



Microstructure of Mozzarella cheese as affected by the immersion freezing in NaCl solutions and by the frozen storage

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ABSTRACT

The freezing of Mozzarella cheese by immersion in NaCl solutions may be an innovative procedure for the dairy industry because it combines conveniently salting and freezing processes. The influence of this type of freezing method and of the frozen storage of samples on the microstructure of Mozzarella cheese was studied. Slabs ($2 \times 10 \times 10 \text{ cm}^3$) were immersed in 23% w/w NaCl solutions (control samples: 4 °C, 90 min; frozen samples: –15 °C, 180 min). Half of the frozen samples were immediately thawed at 4 °C. The other half was stored at –20 °C for 2 months and then was thawed at 4 °C (frozen-stored samples). Samples were stored at 4 °C and assayed at 1, 14 and 41 days. Although the immersion freezing of Mozzarella cheese affects its microstructure, only small differences were observed in the frozen-stored samples compared to control and frozen samples. Those differences consisted on less defined honeycomb appearance and the presence of small cracks at 41 days of ripening in the frozen-stored samples. Therefore, it was considered that the immersion freezing might be useful for the manufacture and commercialization of Mozzarella cheese.

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1. Introduction

Nature or processing defines food structure. Therefore, food technology may preserve, transform, destroy, or create structure (Aguilera, 2002). In the case of Mozzarella cheese, microstructure changes substantially during the first weeks after manufacture (McMahon et al., 1999). As the commercial Mozzarella cheese curd is heated, stretched, and molded, the proteins have the appearance of continuous, interconnected, smooth-walled fibers separated by channels that contain molten fat globules, serum, bacteria, and water-soluble cheese components. However, over time the open channels become occluded with proteins that absorb the free serum, swelled, and encased the fat globules (McMahon et al., 1999). Practically all food properties are structure sensitive (Aguilera, 2002). In the case of Mozzarella cheese, microstructure is one of the major controlling factors of texture and functional properties. Understanding the way microstructure is formed would allow to develop strategies for controlling and improving functional properties of cheese (Joshi et al., 2004).

Prolonging the storage time of Mozzarella cheese, while still maintaining good functionality, can be achieved by cheese freezing, however some studies have shown that freezing increases hardness (Diefes et al., 1993), decreases meltability (Oberg et al., 1992), and causes less free-oil formation (Bertola et al., 1996).

Numerous studies indicate that the effects of freezing, frozen storage or thawing change with cheese variety, process conditions, and ripening time (Chaves et al., 1999; Kuo and Gunasekaran, 2003; Graiver et al., 2004), justifying thorough studies if new alternatives are investigated.

An interesting alternative for fast freezing of cheeses may be freezing by immersion in NaCl solutions (Zorrilla and Rubiolo, 2005a,b; Ribero et al., 2007). In addition to the advantages associated with a fast freezing method, immersion freezing of cheeses would allow decreasing the production time because salting and freezing occur at the same time. Ribero et al. (2007) shown that although the immersion freezing of Mozzarella cheese affects some of the studied viscoelastic parameters, the differences observed between frozen and frozen-stored samples with control samples were small. Microstructural studies may contribute to a better understanding of the effect of this new freezing alternative on Mozzarella cheese characteristics. Therefore, our objective was to evaluate the influence of the freezing by immersion in NaCl solutions and of the frozen storage on the microstructure of Mozzarella cheese.

2. Materials and methods

2.1. Cheese sampling and immersion brine

Unsalted fresh Mozzarella cheeses (3500 g weight, $28 \times 10 \times 10 \text{ cm}^3$ size) were provided by a local factory. The initial

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composition was: 50.2 ± 0.3 (g/100 g cheese) moisture, 22 ± 2 (g/100 g cheese) proteins, 19.7 ± 0.4 (g/100 g cheese) fats, 0.143 ± 0.004 (g/100 g moisture) chlorides in moisture, and 5.43 ± 0.03 pH. Samples were slabs of 2 cm thickness, which were cut perpendicularly to the principal axis of the cheese block. The immersion brine was a NaCl solution of 23% w/w ($0.55\% \text{ Ca}^{+2}$, pH 5.2).

2.2. Treatments

Twenty-one slabs were immersed in the brine at 4 °C and removed at 90 min (control samples) and forty-two slabs were immersed in the brine at –15 °C and removed at 180 min. Samples were carefully wiped with a paper towel. Immersion times were estimated using the mathematical model proposed by Zorrilla and Rubiolo (2005a,b) to ensure similar average salt concentration in cheese samples. Twenty-one frozen samples were thawed at 4 °C immediately after the freezing process was finished (frozen samples), while twenty-one frozen samples were stored at –20 °C for 2 months and then were thawed at 4 °C (frozen-stored samples). After thawing, all samples were packed under vacuum and stored at 4 °C during 6 weeks. Three slabs per treatment were randomly selected at 1, 14, and 41 days of ripening. A slice of $100 \times 20 \times 2 \text{ mm}^3$ obtained from the center of the slab was used to obtain a strip of $20 \times 2 \times 2 \text{ mm}^3$ from the center of the slice, which was used to study the microstructure.

2.3. Chemical analysis

The half of each slab of Mozzarella cheese was used to determine pH and contents of chloride, moisture, and total nitrogen. The pH was determined with an electrode for solid foods (PH Spear, OAKTON Instruments, Vernon Hills, USA) in duplicate. Chloride content was determined as suggested by Fox (1963) with an automatic titrator model DL40 RC (Mettler Instrumente AG, Greifensee, Switzerland) in 5 replicates. Moisture content was determined in a microwave CEM AVC 80 (CEM, Mattheus, USA) in duplicate. Total nitrogen was determined in duplicate using the micro-Kjeldahl method with an automatic digester model 430, a distillation unit model 322, and a control unit model 342 (Büchi, Flawil, Switzerland), and a DL40 RC titrator (Mettler Instrumente AG, Greifensee, Switzerland). Fat content was determined for initial composition in duplicate (International Dairy Federation, 1969).

2.4. Cheese microstructure

Scanning electron microscopy (SEM) was used to examine the microstructure of cheese. Samples were prepared according to the method of Kuo and Gunasekaran (2003). Each strip of $20 \times 2 \times 2 \text{ mm}^3$ was fixed in 2.8% glutaraldehyde in a 0.05 M

sodium phosphate buffer (pH 6) for 48 h at 4 °C. The fixed cheese strips were dehydrated in a graded ethanol series. This consisted of 30 min in each of 25%, 50%, 70%, 80%, 95%, 100%, 100%, and 100% (v/v) ethanol solution. The strips were then defatted three times with chloroform for 30 min. The defatted strips were dehydrated three times with absolute ethanol for 30 min. Strips were then frozen in liquid air and fractured. Therefore, after the cryofracture of strips, the observations by scanning electron microscopy were carried out approximately at the geometrical center of the original slab. The cryofractured specimens were mounted on SEM stubs with silver conducting paint, and dried and coated with gold in argon atmosphere using a laboratory evaporator Veeco VE-300 (Veeco Instruments Inc., Long Island, NY, USA). The specimens were examined in a JEOL JSM-35C scanning electron microscope (JEOL Ltd., Tokyo, Japan) operated at an accelerating voltage of 20 kV. Images were obtained at three different magnifications (X): 120, 400, and 1200.

3. Results and discussion

The chemical composition for each treatment is shown in Table 1. Expected values of moisture content, chloride in moisture content, protein content, and pH were observed (Diefes et al., 1993; Bertola et al., 1996; Chaves et al., 1999; McMahon et al., 1999).

The stretching of the curd in hot water or brine (pasta filata process) during the cooking stage imparts peculiar structural components to Mozzarella cheese (Joshi et al., 2004). Microscopic studies have shown that stretching creates a network of parallel-oriented protein fibers, while large open channels between protein fibers are occupied by water and fat droplets (McMahon et al., 1999). Changes in microstructure and functionality of pasta filata Mozzarella cheese during storage can be attributed not only to the breakdown of α_{s1} -casein and β -casein by residual coagulant and milk plasmin, but also due to the changes in the state of water in cheese and the increase in protein hydration (McMahon et al., 1999). So, while the appearance of the channel walls are textured by numerous circular indentations by 1 day of storage, the interstitial spaces between the fat globules, appear to be completely filled by the protein matrix by 21 days of storage.

The specimens were analyzed at 120X to determine if the cryofracture was correctly carried out. Indeed that magnification allows determining the structural homogeneity of the specimens. The magnifications 400 and 1200X improved the resolution during the observation of the microstructure of the cheese. When the microstructure of cheeses was examined at low magnification and 1 day of ripening, differences between treatments were not observed (Fig. 1). The proteins formed a continuous surface. Channels that contained the serum and fat globules eliminated during the sample preparation, shown smooth edges and had a size from approximately 5 to 20 μm in width (Fig. 2). When increasing the magnification, differences between treatments were not observed

Table 1
Chemical composition of the cheese samples studied^a.

Treatment	Time (days)	Moisture (g/100 g cheese)	Chloride in moisture (g/100 g moisture)	Protein (g/100 g cheese)	pH
Control samples	1	47 ± 1	2.40 ± 0.05	23.9 ± 0.5	5.24 ± 0.04
	14	48.3 ± 0.7	2.43 ± 0.04	22.9 ± 0.6	5.31 ± 0.05
	41	47.7 ± 0.2	2.46 ± 0.02	23.4 ± 0.1	5.27 ± 0.02
Frozen samples	1	49.1 ± 0.2	2.62 ± 0.08	23 ± 1	5.33 ± 0.02
	14	49.9 ± 0.1	2.77 ± 0.09	21.9 ± 0.3	5.253 ± 0.005
	41	49.4 ± 0.3	2.69 ± 0.05	21.9 ± 0.5	5.275 ± 0.008
Frozen-stored samples	1	48.7 ± 0.5	2.71 ± 0.05	22.4 ± 0.5	5.21 ± 0.02
	14	49.8 ± 0.3	2.83 ± 0.02	21.3 ± 0.9	5.257 ± 0.008
	41	49.3 ± 0.3	2.74 ± 0.06	22.1 ± 0.6	5.38 ± 0.02

^a Average values and standard deviations.

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