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Water Buffalo Mozzarella Cheese Stored in Polysaccharide-Based Gels: Correlation Between Prolongation of the Shelf-Life and Physicochemical Parameters

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ABSTRACT

An innovative packaging system has been developed, based on natural gels, that has shown the peculiar characteristic to strongly increase the shelf life of water buffalo Mozzarella cheese. To explain the mechanism of action of the gel, measurements of Ca and Na in the cheese and in the storage liquid were carried out, together with pH determination. A correlation has been found between the constant level of Ca and pH in the cheese and the prolongation of nutritional characteristics; in fact, both parameters diminish significantly in the absence of gel. At the same time, the weight of the cheese in gel remained constant for as long as 30 d. Confocal laser microscopy gave direct evidence of the persistent physical structure of proteins and lipids of Mozzarella when stored in gel.
Key words: Mozzarella cheese, polysaccharide gel

INTRODUCTION

The real water buffalo Mozzarella (**WBM**) cheese suffers from limited shelf life. Today, many researchers focus on the development of new methodologies and innovative materials that increase the shelf life of foods, without affecting their quality (so-called mild technologies).

Shelf life of Mozzarella is strictly linked to the type of raw material used (unpasteurized milk) and to the technology of processing. The product obtained from unpasteurized milk and natural whey can be stored and immersed in its mother solution for 3 to 4 d at a temperature between 4 and 10°C with no loss of its characteristics (translucent external skin, white color, soft and elastic curd, peculiar taste of fresh milk, and wildness aroma; Paonessa, 2004). Beyond such period, the external skin peels off, whereas the shred loses consistency and becomes buttery after the destruction of the overlapping shells structure obtained during high temperature stretching. Only industrial products, obtained with pasteurized milk and selected starter, can maintain a prolonged shelf life, up to 20 d, but the taste is absolutely unsatisfactory when compared with traditional artisan Mozzarella.

In a previous work (Laurienzo et al., 2006) we reported the results relative to innovative packaging systems, based on natural gels, that have shown the peculiar characteristic to increase the shelf life of the WBM cheese, without adding any chemical substance and without thermal procedures. It is in fact common practice of cheesemakers, particularly for export products, to add preservatives such as citric acid or lactic acid and often to sterilize the milk at high temperatures. These procedures inevitably affect sensorial properties of Mozzarella cheese.

The basis of the innovation is first of all related to the choice of material: mixtures of biodegradable and biocompatible polymers belonging to well-known families of natural polysaccharides, already approved for use in the agro-food industry.

The results published have shown that such innovative gel guarantees a prolonged shelf life up to 20 d of the traditional Mozzarella with no influence on the values of pH, the microbiological characteristics, the mechanical properties, and the typical taste. Unfortunately, the analysis of the protein fraction from Mozzarella cheese has not explained the differences in mechanical properties between gel-stored WBM cheese and liquid-stored WBM cheese (Laurienzo et al., 2006).

Hence, we speculated that the different behavior could be attributed to the changes in content of Na and Ca between the 2 types of Mozzarella cheese, following different osmotic exchange of the different storage media (gel and water). In fact, during cheesemaking, the mechanism that regulates curd coagulation is based on an enzymatic modification, from chymosin (enzyme contained in the rennet) of κ-CN, that falls in presence of Ca, forming the curd (Vitagliano, 1976); the pH at

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draining determines the retention of minerals, mainly containing Ca and P, in the cheese curd (Pastorino et al., 2003). Calcium-neutralizing negative charges of CN molecules increase the affinity of rennet for CN micelles and the rate of enzymatic reaction; this charge neutralization facilitates protein-to-protein interactions between CN molecules, which increases the aggregation of renneted micelles (Vitagliano, 1976). The content of Ca then affects the extent and degree of protein aggregation determining the basic structure and texture of cheese (Lucey et al., 2003; Pastorino et al., 2003). It is generally accepted that pH and Ca concentration influence the ability of curd to plasticize in hot water or hot dilute brine (Joshi et al., 2004; Lee et al., 2005; McMahon et al., 2005).

It has been suggested that adding salt promotes Ca solubilization from paracasein in CN pellets and cheese, thus displacing Ca from the protein matrix and into the serum (Pastorino et al., 2003). After permanence in pickle, where salt concentration is 10 to 18% NaCl, WBM cheese has a greater salt content in peripheral zones, whereas down to some millimeters from the surface, the content of NaCl is almost null. In preservation liquid, salt concentration tends to become homogeneous in all the Mozzarella cheese; in fact, from external zones of Mozzarella cheese, salt migrates toward the inner layers, replacing itself with Ca; NaCl promotes microstructural swelling, a concomitant increase in water-holding capacity, and the solubilization of intact CN from the paracasein matrix (Guo et al., 1997). Unfortunately, little information is available on the effects of pH and Ca content, and of their interaction, during storage of Mozzarella cheese in preservation liquid.

The aim of this work was to verify the effectiveness of our innovative packaging system to avoid large changes of content of Na and Ca in Mozzarella cheese during preservation until 30 d, compared with the Mozzarella cheese in preservation liquid, to explain the persistence of mechanical and nutritional properties of Mozzarella in gel.

MATERIALS AND METHODS

Materials

Nitric acid was obtained from Carlo Erba Reagenti, Rodano (Mi), Italy. Water TraceSelectUltra (for trace analysis) was obtained from Fluka, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland. Sodium and Ca (standard for atomic absorption), concentration = 1 mg/mL, in diluted hydrochloric acid, were obtained from Carlo Erba Reagenti. Rhodamine B fluorescent dye was obtained from Sigma Chemical Co. (St. Louis, MO). Lipid-soluble Nile Blue fluorescent dye was obtained from Sigma Chemical Co.

Samples Packaging

The WBM cheese samples used in this study were acquired at local supermarkets. Gel for sample conservation was prepared as described in Laurienzo et al. (2006). The samples in gel and the samples in preservation liquid were stored at 4°C and analyzed at different storage times. Experiments were run in triplicates, and the measurements were performed according to this time schedule (d): 0, 2, 4, 8, 10, 15, 20, 30.

pH Measurements

The pH determinations were carried out by a CRISON 507 pH meter equipped with type 52-00 electrodes and a type 52-32 electrode for penetration analysis. The analysis was carried out on the preservation liquid, on the cheese stored in it, and on the cheese stored in the gel; pH of the gel was neutral.

Samples Mineralization

Cheese samples (5 ± 0.1 g), stored in gel and preservation liquid, were placed in a drying oven at 105°C for 5 h. Samples were weighed for determination of the tenor in DM and carbonized on the Bunsen flame. Successively, samples were placed in a furnace at 550°C for 10 h until they turned to white ash. Following, they were suspended in 3 mL of nitric acid 10% (Carlo Erba Reagenti), taken to a final volume of 25 mL with Water Tracepur Fluka (Sigma-Aldrich Chemie GmbH) and filtered with a syringe filter SFCA membrane 0.45 μm (Millex, Millipore, Bedford, MA), for inorganic trace analysis.

Preservation liquid was filtered with syringe filter SFCA membrane 0.45 μm and analyzed.

Samples Analysis

A spectrophotometer Varian AA-200 (Mulgrave, Victoria, Australia) was used for this work with a flame burner for analysis of Na and Ca concentration.

The hollow cathode metal lamp was used at opportune wavelength: for Ca determination, the lamp was set at 422.7 nm, whereas for Na determination, a value of emission of 589.0 nm was set.

Metal atomic absorption standard solutions with Na and Ca as solutes (Carlo Erba Reagenti, concentration = 1 mg/mL, in diluted hydrochloric acid) were used to prepare the working standard for the programmable sample dispenser. The final concentration of nitric acid in all the solutions was 0.5% vol/vol.

A new rational calibration procedure was selected on the Spectrophotometer AA to assess the amount of

Table 1. The pH value of preservation liquid of water buffalo Mozzarella cheese in preservation liquid and in gel at different times

Conservation time	LP[1]	WBLP[2]	WBgel[3]
0	4.28	5.25	5.25
2	4.53	5.15	5.28
4	4.70	5.05	5.30
6	3.87	5.12	5.27
8	4.34	5.03	5.31
10	4.29	5.01	5.37
15	5.03	5.05	5.47
20	5.54	4.99	5.50
30	4.94	4.89	5.68

[1]LP = preservation liquid.

[2]WBLP = water buffalo Mozzarella cheese in preservation liquid.

[3]WBgel = water buffalo Mozzarella cheese in gel.

metal in the samples. Peak height-measurement mode was used.

Weight Measurements

The samples were weighed at time 0 and during the preservation, according to the protocol.

Confocal Laser Scanning Microscopy

Mozzarella from 3 trials (fresh, 21 d in gel, 21 d in preservation liquid) were examined by confocal laser scanning microscopy.

Sections of Mozzarella, approximately 10 × 10 × 2 mm thick, were cut with a razor blade (at 4°C), placed on a microscope slide, and stained by immersion for 2 min in a 0.1% solution of water-soluble Rhodamine B fluorescent dye (Sigma Chemical Co.). Excess dye was washed off with distilled water. Lipid-soluble Nile Blue fluorescent dye (Sigma Chemical Co.) was then directly applied in solid form to the surface of the Mozzarella. Both of these dyes were used concurrently to observe the fat and aqueous phases of the Mozzarella. The sample preparation procedure was performed over ice to keep the temperature low and minimize fat globule distortion. The Mozzarella samples were observed using confocal laser scanning microscopy (Leica, Heidelberg, Germany), with an argon-HeNe laser in dual-beam fluorescent mode and excitation wavelengths of 647 and 568 nm for the fat and aqueous phase, respectively. The fat globule size in the Mozzarella structure was determined from the microscope images using Image Tool for Windows NT version 2.0

RESULTS AND DISCUSSION

The values of pH are reported in Table 1. The pH of the preservation liquid changes from acid value to a more alkaline value, even though not very monotonically. The pH of Mozzarella cheese, stored in preservation liquid, changes from a value of 5.25 (d 0) to a value of 4.89 (d 30), whereas pH of gel-stored Mozzarella cheese showed regular variations, changing from 5.25 (d 0) to 5.68 (d 30).

In Figures 1, 2, and 3 are reported the values of the content of Ca and Na in Mozzarella cheese and its preservation liquid. It is evident that there is a migration of Ca from Mozzarella cheese to preservation liquid and a migration of Na from preservation liquid to Mozzarella cheese. These drastic changes, together with the observed change of value of pH could be responsible for changes in rheological characteristics of Mozzarella

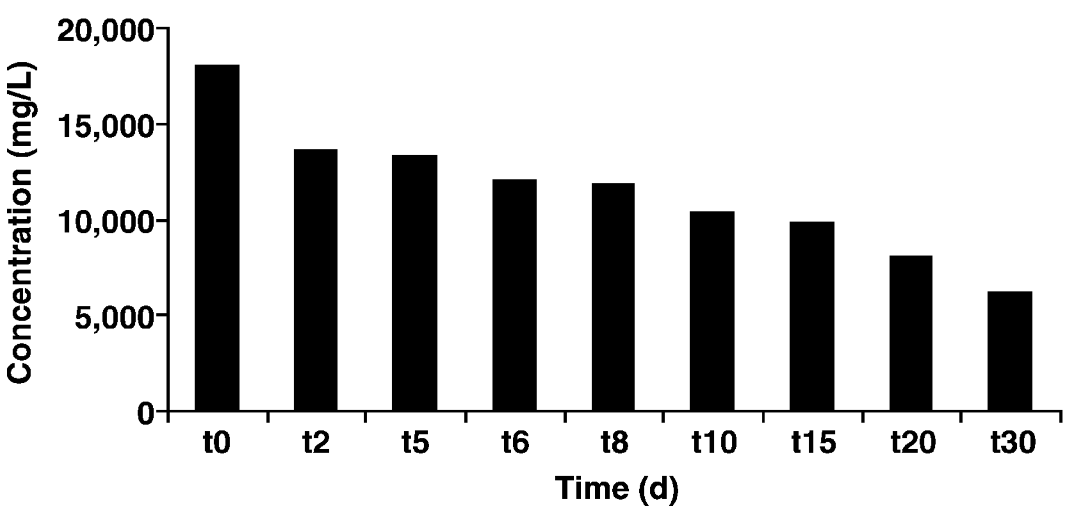

Figure 1. Sodium concentration in preservation liquid (mg/L) relative to conservation days. Standard deviation is less than 0.2%.

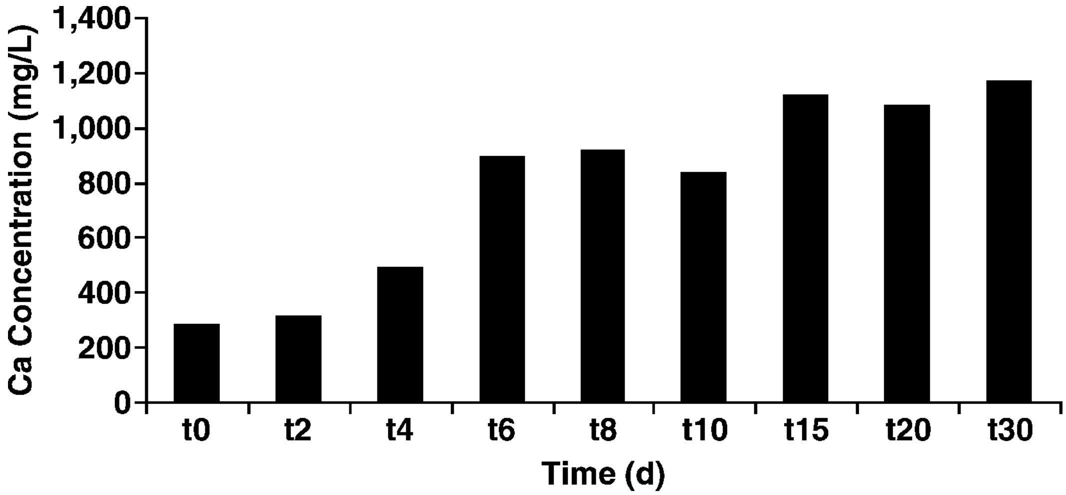

Figure 2. Calcium concentration in preservation liquid (mg/L) relative to conservation days. Standard deviation is less than 0.2%.

cheese; in fact, as reported in literature, stretchability of Mozzarella is influenced by pH and Ca content (Kimura et al., 1992; Guinee et al., 2002; Hassan et al., 2004), whereas an increase in the value of NaCl promotes microstructural hydrating and swelling of the protein matrix (Guo et al., 1997; Pastorino et al., 2003). In particular, a pH reduction increases the ratio between soluble Ca over colloidal Ca, which in turn would increase the degree of hydration of CN (Sood et al., 1979) and reduce its aggregation state, which then increases the susceptibility of CN to hydrolysis (Feeney et al., 2002).

In Figure 4 are reported the values of the content of Ca and Na in Mozzarella cheese stored in gel up to 30 d. As for the pH (Table 1), changes in Ca and Na in these samples are strongly reduced. There are small variations of the 2 elements, due probably to the intrinsic variability of artisan samples; in fact, the changes are in a range of 4,000 to 5,000 mg/kg, whereas, for Mozzarella cheese stored in preservation liquid, these variations are drastic, as shown in Figures 5 and 6.

Stability of pH and of the content of Na and Ca in the gel-stored WBM cheese could explain its almost constant rheological and mechanical characteristics.

As an additional proof of the importance of regulation of the salt exchange, gel-stored and liquid-stored WBM cheeses have been weighed in time, and the results are reported in Table 2; for WBM stored in its preservation liquid, there is an increase in weight from 20 to 35% of the original weight. These differences in the weight could be attributed to the osmotic pressure change that determines the preservation liquid to enter the cheese.

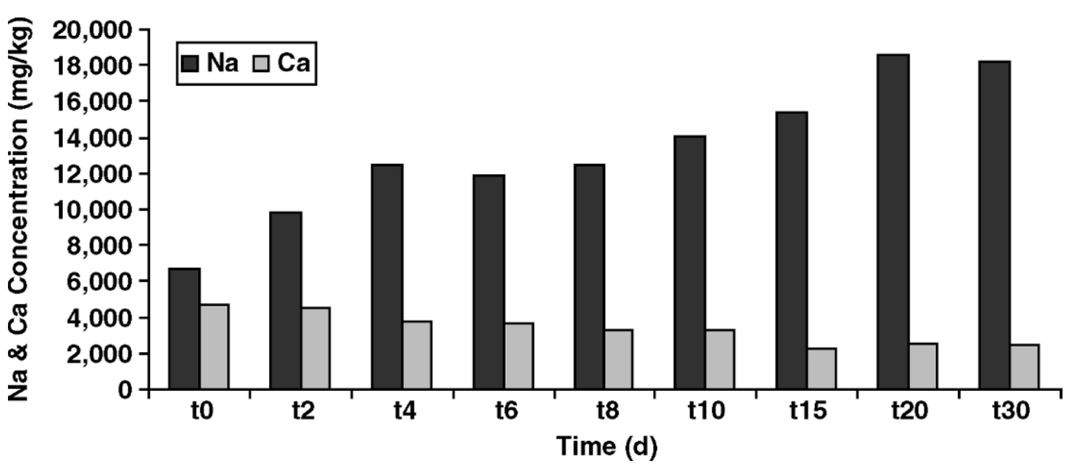

Figure 3. Sodium and Ca concentration in water buffalo cheese (mg/kg) relative to conservation days. Standard deviation is less than 0.2%.

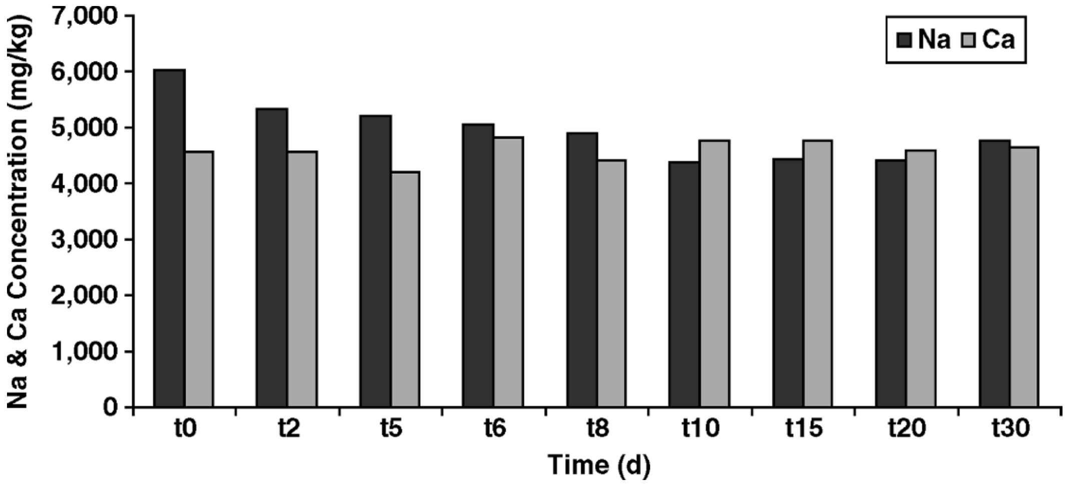

Figure 4. Calcium and Na concentration in water buffalo cheese in gel (mg/kg) relative to conservation days. Standard deviation is less than 0.2%.

For the WBM cheese in polysaccharide gel, this variation ranges from 0.32 to 1.78%, as a consequence of the capacity of gel to release on demand a little amount of water that is absorbed by cheese and helps to preserve an optimal degree of humidity .

The microscopic structure of Mozzarella, in particular the protein network, greatly influences physical properties and sensory attributes. Confocal laser scanning microscopy is considered a powerful tool to study the microstructure of Mozzarella, because the laser scanning penetrates the surface to visualize thin optical sections to obtain 3-dimensional analysis of Mozzarella microstructure without disturbing the internal structure and to measure the size and shape of intact fat globules (Auty et al., 2001). Some evidence indicates that refrigerated storage of Mozzarella cheese caused swelling of the protein matrix and increases in size, area, and perimeter of the fat particles (McMahon et al., 1999; Joshi et al., 2004).

Confocal laser scanning microscopy was hence used in this study to help relate Mozzarella processing conditions to the microstructure and therefore to the final product quality. The relevant results of the analysis are reported in Figure 7.

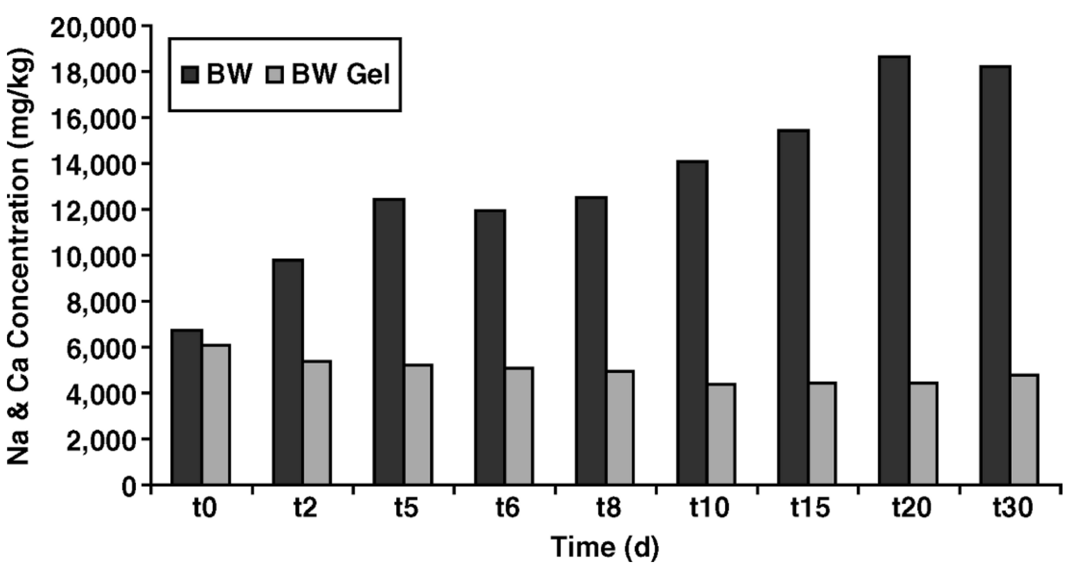

Figure 5. Sodium concentration in water buffalo cheese in preservation liquid and in gel (mg/kg) relative to conservation days. Standard deviation is less than 0.2%.

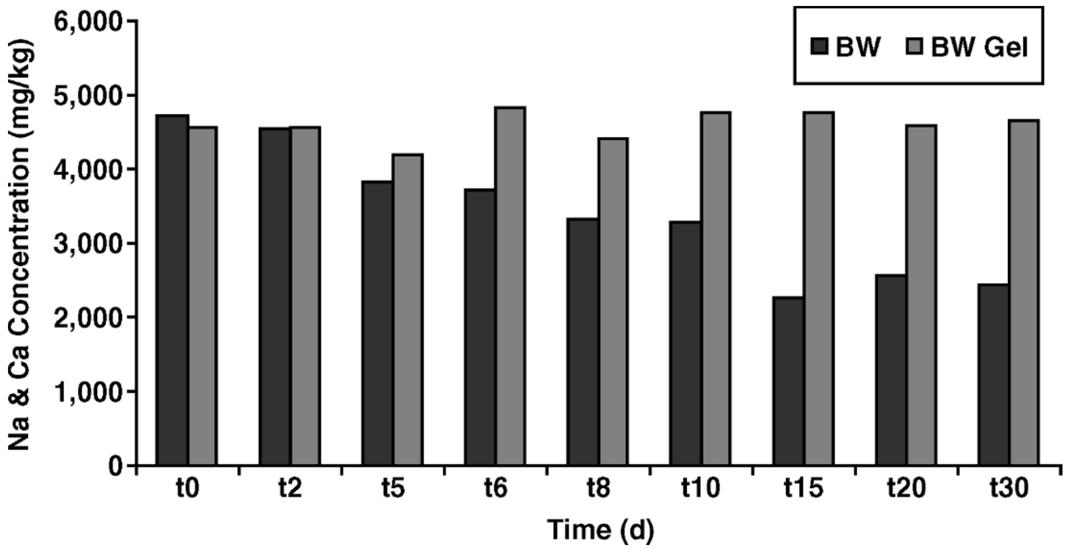

Figure 6. Calcium concentration in water buffalo cheese in preservation liquid and in gel (mg/kg) relative to conservation days. Standard deviation is less than 0.2%.

The storage of Mozzarella for 21 d without gel (Figure 7C) caused an alteration of the microscopic structure of Mozzarella, in particular of the protein network. In fact, the microstructure of the said Mozzarella, compared with the Mozzarella preserved in gel (Figure 7B), indicated that during storage in liquid, there was loss of identity of the protein fibers and swelling of the protein matrix. Also, the fat globules were generally bigger. Importantly, the protein fibers and fat globule microstructure of the Mozzarella after 21 d in gel were similar to that of the fresh Mozzarella (Figure 7A).

Table 2. Weight measurements of Mozzarella water buffalo cheese in preservation liquid and in gel at different times

	Weight (g)			
Samples	Time 0 (d)	Time 2 (d)	Difference (g)	Difference (%)
WBLP[1]	54.64	71.57	+16.93	+30.98
WBgel[2]	55.26	55.44	+0.18	+0.32
		Time 4 (d)		
WBLP	51.14	68.66	+17.52	+34.26
WBgel	58.65	59.42	+0.77	+1.31
		Time 6 (d)		
WBLP	55.05	70.58	+15.69	+28.50
WBgel	53.86	54.82	+0.96	+1.78
		Time 8 (d)		
WBLP	55.36	66.13	+10.77	+19.45
WBgel	52.46	52.76	+0.3	+0.57
		Time 10 (d)		
WBLP	53.05	71.95	+18.9	+35.62
WBgel	51.86	52.78	+0.92	+1.77
		Time 15 (d)		
WBLP	54.38	68.90	+14.52	+26.70
WBgel	51.36	52.00	+0.64	+1.25

[1]WBLP = water buffalo Mozzarella cheese in preservation liquid.

[2]WBgel = water buffalo Mozzarella cheese in gel.

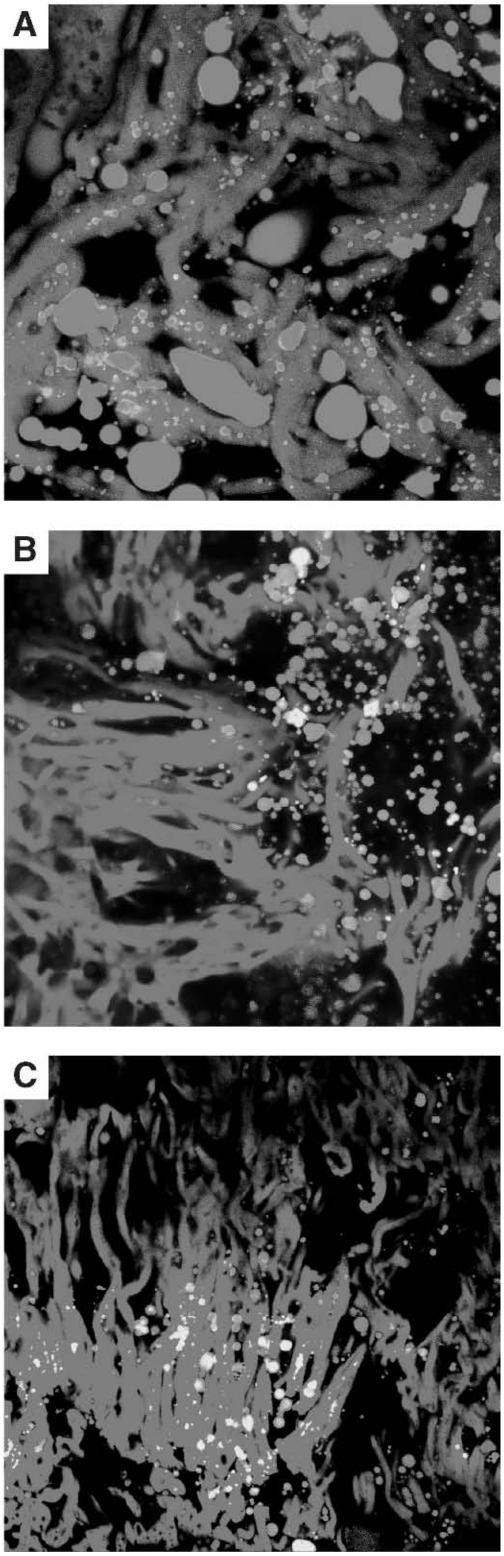

Figure 7. (A) Confocal fluorescence staining of Mozzarella after 21 d of storage in liquid, (B) Mozzarella after 21 d of storage in gel, and (C) fresh Mozzarella. (A) Alteration of the microscopic structure of protein fibers and fat globules is evident compared with (B) and (C). Color figure available online (http://jds.fass.org/content/vol91/issue4/)

CONCLUSIONS

The aim of this work was to explain the ability of an innovative packaging system based on natural gel to delay the loss of nutritional properties of WBM cheese until 30 d, compared with WBM cheese in its preservation liquid as reported in a previous paper (Laurienzo et al., 2006).

We concentrated our investigation on measurements of salts and pH; in fact a correlation between pH, content in Ca, Na content, and structure of Mozzarella cheese has been reported in the literature (Kimura et al., 1992; Kindstedt et al., 2001; Feeney et al., 2002).

Results obtained have confirmed the ability of the polysaccharide gel to avoid drastic change in the content of Ca and Na and in the values of pH that normally occur in liquid-stored Mozzarella cheese within 2 d of production. Strongly reduced changes in both parameters confirm their correlation with the preservation of the mechanical parameters (texture, stretchability, and flowability) in gel, as previously reported. Confocal laser scanning microscopy gave direct evidence of the persistence of physical structure of proteins and lipids in the gel-stored samples.

We can conclude that polysaccharide gel represents an efficient primary packaging to maintain for a prolonged time those parameters that have an effect on the shelf life of Mozzarella cheese.

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