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Use of Chitosan to Prolong Mozzarella Cheese Shelf Life

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ABSTRACT

This study was undertaken to evaluate the feasibility of using chitosan, a natural antimicrobial substance, to improve the preservation of a very perishable cheese. The effectiveness of chitosan to inhibit the growth of spoilage microorganisms in Mozzarella cheese was studied during refrigerated storage. A lactic acid/chitosan solution was added directly to the starter used for Mozzarella cheese manufacturing. Mozzarella cheese samples were stored at 4°C for about 10 d and microbial populations as well as the pH were monitored. Results demonstrated that chitosan inhibited the growth of some spoilage microorganisms such as coliforms, whereas it did not influence the growth of other microorganisms, such as *Micrococcaceae,* and lightly stimulated lactic acid bacteria.
(**Key words:** Mozzarella cheese, chitosan, shelf life, natural antimicrobial substance)

INTRODUCTION

Mozzarella cheese is a typical Mediterranean pasta filata product, cut and manufactured in various shapes, and usually brined. It is characterized by a notable economic relevance because of the steady rise in its production and consumption. It is very popular with Italian consumers, both as a fresh cheese and as an ingredient. Numerous studies have characterized this cheese from a microbiological point of view (Ottogalli et al., 1985; Massa et al., 1992; Altieri et al., 1994), recovering several microbial species, such as *Lactobacillus lactis* subsp. *lactis, L. lactis* subsp. *diacetylactis, L. lactis* subsp. *cremoris, Streptococcus thermophilus,* enterococci such as *Enterococcus faecium* and *E. faecalis, Enterobacteriaceae* such as *Escherichia coli,* some yeasts such as *Debaryomyces hansenii* and *Kluyveromyces marxianus,* and various spoilage psychrophilic microflora (Cantoni et al., 2003a,b; Parisi, 2003a,b). Owing to the variety of microorganisms found in this product, Mozzarella has a rather short shelf life.

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Numerous studies have underlined that fairly often, Mozzarella cheese is spoiled by *Pseudomonas* spp. growing on the cheese surface, mostly coming from water used during manufacture (Cabrini and Neviani, 1983; Cantoni et al., 2003a,b). Another factor limiting Mozzarella cheese shelf life is the presence of coliforms (Rondinini and Garzaroli, 1990; Parisi, 2003a,b). The same studies demonstrated that proteolytic and lipolytic reactions also are of high importance in Mozzarella cheese preservation. At present, Mozzarella cheese shelf life is approximately 5 to 7 d, and efforts are in progress to prolong this shelf life by means of process innovation (Brody, 2001) and raw materials quality improvement. The use of chitosan as an antimicrobial agent to prolong the shelf life of packed Mozzarella cheese could be viable because it is environmentally friendly and relatively inexpensive. This is because chitosan is a deacetylated form of chitin, the second most abundant biopolymer on earth after cellulose. It has 3 types of reactive functional groups: an amino group and both primary and secondary hydroxyl groups (Furusaki et al., 1996). This substance has been of interest in the past few decades because of its potential range of industrial applications, but few efforts have been performed in food applications, in spite of literature evidence regarding its effectiveness in inhibiting microbial growth. Wang (1992) observed complete inactivation of *Staphylococcus aureus* after 2 d of incubation at pH 5.5 in presence of 0.5 to 1.0% chitosan; furthermore, Darmadji and Izumimoto (1994) registered an interesting effect of chitosan in meat preservation, in particular against *E. coli.* Simpson et al. (1997) studied the antimicrobial effect of chitosan on raw shrimp; using different concentrations of chitosan, they observed variations in the shrimp's microbial susceptibility to chitosan. Shahidi et al. (1999) demonstrated antimicrobial effects of water-soluble chitosan on different bacterial species, such as *Bacillus cereus, Proteus vulgaris,* and *E. coli.*

Chitosan is very versatile and it may be used in various applicative areas, as an antimicrobial agent, as an edible film, as an additive, or to improve nutritional quality (Shahidi et al., 1999).

The aim of this work was to test the effectiveness of low-molecular-weight chitosan as a natural antimicrobial additive in Mozzarella cheese. In particular, the experiment aimed to prolong Mozzarella cheese shelf

Table 1. Effect of chitosan against lactic acid bacteria in different control points during Mozzarella cheese manufacture.[1]

Control point	Time (days)	Mozzarella (cfu/g)	Mozzarella with chitosan (cfu/g)	*P*-value
Milk	t_0	$5.60 \times 10^5 \pm 5.65 \times 10^4$		—
Milk + whey	t_1	$4.75 \times 10^7 \pm 2.47 \times 10^7$	$4.05 \times 10^7 \pm 6.36 \times 10^6$	0.76
When pH reached 6.45	t_2	$4.75 \times 10^7 \pm 3.54 \times 10^6$	$4.20 \times 10^7 \pm 8.49 \times 10^6$	0.52
Curd break	t_3	$2.75 \times 10^6 \pm 3.54 \times 10^5$	$2.65 \times 10^6 \pm 4.95 \times 10^5$	0.84

[1]Numbers reported (cfu/g) represent means of duplicate counts. Data are presented ± standard deviation.

life, by using chitosan to inhibit the growth of spoilage bacteria.

MATERIALS AND METHODS

Mozzarella Cheese Manufacture

The Mozzarella cheese used in this work was made according to the following procedure: 50 L of milk (pH 6.7) was put into a boiler and a revitalized *Streptococcus salivarius* subsp. *thermophilus* strain was prepared from a freeze-dried culture (Lyoto ST 5.64, Clerici-Sacco, Como, Italy; optimal temperature = 32 to 45°C; no gas) and was used as graft in 1:10 ratio. When milk acidity reached pH 5.5, temperature was raised to 36°C and 20 mL of liquid rennet was added. After 30 min, the curd was cut and a 5-h ripening was carried out. Afterward, the curd was gently stirred to facilitate whey ejection; in this phase, the curd pH was approximately 4.5. Hot water (82°C) was used to mill and mold the cheese pieces that were worked by hand until they were adequately smooth and elastic (it took approximately 10 min). Then 50-g Mozzarella balls were formed, put into cold water, packaged by hand, and kept in a 12% NaCl (wt/wt) brine for the entire storage time. Plastic bags (nylon/polyethylene, 100 μm; Tecnovac, San Paolo D'Argon, Bergamo, Italy) were used; they were 170 × 250 mm, with properties specified by the manufacturer as follows: test temperature = 23°C; ΔRH (%) = 0.885; water vapor transmission rate = 1.45 $g \cdot m^{-2} \cdot d^{-1}$; water vapor pressure = 22.4 mmHg; permeance = 0.0656 $g \cdot m^{-2} \cdot g^{-1} \cdot mmHg^{-1}$; permeability = $7.31 \cdot 10^{-6}$ $g \cdot m \cdot m^{-2} \cdot day^{-1} \cdot mmHg^{-1}$; $6.43 \cdot 10^{-10}$ $g \cdot cm \cdot cm^{-2} \cdot sec^{-1} \cdot atm^{-1}$. The packaged cheese was stored at 4°C for the entire observation time (9 d). Simultaneously, a modified Mozzarella cheese was manufactured, adding a low-molecular-weight chitosan (85% deacetylation) (Aldrich, Milan, Italy). Briefly, a chitosan solution was prepared into the whey and the modified whey was put into the working milk in order to get a final concentration of 0.075% chitosan. The Mozzarella manufacture was carried out as described above.

Two independent manufacturing processes were performed for normal and modified Mozzarella cheese, and they will be known heretofore as M1 and M2.

Microbiological Analyses

The number of total coliforms and lactic acid bacteria was monitored in 4 different control points, as follows: 1) into milk; 2) into milk + graft and chitosan solution; 3) when pH reached 5.5 (pH useful to cut the curd); and 4) after the ripening time. Moreover, cell loads were determined on the finished Mozzarella cheese and during storage. The following media and the incubation conditions used were: spread plating onto plate count agar (Biolife, Milan, Italy) plates incubated at 37°C for 48 h for total mesophilic bacteria; spread plating onto plate count agar plus 5% skim milk (Biolife) plates incubated at 30°C for 5 d for proteolytic bacteria; spread plating onto trybutyrin agar (Oxoid, Milan, Italy) plates incubated at 30°C for 72 h for lipolytic 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 in-

Table 2. Effect of chitosan against coliforms in different control points during Mozzarella cheese manufacture.[1]

Control point	Time (days)	Mozzarella (cfu/g)	Mozzarella with chitosan (cfu/g)	*P*-value
Milk	t_0	$6.30 \times 10^3 \pm 9.89 \times 10^2$		—
Milk + whey	t_1	$4.35 \times 10^4 \pm 9.19 \times 10^3$	$3.75 \times 10^4 \pm 3.54 \times 10^3$	0.52
When pH reached 6.45	t_2	$2.60 \times 10^4 \pm 5.66 \times 10^3$	$4.75 \times 10^3 \pm 3.53 \times 10^2$	0.12
Curd break	t_3	$2.85 \times 10^3 \pm 1.20 \times 10^3$	$4.05 \times 10^2 \pm 7.7 \times 10$	0.21

[1]Numbers reported (cfu/g) represent means of duplicate counts. Data are presented ± standard deviation.