Innovative Active Packaging Systems to Prolong the Shelf Life of Mozzarella Cheese

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ABSTRACT

In this work the effectiveness of different antimicrobial packaging systems on the microbial quality decay kinetics during storage of Mozzarella cheese was evaluated. Lemon extract, at 3 different concentrations, was used as active agent, in combination with brine and with a gel solution made of sodium alginate. Shelf life tests were run at 15°C to simulate thermal abuse. The cell load of spoilage and dairy functional microorganisms were monitored at regular time intervals during storage. By fitting the experimental data through a modified version of the Gompertz equation, the shelf life of dairy products packaged in the different systems was calculated. Results show an increase in the shelf life of all active packaged Mozzarella cheeses, confirming that the investigated substance may exert an inhibitory effect on the microorganisms responsible for spoilage phenomena without affecting the functional microbiota of the product.

Key words: active packaging, Mozzarella cheese, microbial quality, shelf life

INTRODUCTION

Mozzarella cheese is a member of the pasta filata (stretched curd) cheese family that originated in Italy. A unique cooking and stretching treatment of acid-ripened curd at optimal pH in hot water, which imparts a fibrous structure with superb melting and stretching properties, distinguishes this cheese. The shelf life of the product under brine is very short, approximately from 5 to 7 d (Altieri et al., 2005) due to the chemical, biochemical, and physical changes determined by food-borne spoilage microorganisms and enzymes. It is well known that as a consequence of each change some alterations in the product can occur at the sensorial and nutritional level, depreciating its commercial value

(Bishop and White, 1986; Byrne and Bishop, 1998). The main microbial groups responsible for detrimental phenomena in Mozzarella are *Pseudomonas* spp., coliforms, and various psychrophilic bacteria (Boor, 1997; Altieri et al., 2005).

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 the traditional product beyond the market borders. The main approaches to maintain the food product quality for more time are based on the raw materials quality improvement, on making process innovations, and on the use of suitable storage conditions (de Ruig and van den Berg, 1985; Farkye et al., 1991; Kindstedt, 1993; Brody, 2001).

Dairy researchers have been able to better understand how variations in ingredient selection affect the composition and thus the functional properties of Mozzarella cheese. The influence of ratio of rods to cocci, for example, has been one of the subjects of much interest in the cheese industry because of its effect on proteolytic activity (Yun et al., 1995). The addition of nonfat dry milk is another attractive option for Mozzarella cheese makers to increase the level of nonfat solids of milk (Yun et al., 1998).

Among the process innovations (high-pressure processing, pulsed electric field technology, and irradiation) aimed to inactivate the spoilage microorganisms and enhance the nutritional, sensorial, and microbial characteristics of the product, a good opportunity comes from the use of antimicrobial compounds during milk transformation. The high consumer attention to the food safety aspects justifies increased research interest in using active agents derived from natural sources (animal and vegetable origin), considered suitable for food application, able to reduce the initial microbial count, and able to control the cell growth during the different steps of the product chain (Paster et al., 1990; Stevens et al., 1992; Olasupo et al., 2003). Altieri et al. (2005) successfully tested the effect of chitosan during the process of making Mozzarella cheese. The natural compound was able to control the microbial growth of

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the main spoilage microorganisms, without affecting the functional dairy microbiota. Moreover, no differences in sensorial characteristics were recorded in Mozzarella cheese produced in the presence of the selected active agent.

Efforts to prolong the shelf life of Mozzarella cheese are also in progress by means of the optimization of storage conditions. Industrially produced cheeses are usually frozen and stored to preserve their quality during marketing. Although freezing is effective in extending shelf life and preserving the color, flavor, and nutritive value of many cheeses, it modifies physical properties (Diefes et al., 1993; Kuo and Gunasekaran, 2003). Shredded Cheddar and Mozzarella cheeses were powder coated electrostatically and nonelectrostatically with a mixture of natamycin and powdered cellulose to improve the product shelf life (Elayedath and Barringer, 2002). The authors were able to assess that the drum method of coating is more effective than the belt conveyor, even when electrostatics are used with the belt. Electrostatic coating is also an environmentally friendly process, which reduces exposure of workers to inhalation of dust and risk of dust explosions.

To the best of our knowledge, there is a lack of work on the active packaging systems applied to dairy fresh product, even though the use of a packaging solution exerting antimicrobial activity could present several advantages from safety and commercial points of view. For this reason our attention was focused on the study of the effects of 2 different active packaging systems based on the use of lemon extract as an antimicrobial agent to preserve the quality of Mozzarella cheese. Lemon extract is a natural compound from citrus fruits, well known for their high content in flavonoid compounds as hesperidine, narirutin, naringin, and eriocitrin; terpenes as citral and *p*-cymene; and vitamin C (Mouly et al., 1994; Belletti et al., 2004). In the literature there are interesting applications of lemon extracts to improve the acceptability of products such as meat and vegetables (Ponce et al., 2004; Fernandez-Lopez et al., 2005). Because of the potential use of lemon extract in food preservation, our aim was to study its effect in conjunction with technologies of antimicrobial packaging systems.

MATERIALS AND METHODS

Sample Preparation

Mozzarella cheese samples of about 50 g were kindly provided by Posta la Via (Foggia, Italy). After salting Mozzarella cheese in 12% (wt/wt) aqueous NaCl solution for 4 to 5 h, we proceeded to immerse cheese forms in shipping brine consisting of 2% NaCl solution. Two other active packaging systems were used to store Moz-

zarella cheese, referred in the text as active saline solution and active gel. They were obtained by dissolving the active compound, lemon extract 100% (Spencer Food, Industrial bv, Amsterdam, the Netherlands), in brine at 3 different concentrations: 500, 1,000, and 1,500 ppm. To obtain the active gels, an amount of agar technical (Oxoid, Milan, Italy) was added to the same saline solution before adding lemon extract. The obtained packaged samples were stored at 15°C to simulate thermal abuse during storage. Microbiological analyses were performed every day to determine the quality of the product.

Microbiological Analyses

Ten grams of Mozzarella cheese, packaged in the different systems, was diluted with 90 mL of a sterile saline solution (0.9%) in a stomacher bag and mixed for 1 min with a stomacher (mod. 4153-50, International PBI, Milan, Italy). Decimal dilutions of cheese homogenates were performed, and microbiological counts of mesophilic lactic acid bacilli, lactococci, total bacterial count, total coliforms, and *Pseudomonas* spp. were determined. The following media and the incubation conditions were used: spread plating onto plate count agar (Biolife, Milan, Italy) plates incubated at 30°C for 48 h for total mesophilic bacteria; pour plating in violet red bile agar (Oxoid), with a covering layer of the same medium, incubated at 37°C for 24 h for total coliforms; spread plating onto *Pseudomonas* agar base with selective supplement (Oxoid) plates incubated at 25°C for 48 h for *Pseudomonas* spp.; pour plating in DeMan, Rogosa, and Sharpe agar (MRS, Oxoid) incubated in anaerobic conditions for 48 h at 37°C for mesophilic lactic acid bacilli; and pour plating in M17 agar (Oxoid) incubated at 37°C for 48 h for lactococci.

Chemical Analyses: pH Evaluation

The pH was evaluated in Mozzarella cheese pieces and in the brine-water mix. A Crison pH meter (mod. MicroPH2001, International PBI) was used. Data are the average of 2 replicates.

Shelf Life Calculation

To calculate the shelf life of the packed Mozzarella cheese, the approach proposed by Altieri et al. (2005) was adopted. It is briefly summarized here.

The method consists of using the following equation, obtained by rearranging the Gompertz equation in such a way that the shelf life appears directly as a parameter of the equation relating $\log(\text{cfu/g})$ to the storage time:

$$\log\left(\frac{\text{cfu}}{g}\right) = \left[\log\left(\frac{\text{cfu}}{g}\right)\right]_{\max} - A \cdot \exp\left\{-\exp\left[\left(\mu_{\max} \cdot 2.71\right) \cdot \frac{\lambda - \text{S.L.}}{A} + 1\right]\right\} + A \cdot \exp\left\{-\exp\left[\left(\mu_{\max} \cdot 2.71\right) \cdot \frac{\lambda - t}{A} + 1\right]\right\},$$

where $\left[\log\left(\frac{\text{cfu}}{g}\right)\right]_{\max}$ is the decimal logarithm of the microbial acceptability limit, A is the maximum bacteria growth attained at the stationary phase, $\mu_{\max}$ is the maximal specific growth rate, λ is the lag time (day), and t is the time (day).

By fitting this equation to the experimental data, it was possible to estimate the shelf life as an equation parameter along with its confidence interval.

Statistical Analyses

All analyses were carried out in duplicate. The means and standard deviations were calculated. The confidence intervals of the model's parameters were evaluated as follows: first, a fit was run with the original data; then, using the standard deviation of the data points, 100 additional fits were run on artificial data sets, which were generated by randomly varying the data around the fitted function. From these additional fits, a distribution of values for each parameter was obtained. The sets of data obtained for each parameter were statistically treated to obtain the 95% confidence interval.

RESULTS AND DISCUSSION

To prolong the shelf life of Mozzarella cheese, 2 different active packaging systems were developed, tested, and compared with the standard system consisting of brine solution. Three different concentrations of lemon extract were added to salt solutions with and without adding sodium alginate to obtain, respectively, an active solution and an active gel. Even if the current legal prescriptions impose 4°C as the maximum temperature value allowed for shipping and storing Mozzarella cheese in supermarkets, in this work the product was stored at high temperature to simulate thermal abuse conditions in order to accelerate the detrimental phenomena responsible for unacceptability from the consumer.

Figures 1 and 2 show the evolution during storage of lactic acid bacilli and lactococci viable counts. Because of the similar results obtained at all tested concentrations of active compound, only the experimental data obtained at the lower lemon extract concentration

Figure 1. Time course during storage of lactic acid bacilli concentration in Mozzarella stored in different packaging systems. The curves are the best fit of the reparameterized Gompertz equation to experimental data. (◇) Control sample; (■) sample in gel with 500 ppm of lemon extract; (□) sample in saline solution with 500 ppm of lemon extract.

were reported. In each figure, 3 sets of data are shown, each for the type of packaging investigated. The functional microorganisms grew during storage and as can be inferred from the figures, there are no marked differences between the control sample and those stored using the active developed systems, for lactic acid bacilli and lactococci. These results suggest that the functional dairy microbiota are not affected by the action of lemon extract during the entire period of observation.

A different behavior was observed for coliforms and *Pseudomonas* spp. The control samples showed higher

Figure 2. Time course during storage of lactococci concentration in Mozzarella stored in different packaging systems. The curves are the best fit of reparameterized Gompertz equation to experimental data. (◇) Control sample; (■) sample in gel with 500 ppm of lemon extract; (□) sample in saline solution with 500 ppm of lemon extract.

Figure 3. Time course during storage of *Pseudomonas* spp. in Mozzarella stored in different packaging systems. The curves are the best fit of reparameterized Gompertz equation to experimental data. (◇) Control sample; (■) sample in gel with 1,500 ppm of lemon extract; (□) sample in saline solution with 1,500 ppm of lemon extract.

values of spoilage microbial count than Mozzarella cheese stored in presence of the antimicrobial compound. In particular, the active solution system was more effective in slowing down the microbial growth than the active gel system. The growth of both spoilage microbial groups was slowed by the presence of lemon extract already at the first stage of storage. The results on microbial growth of Mozzarella cheese packaged in the 3 different systems, without lemon extract and with the active agent at higher concentration, were reported in Figures 3 and 4 for *Pseudomonas* spp. and coliforms, respectively. The observed differences between samples with and without active compound suggest that for both

Figure 4. Time course during storage of coliforms in Mozzarella stored in different packaging systems. The curves are the best fit of reparameterized Gompertz equation to experimental data. (○) Control sample; (×) sample in gel with 1,500 ppm of lemon extract; (■) sample in saline solution with 1,500 ppm of lemon extract.

microbial groups lemon extract exerts a great influence on the maximum cell load reached at the stationary phase by both microbial species. Moreover, a prolongation of the lag time was also recorded on coliforms. Because the 2 microbial groups are mainly responsible for quality decay during storage of Mozzarella cheese, cell load data were used to estimate the shelf life of the investigated samples by fitting equation [1] to the experimental data. According to the European Union (1997) the value of $\left[\log\left(\frac{cfu}{g}\right)\right]_{\max}$ was set to 5 for coliforms; for *Pseudomonas* spp. it was set to 6 because at this level of contamination the alterations of the product start to appear (Bishop and White, 1986). The

Table 1. Calculated model parameters referred to *Pseudomonas* spp. in Mozzarella cheese packaged in the 3 different systems at the 3 investigated concentrations of lemon extract

<i>Pseudomonas</i>	A	Shelf life	$\mu_{\max}$	Lag
Control sample	7.77	1.58	4.96	7.01E-16
	7.45–8.08	1.43–1.70	4.60–5.47	4.88E-16–1.06E-15
Active gel, 500 ppm	7.41	2.05	11.10	1.43
	7.13–7.88	2.00–2.11	9.64–13.77	1.32–1.53
Active solution, 500 ppm	7.50	2.45	7.81	1.56
	7.13–8.00	2.24–2.55	6.77–14.09	1.49–1.76
Control sample	5.17	1.24	3.06	0.00
	4.67–5.54	1.07–1.44	2.53–3.70	0.00–0.00
Active gel, 1,000 ppm	5.31	2.21	1.6955	0.00
	4.84–5.88	1.99–2.40	1.51–1.92	0.00–0.00
Active solution, 1,000 ppm	5.16	3.12	1.44	0.73
	4.36–5.92	2.78–3.46	1.14–1.84	0.28–1.22
Control sample	7.25	1.37	3.25	0.00
	6.81–7.78	1.22–1.50	2.97–3.68	0.00–0.00
Active gel, 1,500 ppm	6.11	2.20	2.04	0.00
	5.71–8.55	2.02–2.38	1.86–2.27	0.00–0.00
Active solution, 1,500 ppm	5.70	2.99	1.50	0.00
	5.14–6.08	2.73–3.26	1.34–1.77	0.00–0.00

Table 2. Calculated model parameters referred to coliforms in Mozzarella cheese packaged in the 3 different systems at the 3 investigated concentrations of lemon extract

Coliforms	A	Shelf life	$\mu_{\max}$	Lag
Control sample	7.02	1.77	3.47	0.00
Active gel, 500 ppm	6.77–7.37	1.44–2.07	2.96–4.29	0.00–0.00
	5.49	2.23	8.44	1.42
Active solution, 500 ppm	5.22–5.66	2.12–2.36	7.11–11.11	1.30–1.58
	5.14	2.61	7.39	1.43
Control sample	5.00–5.30	2.33–4.65	6.26–17.45	1.33–1.75
	8.17	1.78	3.29	0.00
Active gel, 1,000 ppm	7.68–8.54	1.67–1.83	3.17–3.49	0.00–0.00
	7.77	2.15	3.29	0.54
Active solution, 1,000 ppm	7.32–8.21	1.99–2.30	2.90–3.71	0.44–0.61
	7.28	2.03	14.01	1.65
Control sample	6.96–7.78	1.98–2.06	12.46–15.38	1.57–1.69
	7.15	1.77	1.78	0.00
Active gel, 1,500 ppm	6.65–7.686	1.65–1.91	1.58–1.96	0.00–0.00
	4.08	1.82	3.74	1.07
Active solution, 1,500 ppm	3.75–4.38	1.62–2.00	2.88–33.56	0.927–1.91
	3.50	2.2	3.08	1.23
	3.24–3.8	2.01–2.48	2.40–30.13	0.97–1.92

curves shown in Figures 3 and 4 result from the best fitting, whereas the model parameter obtained as well as the confidence intervals are reported in Tables 1 and 2 for *Pseudomonas* spp. and coliforms, respectively. As shown in the above tables, the 2 studied active packaging systems slowed the growth of the spoilage microbiota during Mozzarella storage, leading to a slight increase in the shelf life of the investigated cheese, which is approximately 1 d. Because the confidence intervals of the shelf life values do not superimpose on each other, it is possible to consider statistically significant the differences recorded between each lemon-free packaging solution and the 2 corresponding active packaging systems.

It is worth noting that, in the case of coliforms, the increase of lemon extract concentration does not significantly influence the shelf life of the packed product; whereas in the case of *Pseudomonas*, the concentration of the antimicrobial compound seems to exert a certain influence on the shelf life of the investigated product. In fact, in this last case the longer shelf life was obtained when the level of lemon extract was higher. Among the investigated packaging systems the best performances were obtained by using the active solution for both microorganisms. It is reasonable to suppose that the mobility of the active compound in the water saline solution is higher than in the gel, leading to a higher availability of the antimicrobial agent into the brine solution than in the active gel.

For all samples, no differences were detected in the evolution of pH among the investigated samples. During the entire period of observation the pH ranged around 5 into the Mozzarella cheese, packaged according to the 3 systems. The values of pH into the brine of all packaged Mozzarella samples were de-

creased during time and ranged from 7 to 5, independently from the lemon extract concentrations. This evidence confirmed that pH never became a limiting factor for the studied bacterial populations, suggesting that the above difference in the microbial growth among the samples cannot be ascribed to differences in the pH.

To evaluate the influence of lemon extract on the sensorial properties of the cheese, 5 trained people performed a simple and unstructured sensory evaluation every time the samples were opened for laboratory analyses. According to this test, no differences between lemon and lemon-free packaged samples were recognized, corroborating the hypothesis that the tested concentrations of the active compound do not influence the sensorial characteristics of Mozzarella cheese.

CONCLUSIONS

The effectiveness of antimicrobial packaging systems on the microbial quality decay kinetics of Mozzarella cheese was evaluated. Lemon extract at 3 different concentrations was used as the active agent. Shelf-life tests were run at 15°C to monitor the cell load of spoilage and dairy functional microorganisms during storage. Under the tested conditions the results show an increase in the shelf life of all active packaged Mozzarella cheeses, confirming that the investigated substance may exert an inhibitory effect on the growth of spoilage microorganisms such as coliforms and *Pseudomonas* spp. without affecting the functional dairy microbiota and the sensorial characteristics of the product. The developed active packaging proposed in this work could be advantageously used to prolong the shelf life of Mozzarella, allowing for its distribution beyond market borders.

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