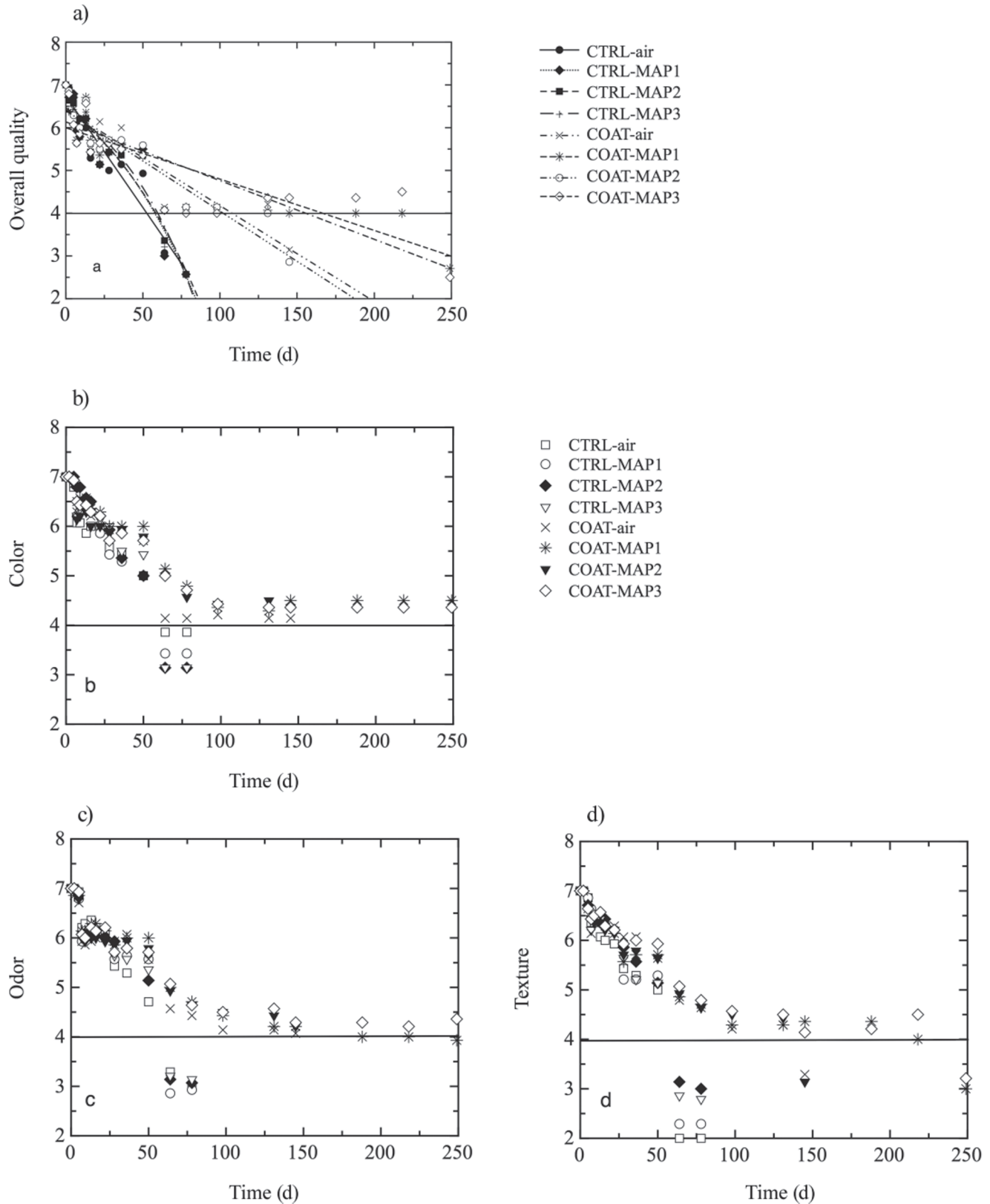


Figure 1. Evolution of overall quality (a), color (b), odor (c), and texture (d) of low-moisture Mozzarella cheese stored at 4°C. CTRL = control; MAP1 = modified atmosphere packaging (MAP) 1 (75% CO₂ and 25% N₂); MAP2 = 25% CO₂ and 75% N₂; MAP3 = 50% CO₂ and 50% N₂; COAT = coating treatment; air = ordinary atmosphere.

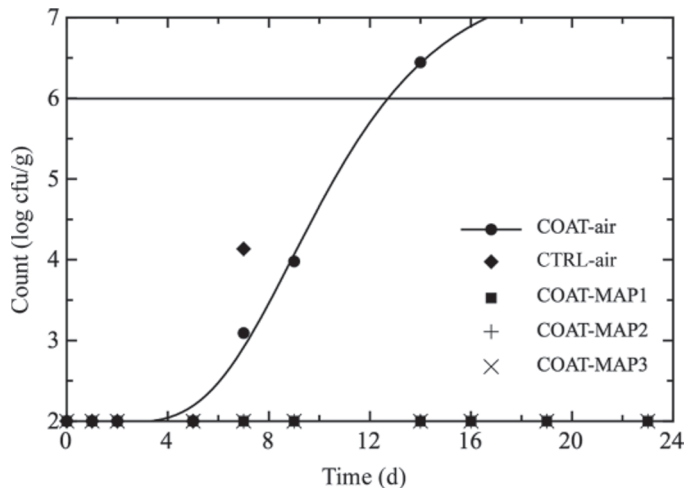


Figure 2. Evolution of *Pseudomonas* spp. cell load for low-moisture Mozzarella cheese stored at 8°C. COAT = coating treatment; air = ordinary atmosphere; CTRL = control; MAP1 = modified atmosphere packaging (MAP) 1 (75% CO₂ and 25% N₂); MAP2 = 25% CO₂ and 75% N₂; MAP3 = 50% CO₂ and 50% N₂.

to inhibit the growth of *Pseudomonas* spp. for the entire storage time. Gram-negative bacteria are generally more sensitive to CO₂ than the gram-positive ones (Rosenthal et al., 1991). The results confirm data from the literature on the effect of active coating in combination of MAP on the spoilage microorganisms of dairy product (Conte et al., 2009; Del Nobile et al., 2009). *Enterobacteriaceae* were not detected in any samples during the entire storage period.

From the overall quality point of view, CTRL-air and COAT-air samples did not reach the SAL for the period in which they were monitored (Figure 3). As mentioned before, the analyses for these samples were stopped because the microbiological threshold limit was reached. The adopted packaging strategy allowed prolonging the overall quality of the LM Mozzarella cheese. In particular, the samples packaged under high CO₂ concentration (COAT-MAP1) reached the overall quality threshold value first, followed by the samples under low CO₂ levels (COAT-MAP2 and COAT-MAP3).

The MAL^{*Pseudomonas*}, SAL^{OQ}, and shelf-life values for LM Mozzarella cheese stored at 8°C are reported in Table 3. The data highlight that the limiting factor for the shelf life of CTRL-air was the appearance of visual molds on the surface. For these samples, a shelf-life value of about 8.0 d was obtained. The microbiological quality was the factor that limited the shelf life of samples subjected at coating treatment under air, with a value of about 13 d. Active coating treatment in combination with MAP was able to improve the product shelf life until obtaining a value of about 37 d for COAT-MAP1 and 40 d for COAT-MAP2 and COAT-

MAP3 samples. In this case, the sensory quality was the parameter that compromised the product shelf life, whereas the *Pseudomonas* spp. growth and the molds did not represent a limiting factor. Therefore, the use of MAP highlighted the higher inhibitory effect on the growth of molds, as stated by Michniewicz (1998). Data confirm the effect of potassium sorbate incorporated into edible coatings as an antimicrobial agent against microbial spoilage and molds such as *Penicillium* spp. and *Aspergillus* spp. (Mehyar et al., 2011; Sayanjali et al., 2011).

LM Mozzarella Cheese Stored at 14°C

The evolution of TVC, LAB, and lactococci in LM Mozzarella cheese samples did not show differences among samples during the entire storage time (data not shown). In particular, the initial cell load for TVC and LAB was about 5.0 log cfu/g and increased until reaching a final value of about 8.0 log cfu/g. For lactococci, the microbial counts ranged from a value of 7.0 to 8.0 log cfu/g at the end of storage.

Figure 4 shows the evolution of *Pseudomonas* spp. cell load for the LM Mozzarella cheese stored at 14°C. Temperature was the most important variable for the microbiological quality of the product. This is highlighted by the faster increase in spoilage microorganisms cell load compared with that of the samples stored at lower temperature. Specifically, CTRL-air samples reached the microbiological threshold value first, followed by the COAT-air samples. Coated samples under modified atmosphere conditions did not exceed the

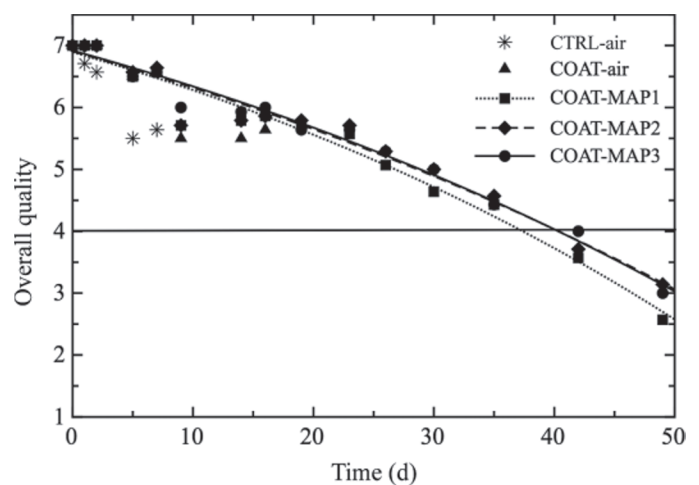


Figure 3. Evolution of overall quality of low-moisture Mozzarella cheese stored at 8°C. CTRL = control; air = ordinary atmosphere; COAT = coating treatment; MAP1 = modified atmosphere packaging (MAP) 1 (75% CO₂ and 25% N₂); MAP2 = 25% CO₂ and 75% N₂; MAP3 = 50% CO₂ and 50% N₂.

Table 3. Shelf life of low-moisture Mozzarella cheese stored at 8°C¹

Sample ²	MAL ^{<i>Pseudomonas</i>} (d)	VMT (d)	SAL ^{OQ} (d)	Shelf life (d)
CTRL-air	>7	8.00 ± 0.00	>7	8.00 ± 0.00 ^a
COAT-air	12.72 ± 0.40	>14	>14	12.72 ± 0.40 ^b
COAT-MAP1	>49	>49	37.38 ± 1.48 ^a	37.38 ± 1.48 ^c
COAT-MAP2	>49	>49	40.35 ± 1.71 ^a	40.35 ± 1.71 ^d
COAT-MAP3	>49	>49	40.33 ± 1.18 ^a	40.33 ± 1.18 ^d

^{a-d}Data within a column with different superscript letters are significantly different ($P < 0.05$).

¹MAL = microbiological acceptability limit; VMT = visual molds time; SAL^{OQ} = sensorial acceptability limit in terms of overall quality.

²CTRL-air = control in ordinary atmosphere; COAT-air = coating in ordinary atmosphere; COAT-MAP = coating in modified atmosphere packaging. MAP1 = 75% CO₂ and 25% N₂; MAP2 = 25% CO₂ and 75% N₂; MAP3 = 50% CO₂ and 50% N₂.

limit value during the entire storage period. In particular, the cell load increased during the first 4 d and then a decrease was observed. The MAL for all samples are reported in Table 4. The MAL^{*Pseudomonas*} value was about 1.0 and 2.0 d for control and active coating samples, respectively. The adopted strategy in this study was able to improve the microbiological quality of product stored under thermal abuse. The MAL^{*Pseudomonas*} value for coated samples packaged in MAP was higher than the monitored period. Moreover, the inhibitory effect on the growth of molds was also observed. *Enterobacteriaceae* were not detected in any samples during the entire storage period.

From a sensory point of view, the combination of active coating and MAP was able to improve the quality of product (Figure 5). In fact, CTRL-air and COAT-air rapidly reached the threshold value with respect to the COAT-MAP samples. In particular, an SAL^{OQ} value of about 2.32 and 4.00 d was observed for CTRL-air and COAT-air, respectively (Table 4). On the other hand, coated samples packaged under MAP showed an SAL^{OQ} value of about 11 d. Moreover, no significant differences between different MAP was observed.

The shelf life for LM Mozzarella cheese stored at 14°C is reported in Table 4. The microbiological quality was the factor limiting the shelf life of control and ac-

tive coating samples packaged in air. For these samples, a shelf-life value of about 1.0 and 2.0 d, respectively, was observed, whereas a shelf life of 11 d for coated samples was reached. With regard to the VMT parameter, no visual molds appeared during the storage time for all samples. The data confirmed the antimicrobial and antifungal effect of potassium sorbate and MAP on improving the microbiological quality and safety of the product under thermal abuse conditions.

CONCLUSIONS

The active coating combined with packaging in modified atmosphere were used to prolong the shelf life of LM Mozzarella cheese. Microbial and sensorial properties were monitored to determine the quality loss during storage at 4, 8, and 14°C. Results highlighted that the approach used in this study was effectively able to prolong the shelf life of the product by inhibiting the spoilage microorganisms and molds. The sensory quality was also improved due the ability of coating to prevent excessive dehydration from the product surface. Moreover, it should be emphasized the importance of this strategy under thermal abuse conditions, as it is able to enhance the safety of the product. Therefore, the combination of active coating and MAP represents

Table 4. Shelf life of low-moisture Mozzarella cheese stored at 14°C¹

Sample ²	MAL ^{<i>Pseudomonas</i>} (d)	VMT (d)	SAL ^{OQ} (d)	Shelf life (d)
CTRL-air	1.16 ± 0.04 ^a	>8	2.32 ± 0.01 ^a	1.16 ± 0.04 ^a
COAT-air	2.16 ± 0.07 ^b	>8	4.02 ± 0.86 ^b	2.16 ± 0.07 ^a
COAT-MAP1	>13	>13	10.93 ± 0.50 ^c	10.93 ± 0.50 ^b
COAT-MAP2	>13	>13	11.04 ± 1.36 ^c	11.04 ± 1.36 ^b
COAT-MAP3	>13	>13	11.53 ± 0.70 ^c	11.53 ± 0.70 ^b

^{a-c}Data within a column with different superscript letters are significantly different ($P < 0.05$).

²MAL = microbiological acceptability limit; VMT = visual molds time; SAL^{OQ} = sensorial acceptability limit in terms of overall quality.

²CTRL-air = control in ordinary atmosphere; COAT-air = coating in ordinary atmosphere; COAT-MAP = coating in modified atmosphere packaging. MAP1 = 75% CO₂ and 25% N₂; MAP2 = 25% CO₂ and 75% N₂; MAP3 = 50% CO₂ and 50% N₂.

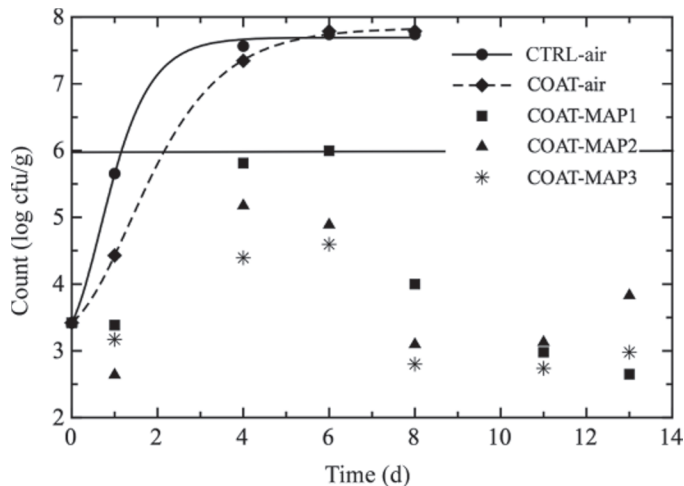


Figure 4. Evolution of *Pseudomonas* spp. cell load for low-moisture Mozzarella cheese stored at 14°C. CTRL = control; air = ordinary atmosphere; COAT = coating treatment; MAP1 = modified atmosphere packaging (MAP) 1 (75% CO₂ and 25% N₂); MAP2 = 25% CO₂ and 75% N₂; MAP3 = 50% CO₂ and 50% N₂.

a strategic solution to prolong the shelf life of LM Mozzarella cheese. In addition, edible coatings guarantee a considerably lower packaging weight, enhance product appearance, and ensure its safety. Moreover, the simplicity and relative economy could be very beneficial and of commercial importance to the dairy industry.

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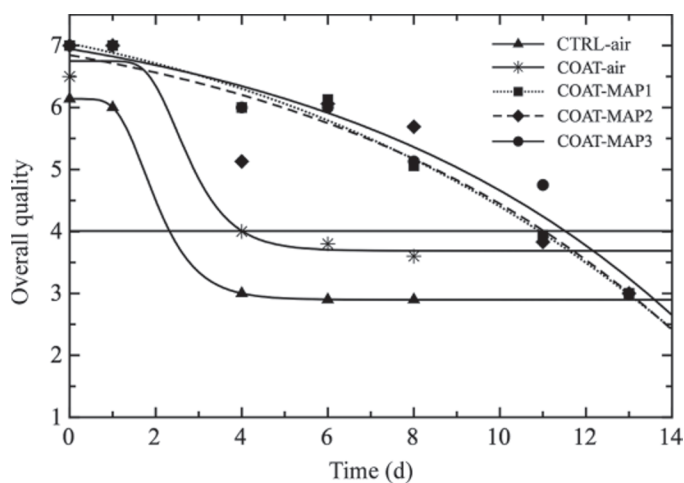


Figure 5. Evolution of overall quality of low-moisture Mozzarella cheese stored at 14°C. CTRL = control; air = ordinary atmosphere; COAT = coating treatment; MAP1 = modified atmosphere packaging (MAP) 1 (75% CO₂ and 25% N₂); MAP2 = 25% CO₂ and 75% N₂; MAP3 = 50% CO₂ and 50% N₂.

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