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Original article

Influence of medium-concentration factor microfiltration treatment on the characteristics of low-moisture Mozzarella cheese

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Abstract – To study the chemical properties, the initial recovery of milk components, oiling off, and the textural properties of the cheese during a 60-day refrigerated storage period, skim milk was microfiltered through a 1.4-μm ceramic membrane to remove bacteria, and then low-moisture Mozzarella cheese was manufactured from medium-concentration factor (CF) microfiltration (MF) (0.1 μm of pore size, CF = 2.3) retentate with added cream. The changes in expressible serum (ES) were also studied. Three cheese-making trials were conducted on Mozzarella cheese milk using two different methods: MF and control. Changes in the levels of proteolysis patterns were monitored using pH 4.6-soluble nitrogen (SN), initially higher for microfiltration Mozzarella cheese (MMC) because of the higher content of caseinomacropeptide, and 12% trichloroacetic acid TCA-SN, and using SDS-PAGE. The levels of the secondary proteolysis (12% TCA-SN) were significantly higher in control Mozzarella cheese (CMC). The CMC showed a greater release of free oil at the beginning of aging time but this release was always lower than that observed for MMC. The textural characteristics (hardness, springiness, and cohesiveness) of both cheeses have significant differences, highly correlated to proteolysis indicators, during the ripening time. It is suggested that the differences in composition, proteolysis, oiling off, and textural characteristics in both the cheese and the ES attributes arise mainly as a result of the double content of calcium salts in 0.1-μm MF cheese issued from milk enriched with 2.3 times casein content. Indeed, the high content in calcium salts is known to inhibit autolysis of mesophilic lactic starter by increasing the buffering capacity of cheese aqueous phase, and then causing a slowdown of proteolysis. It likely leads to a different structuration of fat in Mozzarella cheese causing higher oiling off. The results obtained in this study show the possibility of making Mozzarella cheese from milk that has 2.3 times more micellar casein (50 $g \cdot kg^{-1}$), and to obtain products similar to those obtained from usual milk, adjustment of calcium salt mineralization is required.

microfiltration / Mozzarella cheese / oiling off / texture profile analysis / expressible serum

摘要 – 中等浓缩因子的微滤处理对低水分 Mozzarella 干酪特性影响摘要。脱脂乳经 1.4 μm 的陶瓷膜除菌后，采用 0.1 μm 陶瓷膜浓缩 2.3 倍并加入稀奶油复配以生产低水分 Mozzarella 干酪，并对其 60 d 成熟期内的化学、乳成分回收、游离油析出、质构及液相等特性进行了研究。两种不同的处理方式（微滤组及对照组）重复三次生产干酪。采用 pH 4.6-SN、12% TCA-SN 及 SDS-PAGE 研究了蛋白降解过程。微滤组 Mozzarella 干酪由于酪蛋白巨肽含量较高而使得初级蛋白降解 (pH 4.6-SN) 高于对照组 Mozzarella 干酪，而二级蛋白降解低于对照组

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Mozzarella 干酪。成熟初期对照组 Mozzarella 干酪游离油的析出较多，且一直高于微滤组 Mozzarella 干酪。成熟期间两组干酪的质构特性（硬度、弹性、粘聚性）差异显著，且其与蛋白降解程度息息相关。两组干酪间的成分、蛋白降解、游离油析出、质构特性及液相成分的差异主要是由于 0.1 μm 陶瓷膜浓缩 2.3 倍使得钙盐浓度增加两倍所引起的。钙盐浓度的增加使得液相部分的缓冲能力增加，抑制了中温发酵剂的分解作用，从而降低了蛋白降解程度。 脂肪结构化的差异是造成游离油析出差异的主要原因。 本文的研究结果表明浓缩 2.3 倍生产 Mozzarella 干酪只要调节钙盐浓度就能够获得与牛乳直接加工生产质量相近的 Mozzarella 干酪。

微滤 / Mozzarella 干酪 / 游离油析出 / 质构剖面分析 / 液相

Résumé – Concentration en caséine micellaire du lait de fabrication par microfiltration et conséquences sur les caractéristiques du fromage Mozzarella. Après élimination des bactéries contaminantes par microfiltration à l'aide d'une membrane céramique 1,4 μm, le fromage Mozzarella à extrait sec élevé était fabriqué à partir de lait concentré 2,3 fois par microfiltration sur membrane 0,1 μm, standardisé en matière grasse par addition de crème. Les fromages obtenus (MMC) ont été caractérisés, par rapport à des témoins (CMC) fabriqués à l'aide de lait seulement épuré par MF 1,4 μm, au plan des rendements de transformation, de leurs paramètres physico-chimiques, de leur exsudation de matière grasse, de leurs propriétés texturales et des variations de composition de leur phase aqueuse interne pendant 60 jours de conservation au froid. Trois expérimentations ont été réalisées. Les cinétiques de protéolyse étaient appréciées par la mesure de l'azote soluble à pH 4,6, initialement supérieure pour les fromages MMC probablement en raison de leur teneur plus élevée en caséinomacropeptide, de l'azote soluble dans le TCA 12 % et par électrophorèse en gel d'acrylamide. Elles montraient une protéolyse secondaire significativement supérieure dans les fromages CMC. L'exsudation de matière grasse des fromages s'accroissait durant l'affinage, elle était hautement corrélée avec les indicateurs de protéolyse mais restait toujours supérieure pour les fromages MMC. Les caractéristiques de texture des deux types de fromage étaient significativement différentes : fermeté et cohésion constamment plus élevées pour les fromages MMC, élasticité plus élevée en début d'affinage pour les fromages CMC mais quasiment identique au 30ème jour d'affinage. Les différences observées entre les deux types de fromage, tant au plan de leurs caractéristiques initiales qu'au cours de l'évolution de celles-ci lors de l'affinage, découlent très certainement de la minéralisation calcique deux fois plus élevée des fromages MMC, minéralisation calcique inhibant les mécanismes d'autolyse des bactéries mésophiles du levain et donc retardant la protéolyse, structurant différemment la matière grasse du caillé et conduisant à une exsudation plus élevée de la matière grasse. Il en résulte que si les résultats obtenus au cours de cette étude démontrent la possibilité de transformer en fromage Mozzarella du lait à teneur en caséine supérieure à 50 $g \cdot kg^{-1}$ (enrichissement par un facteur de 2,3), l'obtention de produits similaires aux produits obtenus à partir de lait non enrichi en caséine requiert un ajustement de leur minéralisation calcique.

microfiltration / Mozzarella / exsudation / texture / sérum expressible

Abbreviations: **MF** = microfiltration, **UF** = ultrafiltration, **TPA** = texture profile analysis, **CF** = concentration factor, **ES** = expressible serum, **CMC** = control Mozzarella cheese, **MMC** = microfiltration Mozzarella cheese.

1. INTRODUCTION

For centuries, milk for cheese-making was not subjected to pretreatment before processing, and many cheese varieties worldwide are still made from raw milk. However, due to some reasons, such as safety, quality consistency, and increased yield, most of the cheese-making processes today involve the treatment of milk by membrane technology or other such treatments prior to the addition of starter culture or coagulant [12, 13, 21, 33]. Within the food industry, dairy applications probably

account for the largest share of the installed membrane capacity. However, in China, the use of membrane technology is not, as of yet, a normal practice. Nevertheless, for China, the widespread inclusion of dairy product in human diet requires new efficient processing methods to be developed to increase the consumption of milk or milk products.

Ultrafiltration (UF) technology was the first membrane technology proposed in the dairy industry for cheese-making [23]. It has made rapid progress because of its potential benefits including higher plant capacity, increased cheese yield, homogeneity of the quality of cheese products, and decreased rennet usage compared to traditional practices. It is now employed for the production of many cheese varieties with specific adjustment of the general parameters to take into account the physicochemical properties of the UF retentates, resulting from the whey protein content and the concentration of micellar calcium [27].

Microfiltration (MF) technology has not only been used in the dairy industry for the removal of bacteria from milk, but also has been used for casein enrichment [5, 28, 33] through the use of MF membranes having different pore sizes. The use of 0.1-μm MF membrane can selectively separate whey proteins along with soluble salts and lactose. The resulting MF permeate named "ideal whey" [5] because of its composition and its characteristics (sterile, native components with highly functional properties, and no fat) has a high added value potential market in comparison to classical whey as described by Maubois et al. [22]. This economic advantage in addition to the technological benefits of the use of casein-enriched cheese milk explains the fast implementation of 0.1-μm MF technology in the European and the US cheese-making industries [28, 32].

The consumption of Mozzarella cheese has greatly increased in China due to the increase in the popularity of pizza and related foods. In this study, the manufacture of a low-moisture Mozzarella cheese is presented, in which the cheese milk was first pretreated using 1.4-μm MF to remove bacteria and then enriched 2.3 times in micellar casein using 0.1-μm MF. As far as we know, although low, medium, and high concentrations of cheese milk using UF for Cheddar and Mozzarella cheese [27] and low (20% more) [28] and high enrichment in micellar casein with preacidification for Mozzarella cheese [1] have been reported, medium-concentration factor (CF) MF milk for cheese-making has not been reported. The consequences of both these pretreatments on the cheese composition, milk component recovery, proteolysis, oiling off, and textural characteristics were determined and compared to those of a control cheese manufactured from normal cheese milk. This study also reports on the changes in the aqueous phase of the cheese occurring during a 10-day ripening time.

2. MATERIALS AND METHODS

2.1. Cheese milk

Fresh raw milk was obtained from a Beijing local dairy farm and was preheated to 50 °C for the separation of cream. The skim milk was then microfiltered at 50 °C through a ceramic membrane system equipped with a 1.4-μm membrane and separated into a bacteria-poor permeate and a bacteria-rich retentate. The microfiltered skim milk was stored in a bulk tank, maintained at 50 °C for at least 1 h, and then transferred to another ceramic MF system equipped with three 0.1-μm ceramic membranes "Isoflux®" (23 channels, 0.35 m^2 in membrane surface area, 25 mm in outer diameter, and 1178 mm in length per ceramic membrane) made in Hydrotech Sep. Technol. Ltd.

The system was operated in a uniform transmembrane pressure mode of 50 kPa with the cross-flow velocity maintained at 5 $m \cdot s^{-1}$. Milk was concentrated on a volume basis to reach a CF of 2.3.

2.2. Cheese manufacture

2.2.1. Cheese preparation

To study the effect of MF treatment on the characteristics of Mozzarella cheese, three separate cheese-making trials were undertaken.

Cheese milk was standardized to 0.7 ± 0.1% casein/fat by mixing MF skim milk and cream. The cream was separated at 50 °C (fat content 40–43%) and then sterilized at 95 °C for 5 min prior to mixing. The skim milk without 0.1-μm MF was used as control cheese milk. All milks were maintained at 4 °C from 8 to 12 h until cheese processing.

2.2.2. Cheese-making process

Mozzarella cheese was manufactured by a combination of dry-salting and brine-salting of the curd as previously described [31]. Both fat standardized (with fat content adjusted to casein contents) milks were batch-pasteurized at 64 °C for 30 min in a stainless steel vat placed in a water bath, cooled to 37 °C. The starter culture (Hansen's TCC) (0.1% milk weight for control cheese milk and 0.2% for MF cheese milk) was added and left for 40 min; rennet powder was then added (0.003% milk weight for control cheese milk and 0.0015% for MF cheese milk) to milk. The milk was agitated for 1 min and set for 40 min after the addition of the rennet. The coagulum was cut into 0.6-cm cubes when firm and allowed to cure for 5 min. The curd was heated from 37 to 42 °C for more than 15 min with continuous stirring. At the end of cooking, the whey (pH 5.9) was drained. Dry salt (2% w/w) was added to the curd and turned every 10 min. When the pH had reached 5.3, the curd was stretched and kneaded by hand in multidirection for 5 min in salt water (0.5% w/v) at 60–70 °C. The resulting curd was vacuum-packaged and stored at 4 °C until analysis.

2.2.3. Compositional analysis

Skim milk, retentate, and permeate were analyzed in triplicate for composition. Total solids (TSs), protein, fat, lactose, casein, and non-casein nitrogen were quantified according to the AOAC procedures [2]. The pH of the milk, whey, and curd was monitored during cheese-making; moisture, fat, protein, salt, and calcium contents of fresh cheese were determined in triplicate at day 7 as previously described [31].

2.2.4. Measuring component recoveries and cheese yield

Whey and stretching water were collected, filtered through a cheese cloth to exclude curd particles, and weighed as described by Fenelon and Guinee [6]. The bulk whey comprised the whey collected during whey drainage and curd cheddaring; the whey and the stretching water were stirred and heated to 50 °C before measurements.

The percentage of milk protein recovery in whey (%PRW) was calculated using the following formula:

$$\%\mathrm{PRW} = \frac{100 \times (\text{weight of whey}) \times (\text{protein in the whey}, x\%, \text{wt/wt})}{(\text{weight of cheese milk}) \times (\text{protein in the cheese milk}, x\%, \text{wt/wt})}.$$

The percentage of milk protein recoveries in stretching water (%PRS) was calculated using the following formula:

$$\%\mathrm{PRS} = \frac{100 \times (\text{weight of stretching water}) \times (\text{protein in the stretching water}, x\%, \text{wt/wt})}{(\text{weight of cheese milk}) \times (\text{protein in the cheese milk}, x\%, \text{wt/wt})}.$$

The percentage of milk protein recoveries in cheese (%PRC) was calculated using the following formula:

$$\%\mathrm{PRC} = \frac{100 \times (\text{weight of cheese}) \times (\text{protein in the cheese}, x\%, \text{wt/wt})}{(\text{weight of cheese milk}) \times (\text{protein in the cheese milk}, x\%, \text{wt/wt})}.$$

The percentage of fat and calcium recoveries in whey, stretching water, and cheese was calculated using the same formula as above.

2.3. Evaluation of proteolysis

The amounts of nitrogen were determined after 1-, 15-, 30-, 45-, and 60-day storage of cheese in pH 4.6 buffer and in 12% trichloroacetic acid (TCA), to evaluate the evolution of proteolysis [25] using the method of Kuchroo [21] and Polychroniadou [30]. The results were expressed as percentage total nitrogen (TN) content of the cheese.

Urea gel electrophoresis was performed on the insoluble fractions of the control and the MF cheese samples stored in pH 4.6 buffer, after 1-, 15-, 30-, 45-, and 60-day aging time, using a Protean II vertical slab gel unit (Bio-Rad Laboratories Ltd.) and the stacking gel system described in the method of Blakesley and Boezi [3]. The changes in the major components during storage and the intensity of individual casein bands (α_{s1} + α_{s2} and β-casein) remaining at each storage time were calculated using Gel-Pro analyzer V.3.0 software and were expressed as a percentage of casein exited in the first-day cheese (100%).

2.4. Oiling off

The oiling off of control cheese and MF cheese was determined by the modified centrifugation method [18] as follows: 3 g of grated cheese was placed in a Van Gulik butyrometer that was filled with water and placed in a water bath at 65 °C for 20 min. The cheese was then centrifuged at 65 °C for 2 min in a vertical position. Determination of exuded oil was done by taking the reading on the butyrometer and was expressed as per 100 g of cheese. Tests were conducted in duplicate.

2.5. Texture measurement

Texture profile analysis (TPA) [8] was conducted on the control and the MF cheese samples using the double compression test. The TPA was performed using a P/50 probe on an Instron Universal Testing Machine (Model TA-XT Plus, Instron, UK) with a full-scale load of 5 kg and a crosshead speed of 12.7 cm·min^{-1}. Four test portions (1.0 cm in diameter × 1.5 cm high) of cheese for TPA were cut from the cheese cylinder using a cork borer and a parallel wire cutter, and they were held at room temperature for half an hour before TPA determination.

To start the test, one test portion with a flat surface was placed in the center of the base plate of the texture analyzer. The direction of the fibers in the cheese samples was perpendicular to the surface of the base plate. Each sample was compressed twice (providing 50% compression) by sending a crosshead down a vertical column with a speed of 1 mm·s^{-1} both times, causing a flat

plate to deform the sample placed on the lower plate of the universal testing machine. Four TPA measurements were taken for each treatment at each aging time. Three TPA parameters (hardness, springiness, and cohesiveness) were calculated at each storage time. Hardness is the force necessary to attain a given deformation, cohesiveness is the strength of the internal bonds making up the body of the product, and springiness is the distance recovered by the sample during the time between the end of the first bite and the start of the second bite (originally known as "elasticity": the rate at which a deformed material goes back to its undeformed condition after the deforming force has been removed).

2.6. Extraction of cheese expressible serum

The extraction procedure was determined by an equipment similar to Morris et al. [27]. A stainless steel mold was designed and fabricated at Beijing Sanyuan Food Co. Ltd., Hengtai Workshop. It consisted of a collection vessel, a perforated vessel, an outer cover, and a heavy ram. The procedure of extraction was similar to Salvat-Brunaud et al. [34] and Hassan et al. [10]. Freshly grated cheese (800 g) was thoroughly mixed with 1000 g of washed sea sand and was placed in stainless steel mold lined with cheesecloth. The cheese-sand mixture was subjected to hydraulic pressure at room temperature. The pressure was increased gradually over 1 h up to a maximum of 8 MPa, and liquid fat and expressible serum (ES) were collected in a graduated cylinder until the flow of liquid stopped completely. Identical pressing conditions were used regardless of the ripening stage of the Mozzarella cheese. The liquid fat and the ES was transferred into a beaker. Separate layers of liquid fat and juice were visible. The beaker was stored at 4 °C for half an hour to allow the liquid fat to solidify. A hole was made in the solid fat layer using a knife, and the ES was decanted through the hole. The ES was then centrifuged at 3000× g for 10 min at 4 °C to remove any extraneous fat and curd particles. The volume of ES was then measured to the nearest 0.5 mL. The resulting ES was analyzed for Ca, Mg, and P contents using flame atomic absorption spectroscopy at the Department of Food Science, China Agriculture University using the method described by AOAC [2].

2.7. Statistical analysis

A complete experimental design was used to study the following factors: (1) milk treatment (control and MF), (2) storage time, and (3) block (each block representing a day of manufacture). The experiment was randomized in blocks. The statistical analysis was carried out using version 6.11 of SAS System statistical package (SAS Institute, Inc., Cary, NC). The results were analyzed using the General Linear Models Procedure to check the individual effects of the factors studied (block, time, and treatment) as well as the interaction between them. Significance of differences was defined at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Cheese milk

Mean compositions (three experiments) of 0.1-μm MF retentate, 1.4-μm MF permeate, control, and MF cheese milk are given in Table I. The removal of bacteria by the 1.4-μm membrane did not modify skim milk composition. As described by Fauquant et al. [5] and Saboya and Maubois [33], MF processing using 0.1-μm membrane of skim milk prior to cheese-making allows water, lactose, most of the whey proteins [13], soluble minerals, and non-protein nitrogen to pass through the membrane leading to an "ideal whey" [5]. Lactose

Table I. Compositional analysis of raw skim milk, 1.4-μm MF permeate and 0.1-μm MF retentate (CF = 2.3).

Component (%)	Raw skim milk	1.4-μm permeate	0.1-μm retentate	Control cheese milk	MF cheese milk
TS	8.62	8.60	11.14	12.12	17.80
Protein	3.21	3.19	5.79	3.13	5.15
Casein/TN	0.76	0.76	0.87	0.76	0.86
Fat	0.02	0.01	0.20	3.44	6.33
Lactose	4.65	4.65	4.53	4.59	4.45
Calcium	0.11	0.11	0.24	0.11	0.22

Table II. Composition of CMC and MMC.

Component ($g \cdot 100\ g^{-1}$)	CMC	MMC	Significant level
Protein	23.58 (0.31)[a]	24.00 (0.20)	NS
Fat	23.13 (0.20)	22.98 (0.59)	NS
Moisture	46.73 (0.43)	46.35 (0.68)	*
Salt	1.42 (0.05)	1.40 (0.08)	NS
Calcium	1.00 (0.01)	1.30 (0.02)	**
Phosphate	0.42 (0.03)	0.54 (0.04)	**
pH (day 1)	5.30	5.32	NS
FDM (%)[b]	43.42	42.83	
MNFS (%)[c]	60.79	60.18	
S/M (%)[d]	3.04	3.02	

[a] Numbers in parentheses represent standard deviation of the mean values.
[b] FDM: fat content on a dry matter weight basis.
[c] MNFS: moisture in the nonfat substance of the cheese.
[d] S/M: salt in the moisture phase of the cheese.
* $0.01 < P \leq 0.5$; ** $P \leq 0.01$.

concentration expressed per 100 g of the product decreased, but its concentration expressed per 100 g of water remained unchanged. Micellar casein, with the bound calcium salts, on the contrary, is retained by the membrane and consequently, the TS, the protein content, and the casein/total protein ratio (from 0.76 to 0.87) as well as the calcium content (from 0.11 to 0.24) increased according to the CF. The addition of cream to the 0.1-μm MF retentate lowered the protein and the casein/TN ratio as well as lactose and calcium contents. The casein/fat ratio was 0.70 for both the control cheese milk and the casein-enriched cheese milk.

3.2. Cheese compositional analysis

As described by Mistry and Maubois [26], in spite of the addition of half the amount of rennet to the control cheese milk, a more rapid casein aggregation and a much firmer final curd were observed during the coagulation of 0.1-μm MF cheese milk. The biochemical components of Mozzarella cheese made from either control Mozzarella cheese (CMC) milk or microfiltration Mozzarella cheese (MMC) milk are presented in Table II.

The compositions of both series of cheeses complied with the United States'

Table III. Composition recovery of cheese, whey, stretching water, and total component.

Item	Recovery (%)	CMC	MMC
Cheese	Protein	76.86	88.42
	Fat	63.57	60.57
	Calcium	79.06	80.03
Whey	Protein	22.09	10.98
	Fat	15.30	17.40
	Calcium	20.02	18.88
Stretching water	Protein	0.50	0.59
	Fat	20.23	22.53
	Calcium	0.70	0.19
Total	Protein	99.45	99.99
	Fat	99.10	100.5
	Calcium	99.78	99.10

definition for low-moisture Mozzarella cheese [17]. The protein content in MMC was higher than that of CMC, while the fat content was lower, but the differences were not significant. As expected from the data in the literature, Ca, P, and moisture contents of both series of cheeses showed significant differences which likely induce functional differences between CMC and MMC found in the later analysis.

3.3. Recovery of components

From the weights of cheese milk, whey, stretching water, and cheese for each vat and their respective fat, protein, and calcium contents, the actual percentage of protein, fat, and calcium that were recovered in the cheese, whey, and stretching water was calculated and the results are given in Table III.

The quality and the precision of the realized measurements were satisfactory because almost 100% of each component of cheese milk was recovered in the three resulting co-products. In the MMC, as 56.5% of the whey proteins were previously removed in the "ideal whey," the recovery of proteins in cheese is much higher (+11.5%) than that in the CMC since it mainly concerns casein. Protein recovery in whey for MF cheese-making is only 50% of the recovery for control cheese-making. It is likely that the fine protein composition in both wheys was different with more κ-GMP (likely 2.3 times) in the MF whey. It would be interesting to determine if this κ-GMP, which is a highly soluble molecule, were responsible for the increase (+0.09%) in the protein content of MMC stretching water. On the other hand, the recovery of fat in MF during cheese-making appeared slightly lower (−3.0%) than that in control cheese-making. Such a decrease previously observed and attributed to the cream separation and recombination done for MF cheese milk [13] could have been avoided if the cream had been added to 1.4-μm MF skim milk before 0.1-μm MF treatment [23, 28, 33]. If the recovery percentage of the total calcium salts was close for both the cheese-making experiments, then the results for whey and stretching water given in Table III indicated that there was less soluble calcium salts in MMC, probably because of a different solubilization of micellar calcium salts.

3.4. Proteolysis

Figure 1 shows the variation in the pH 4.6-soluble nitrogen to total nitrogen (SN/TN) ratios determined in both cheese categories. As expected, there was a common increase in this nitrogen form during cheese ripening. Initially constituted by whey proteins and κ-GMP, this form was progressively enriched by the release of small and large peptides produced by the action of rennet and starter proteases and peptidases hydrolyzing the cheese casein matrix [19]. The higher content at pH 4.6-SN observed in MMC during ripening compared to CMC did not agree with the reported retarded proteolysis in cheese made from protein-enriched cheese milk [27]. Further work is required for identifying the casein fragments causing this increase. One possibility could be that the κ-GMP molecule is

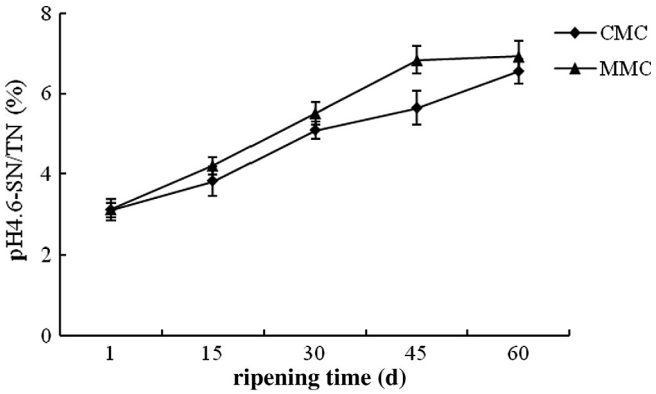

Figure 1. Changes in pH 4.6-SN of CMC and MMC during ripening.

highly resistant to the action of milk and starter proteases [27].

The 12% TCA-SN/TN ratios which measure both the initial milk NPN and the content of very small peptides and amino acids released by peptidases from the curd increased significantly with aging time (Fig. 2) for both series of cheeses. As κ-GMP does not belong to this fraction, the significant increase seen for MMC after 15 days of aging may indicate that the lysis of the starter culture cells which releases peptidases into the cheese juice was higher in this cheese. This hypothesis is related to the higher buffering capacity of MMC inhibiting autolysis of mesophilic lactic starters [32]. Nevertheless, no difference in this nitrogen fraction was observed at 60 days of ripening time, which indicated a similar level of proteolysis.

Urea-PAGE, despite being less sensitive than pH 4.6-SN for detecting the changes in the level of proteolysis, confirmed the trends shown in Figure 1. The data presented in Figure 3 show that the amount of residual α_s-CN and β-CN decreased with aging time at 4 °C for both CMC and MMC but in agreement with the measurements of proteolysis, the residual casein in MMC was always higher than that in CMC (Fig. 3). As expected [19], α_s-CN was hydrolyzed faster than β-CN in both cheeses, with lower levels of proteolysis detected for the MMC at all stages of ripening.

As shown in Table IV, the pH 4.6-SN and the TCA-SN expressed as a percentage of TN were both significantly influenced ($P < 0.01$) by treatment, aging time, and their interaction (treatment × aging time).

The results obtained on the evolution of proteolysis in this study have confirmed the previously published results on cheeses made from cheese milks enriched in proteins either by 0.1-μm MF or by UF [27]. The increase of buffering capacity resulting from the concomitant concentration of calcium salts bound to caseins leads to the inhibition of autolysis of mesophilic starters. The release of proteases and peptidases into the cheese juice was slower as was the hydrolysis of the casein matrix. This low level of proteolysis could be an advantage for cheese varieties, such as Minas frescal [32] or Mozzarella, for which consumers expect a fresh lactic flavor and stability in the functionality during shelf life. For other varieties, adjustment of technological cheese-making parameters, such as the use of the thermophilic starters, partial pre-removal of calcium salts [29], or the addition of attenuated or broken starter cells [33], have to be performed to obtain similar proteolytic patterns.

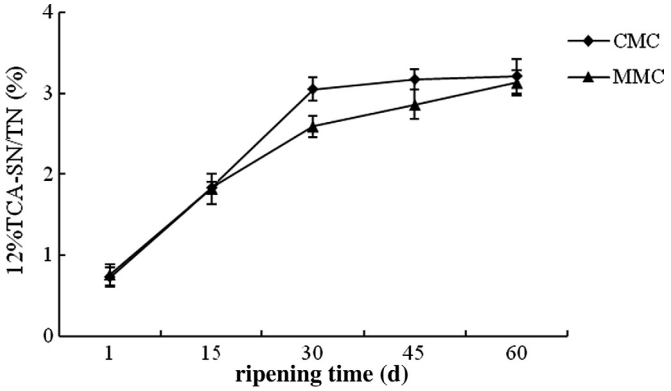

Figure 2. Changes in 12% TCA-SN of CMC and MMC during ripening.

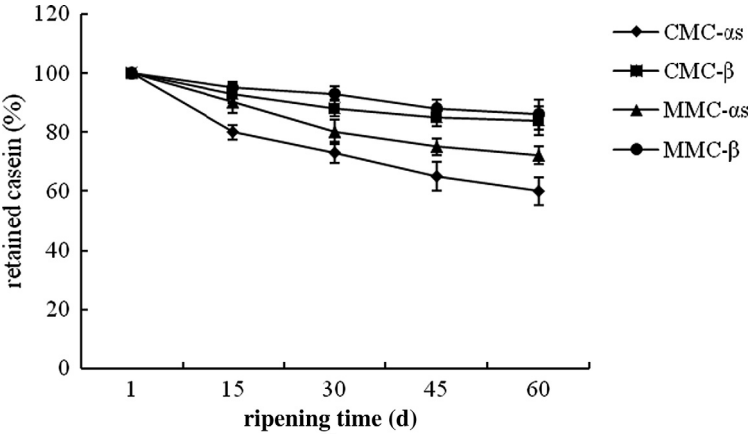

Figure 3. Changes in the major casein of CMC and MMC during ripening.

3.5. Oiling off

The degree of oiling off, shown in Figure 4, was increasing with aging with a highly positive correlation (R^2 = 0.98) with the indicators of proteolysis. It also exhibited constant significant differences (one-way analysis of variance (ANOVA) of the results) between the two treatments (control and MF) with a greater release in MMC in which the fat material appeared to be less entrapped. These results were consistent with the results of Pastorino et al. [29] who found that a higher calcium content of milk used to make a low-moisture part-skim Mozzarella cheese decreased the meltability. They also confirmed the observations of Maubois and Kosikowski [23] who have shown the necessity of adjusting the level of colloidal calcium in the cheese curd to obtain satisfactory stretching and melting properties. Indeed, the distribution of casein-associated calcium within the casein matrix and the cheese juice is a key factor that modulates the structure of

Table IV. Mean square and probabilities for indices of proteolysis changes in Mozzarella cheese during ripening.

Factor		pH 4.6-SN		12% TCA-SN		α_s-CN		β-CN	
	DF	*P*	Ms	*P*	Ms	*P*	Ms	*P*	Ms
Block	2	0.2504	0.0058	0.2114	0.0051	0.6457	1.2333	0.4279	2.1000
Treatment	1	< 0.0001*	1.6427	< 0.0001*	0.2448	< 0.0001*	480.0000	< 0.0001*	326.7000
Time	4	< 0.0001*	13.8602	< 0.0001*	6.2516	< 0.0001*	1747.7167	< 0.0001*	601.3333
Interaction (treatment × time)	4	< 0.0001*	0.2849	0.0011*	0.0742	< 0.0001*	78.7500	< 0.0001*	38.7000
Error	18								
R^2		0.9988		0.9979		0.9937		0.9855	

Treatments: control and MF.
DF: degree of freedom.
P: probabilities.
Ms: mean square.
* Statistically significant at $P = 0.01$.

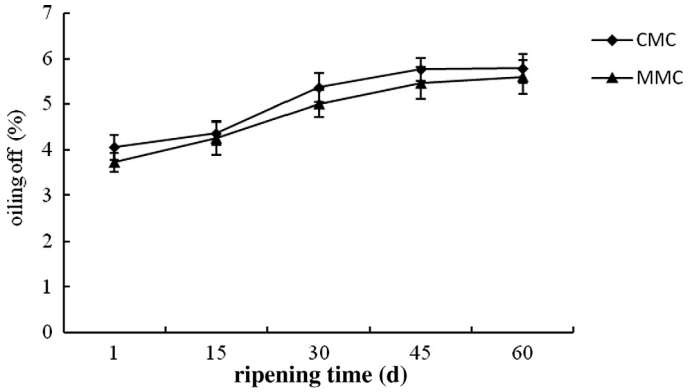

Figure 4. Changes in oiling off of CMC and MMC during ripening.

the casein matrix and consequently the entrapment of the fat as well as the solvation of casein and the stretching ability of the casein network [20].

3.6. Texture

The changes in the three textural parameters determined during ripening of both cheeses (hardness, springiness, and cohesiveness) are shown in Figure 5 (data are the mean values ± standard deviation).

The hardness of MMC was always higher than that of CMC, but the differences between the two cheeses appeared to decrease with aging. Both cheeses showed a decrease with ripening time. Significant hardness changes in CMC were found during the first 15 days, whereas that of MMC significantly decreased during the first 30 days of aging ($P < 0.05$). Then, no significant decrease was observed for both cheeses up to 60 days of ripening. The springiness of both cheeses did not change

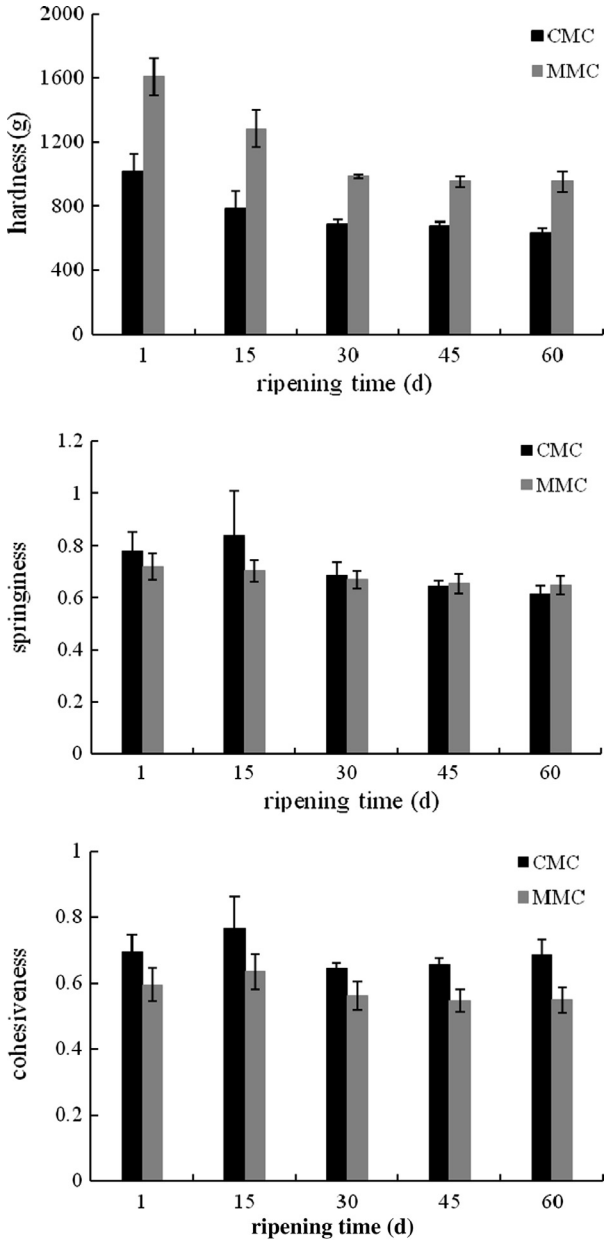

Figure 5. Changes in the texture of CMC and MMC during ripening.

significantly during the 60-day storage time, but an increase took place for CMC at 15 days (Fig. 5). There was a tendency of springiness values of both cheeses to decrease with ripening time, but no substantial changes were found. As shown

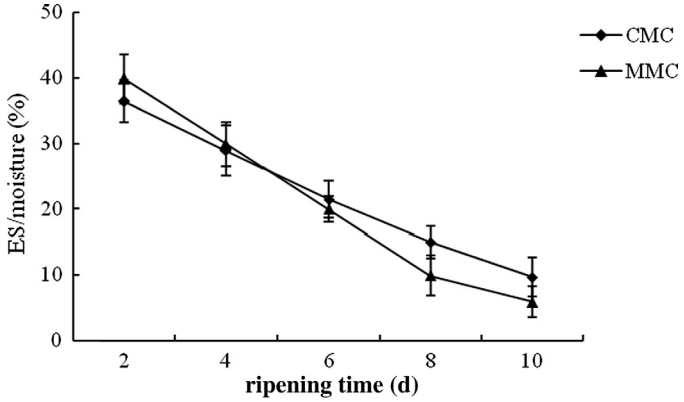

Figure 6. Changes in ES/total moisture during 10-day refrigeration time.

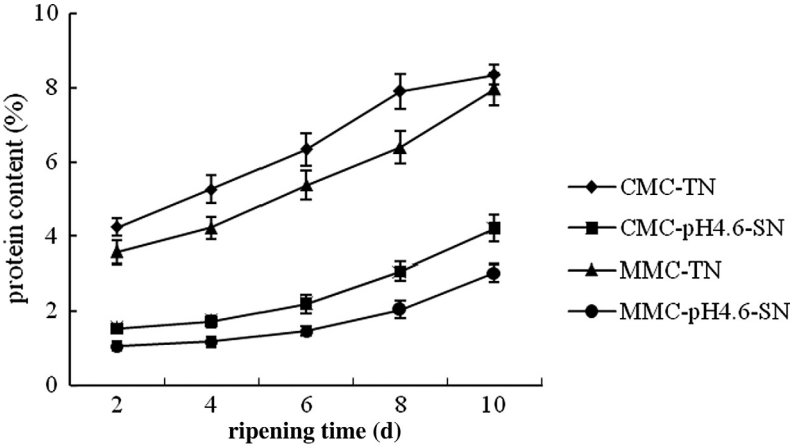

Figure 7. Changes of TN and pH 4.6-SN in ES during 10-day refrigeration time.

in Figure 5, cohesiveness value for CMC and MMC had no obvious changes during 60 days of storage time. This textural attribute showed the lowest inverse relationship with oiling off characteristics ($R^2 = -0.49$).

The textural properties of both cheeses changed during storage time and were influenced by the treatments applied to cheese milks. As hypothesized for the differences observed in proteolysis and oiling off for CMC and MMC, it is likely that the differences in the texture resulted from the initial status of calcium salts in the cheese. It is recognized that calcium plays (at least 50%) a significant role in the functionality (melting and flow properties) and texture of Mozzarella cheese [7, 14–16]. Then, during refrigerated storage, the extent of

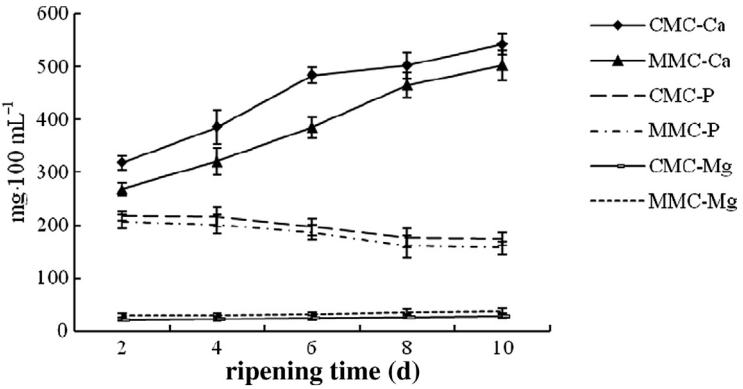

Figure 8. Changes of Ca, Mg, and P in ES during 10-day refrigeration time.

proteolysis as well as the proportion of micellar and soluble calcium will determine the textural characteristics of the cheeses.

3.7. Changes in the composition of ES

Water in cheese is either tightly bound to the protein matrix and, therefore, is unavailable as a solvent or it constitutes the initial aqueous phase of the cheese curd. The amount of aqueous phase extracted ES from Mozzarella cheese during 10-day ripening time is shown in Figure 6. When expressed on the basis of ES/100 g cheese to moisture/100 g cheese, the control cheese declined (from 36.38% to 9.63%) a little more slowly than the MF cheese (from 39.82% to 5.89%) during the 10-day ripening time. By day 10, very little ES was extracted. This trend was similar to that reported by Guo and Kindstedt [9].

TN and pH 4.6-SN contents in ES are shown in Figure 7. The concentration of TN ranged from 3.56% to 4.24% on day 2 and increased to 7.96% and 8.35% by day 10 of ripening time for MMC and CMC, respectively. These values indicate a rapid release of large casein fragments from the curd matrix and are within the range of those observed by De Freitas et al. [4]. The concentration of both nitrogen increased during aging time, but at a lower rate of pH 4.6-SN (1.05–3.02% and 1.55–4.21%) compared to TN (data confirmed by one-way ANOVA) The results obtained agreed with the significant lower extent of proteolysis in MMC.

The Ca, Mg, and P contents of ES in CMC and in MMC during ripening time are shown in Figure 8. During storage time, there was a little decrease of P in ES for both cheeses indicating some calcium phosphate salt insolubilization as seen by De Freitas et al. [4]. The concentration of Ca and Mg increased for both ES during the aging time. The results obtained for Ca in MMC strongly confirms the hypothesis of a higher buffering capacity in their aqueous phase and the consequences for proteolysis and oiling off. Nevertheless, further research is required for a deeper investigation of the components present in ES during aging, such as the identification of peptides and other nitrogen components, minerals, and residual lactose.

4. CONCLUSIONS

The use of control and MF cheese milks for manufacturing low-moisture Mozzarella

cheese was studied. This work demonstrates that the 2.3 times the increase in micellar casein concentration of cheese milk allows the production of cheese that has satisfactory characteristics. The protein recovery in MMC was higher than that in CMC, but the fat recovery was a little lower. The primary proteolysis in MMC was a little higher than that in CMC, but the opposite was observed in the level of secondary proteolysis. There were significant differences in oiling off between CMC and MMC by aging for 5–6 weeks, likely because the structuration of fat material in the respective cheese milks and in the resulting cheeses was not similar. The differences in physicochemical and functional attributes in both cheese and ES between CMC and MMC were ascribed to intrinsic differences in casein and bound micellar Ca of 0.1-μm MF cheese milk. As it was for cheese made from UF cheese milk, the adjustment of technological parameters is required for obtaining the right proportion of total and soluble calcium salts as well as a good structuration of fat in the curd matrix.

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