Effect of whey pH at drainage on physicochemical, biochemical, microbiological, and sensory properties of Mozzarella cheese made from buffalo milk during refrigerated storage

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ABSTRACT

The objective of this research was to determine the effect of drainage pH on physicochemical, biochemical, microbiological and sensory properties of Mozzarella cheese made from buffalo milk during refrigerated storage. Four vats of cheese were made at 4 different whey drainage pH (6.2, 5.9, 5.6, and 5.2). Lower drainage pH caused higher pH 4.4-soluble N and pH 4.4-soluble N:total N. Interaction of drainage pH at d 1 and 30 of storage on all soluble nitrogen fractions was significant. Degradation of caseins in samples made at a drainage pH of 6.2 was lower than that of other cheese samples. The decreasing whey drainage pH significantly increased counts of thermophilic and mesophilic lactobacilli of the samples during refrigerated storage. No coliforms or *Escherichia coli* were detected in the cheeses. The average sensory property scores of all cheese samples were very close, and, as expected, storage time had a negative effect on all sensory scores.

Key words: Mozzarella cheese, drainage, pH, proteolysis

INTRODUCTION

Cheese pH directly influences the structural and rheological properties, presumably because chemical interactions between key structural components are pH-dependent (Lawrence et al., 1987). The pH is particularly important in Mozzarella and other pasta filata cheeses because it influences the ability of the curd to plasticize and stretch in hot water (Kimura et al., 1992). The total calcium content of the curd, which is controlled mainly by the pH of the milk at setting and that of the curd at whey drainage, determines the curd pH at which plasticization is possible. In the normal manufacture of Mozzarella, the milk is typically set at pH 6.55, the whey is drained at pH 6.15, and the ideal pH for plasticizing cheddared curd is about 5.15

(Fox et al., 2000). Metzger et al. (2001a) reported that preacidification reduced the total calcium content of low-fat Mozzarella cheese and given equal milk pH at rennet addition and equal pH at whey draining, citric acid caused larger reductions in cheese calcium content than did acetic acid. In conventional manufacture of pasta filata cheeses, the curd pH is allowed to decrease to a value typically in the range of approximately 5.4 to 5.1 before plasticization. The reduction in curd pH promotes several physicochemical conditions that are conducive to the flow of the curd during plasticization: solubilization of micellar calcium phosphate, an increase in the ratio of soluble to colloidal Ca, and an increase in para-casein hydration (Guinee et al., 2002). The amount of water-insoluble calcium may be more important than total calcium with respect to unmelted cheese hardness and postmelt chewiness (Metzger et al., 2001b). In general, reducing the pH of cheese from 5.8 to 5.4 increases the ratio of soluble to colloidal calcium, an occurrence that would be expected to increase the degree of casein hydration. Moreover, the hydration of para-casein in dilute model systems increases with decreasing pH in the range of 6 to 5.3 (Sheehan and Guinee, 2004).

Drainage is an important step in cheese making. The pH of the whey is related to the pH of the cheese curd in the whey, but the curd pH is usually slightly lower than the whey pH. Whey pH is more commonly measured for routine quality control because a representative sample of whey is easier to obtain than a sample of curd at this point in cheesemaking. Variations in draining pH can also affect the retention of coagulant in the curd (Holmes et al., 1977). Mozzarella cheese undergoes proteolytic changes during refrigerated storage. These proteolytic changes affect the functional properties, such as melting and stretching characteristics. Draining pH is, therefore, one of the manufacturing variables that can affect the quality of Mozzarella cheese (Yun et al., 1995). Several studies have reported on the effects of different pH values on the functional properties of Mozzarella cheeses made from cow's milk (Yun et al., 1993; Joshi et al., 2002, 2004). A limited number of

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studies exist on water buffalo milk Mozzarella cheeses (Ghosh and Singh, 1996; Mauriello et al., 2003), and no research has been done on the effect of acid development by starter culture on the physicochemical and biochemical properties of buffalo Mozzarella cheeses.

The purpose of this investigation was to examine the effect of draining pH on physicochemical, biochemical, microbiological, and sensory properties of Mozzarella cheese during storage.

MATERIALS AND METHODS

Materials

Water buffalo milk was obtained from farmers in Kizilirmak Delta in Bafra, Turkey. Thermophilic cheese culture consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (TCC-4, DVS) and calf rennet (Naturen Standard Plus 175) were obtained from Peyma Chr. Hansen (Istanbul, Turkey). Mozzarella cheeses were manufactured at the dairy plant of the Food Engineering Department, Faculty of Engineering, Ondokuz Mayıs University (Samsun, Turkey).

Cheese Manufacture

The procedure followed by Yazici and Akbulut (2007) was used for the production of Mozzarella cheeses. Four vats of Mozzarella cheese were made on the same day using water buffalo milk. The manufacturing procedures for the 4 cheeses differed with respect to the pH of the milk at starter addition, the pH of the milk at setting (rennet addition), and the pH of the curd at whey drainage (Figure 1). Skim milk was obtained using a cream separator (4500 rpm) and then whole milk (6.8% fat) and skim milk (0.5% fat) were recombined to achieve 4.8% fat content. Then, the buffalo milk (4.8% fat) was heated at 72°C for 2 to 3 s, and divided into stainless steel-jacketed vats after cooling to 37°C. The buffalo milk in each vat was inoculated with the thermophilic starter culture. After ripening to the desired pH for each vat (6.4, 6.2, 5.9, and 5.6), the milk was cooled to 32°C and set. The coagulum was cut when it attained firmness sufficient to withstand cutting. The curd/whey mixture in each vat was allowed to heal and heated to 37°C until the pH of the curd decreased to the desired pH. After whey drainage, the curds were cheddared until pH 5.2 and milled. The milled curd was dry salted at a level of 2.5% (wt/wt) and hand stretched in 82°C water for a period of 10 min (2.5 kg of water/kg of curd) and then placed in 2-kg stainless steel molds. After cooling to room temperature for 6 h, the molds were refrigerated at 4°C for 12 h, vacuum packed, and

stored at 4°C. Cheese making was replicated 3 times for a total of 12 vats.

Physicochemical Analyses

Cheese samples were analyzed for pH, fat, total nitrogen (TN), salt (Mohr method), and total solids according to the methods described by Bradley et al. (1992). The pH was measured on cheese slurry prepared from 10 g of cheese and 10 g of distilled water (Ghosh and Singh, 1996). Acidity of cheese was determined by a titrimetric method (AOAC, 2000). Salt contents of stretchwater were determined according to the Mohr method (Bradley et al., 1992).

Biochemical Analyses

Each cheese sampled was analyzed to evaluate proteolysis during maturation. For this evaluation, the nitrogen content of cheese fractions was determined following the method of Ardö and Polychroniadou (1999). The citrate buffer extract was obtained by adding 50 mL of 0.5 M sodium citrate to 10 g of cheese. The mix was stirred to homogeneity using a stomacher (1 h) and made up to 200 mL with distilled water.

The pH 4.4-soluble N (SN) fraction was obtained by adding 16 mL of 1 M HCl to 80 mL of the citrate buffer extract. The pH was adjusted to 4.4 with 1 M HCl, made up to 100 mL with distilled water and centrifuged ($3,000 \times g$ for 30 min at 4°C); pH 4.4-SN was determined in duplicate in 5 mL of the supernatant.

The 12% TCA-SN fraction was obtained by adding 50 mL of 24% TCA to 50 mL of the pH 4.4-SN fraction and left overnight (4°C). It was then centrifuged ($3,000 \times g$ for 30 min at 4°C) and 12% TCA-SN was determined in duplicate in 10 mL of the supernatant.

The 2.5% phosphotungstic acid (PTA)-SN fraction was obtained by adding 25 mL of 10% PTA to 40 mL of the citrate buffer extract and made up to 100 mL with 25% H₂SO₄. It was left overnight (4°C) and then filtered (Whatman 542); 2.5% PTA-SN was determined in duplicate in 10 mL of the filtrate.

SDS-PAGE

The procedure used by Yazici and Dervisoglu (2003) was followed to prepare the cheese samples and SDS-PAGE gels as well as for the electrophoresis running conditions and evaluation of the electrophoretograms. Modifications were that the final protein concentration in the sample preparation was 0.3% instead of 0.25%. Scanning evaluation of electrophoretograms was performed using an Avegene densitometer (Pacific

Figure 1. Mozzarella cheese manufacturing process.

Image Electronics Co. Ltd., Taipei, Taiwan). The reference standards illustrate the positions of the α_s - and β -casein fractions. γ -Caseins and degradation products (with intermediate and low molecular weight) were tentatively identified according to Marshall et al. (1988), Daigle et al. (1999), Umpierrez et al. (1999), Metzger et al. (2000), Park (2001), and Attaie (2005). Total Lab 100 software (Nonlinear Dynamics Ltd., Newcastle upon Tyne, UK) was used to determine the area and intensity of each band on the resulting gel film. The product of these values was then used to calculate the band density.

Microbiological Analyses

Microbiological analyses were performed using the following media and conditions: plate count agar (Merck, Darmstadt, Germany) for aerobic mesophilic bacteria at 30°C for 48 h (Houghtby et al., 1992) and for psychrotrophic bacteria at 7°C for 10 d (Frank et al., 1992); Rogosa agar (Merck) acidified with acetic acid to pH 5.4 for mesophilic (30°C for 48 h) and thermophilic lactobacilli at 42°C for 48 h under anaerobiosis; M17 agar (Merck) for mesophilic (30°C for 48 h) and thermophilic lactococci at 42°C for 48 h (Dahl et al., 2000; Gobbetti et al., 2002); violet red bile agar (Merck) for coliform bacteria at 35°C for 24 h (Christen et al., 1992); yeast glucose chloramphenicol agar (Merck) for yeasts and molds at 30°C for 72 h (Manolopoulou et al., 2003); and eosin methylene blue (Merck) agar for *Escherichia coli* at 35°C 24 h (Vanderzant and Splittstoesser, 1992).

Sensory Analyses

Cheese samples were organoleptically examined by a group of 8 to 12 panelists at the University of Ondokuz Mayıs Food Engineering Department, according to the method modified from Coppola et al. (1990), with maximum scores of 5, 5, and 10 for appearance and color; body and texture; and flavor, respectively. Panelists were asked to note any of the following defects: unnatural color, dull color, and uneven surface for appearance and color; rough, lack of stretching, pasty, sticky, and hard and soft for body and texture; and salty, rancid, lack of flavor, cooked flavor, and unclean attributes for flavor.

Statistical Analysis

Statistical analysis of data for effects of the factors on pH, acidity, total solids, protein, salt, SN fractions, microbiological analyses, and sensory properties was performed by one-way and 2-factor randomized complete block design using SPSS 12.0 statistical software

(SPSS Inc., Chicago, IL). The factors were cheese type (whey drainage pH 6.4, 6.2, 5.9, and 5.6) and storage time (1, 30, and 60 d). The mean differences were analyzed using Tukey multiple-range test at the 5% significance level.

RESULTS AND DISCUSSION

Physicochemical Properties

The composition of the cheeses is summarized in Table 1. The pH values of all samples declined significantly throughout storage ($P < 0.05$). Compared with the other cheeses, the samples with whey draining pH 6.4 had a significantly higher pH values. The pH values of samples with whey draining pH 6.2, 5.9, and 5.6 on d 30 and 60 were not significantly different ($P > 0.05$). Titratable acidity was not significantly affected by storage time ($P > 0.05$). Total nitrogen was not significantly affected by storage time ($P > 0.05$). Samples with whey draining pH 6.4 and 6.2 were not significantly different during storage ($P > 0.05$).

Table 2 shows the results of the total solids, fat, fat in DM, and salt contents of Mozzarella cheese samples as determined on d 1 of storage. Whey draining pH 6.4 samples showed higher total solids content. This result is agreement with the results of previous studies (Yun et al., 1995; Sheehan and Guinee, 2004). Whey draining pH 5.6 samples had a significantly higher salt content than the other cheeses ($P < 0.05$).

SN Fractions

Table 3 shows the results of the evaluation of proteolysis by analysis of the nitrogen content in fractions of a citrate dispersion of cheese samples, taken at 3 stages throughout storage. The levels of all SN fractions generally increased in the cheeses during storage. The ratio of SN to TN (pH 4.4-SN/TN) is considered an index of the extent of proteolysis in cheese. The pH 4.4-SN fraction included all the nitrogen components of cheese (soluble proteins, peptides, amino acids, amines, urea, ammonia) but excluded all the caseins and their primary large breakdown products that precipitate at pH 4.4 (molecular mass $>15,000$ Da). The SN compounds are mainly produced by the action of the coagulant (Roseiro et al., 2003). In generally, lower draining pH caused higher pH 4.4-SN and pH 4.4-SN/TN levels in our cheeses. This effect was also observed by other authors for Mozzarella (Yun et al., 1995; Sheehan and Guinee, 2004), and our results were similar to the results obtained by these authors.

The 12% TCA-SN fraction contains small peptides between 2 to 20 amino acid residues as well as free

Table 1. Effect of whey pH at drainage on chemical composition (means $\pm$ SD)

Property	Whey draining pH	Storage time (d)		
		1	30	60
pH	6.4	5.2 $\pm$ 0.01 ^{a,B}	5.29 $\pm$ 0.03 ^{a,A}	5.17 $\pm$ 0.03 ^{a,B}
	6.2	5.18 $\pm$ 0.02 ^{ab,A}	5.13 $\pm$ 0.02 ^{b,A}	4.96 $\pm$ 0.05 ^{b,B}
	5.9	5.15 $\pm$ 0.01 ^{b,A}	5.08 $\pm$ 0.06 ^{b,A}	4.88 $\pm$ 0.04 ^{b,B}
	5.6	5.09 $\pm$ 0.02 ^{c,A}	5.07 $\pm$ 0.03 ^{b,A}	4.9 $\pm$ 0.03 ^{b,B}
Titratable acidity (%)	6.4	0.52 $\pm$ 0.06 ^{c,A}	0.54 $\pm$ 0.05 ^{b,A}	0.6 $\pm$ 0.04 ^{b,A}
	6.2	0.62 $\pm$ 0.06 ^{bc,A}	0.68 $\pm$ 0.03 ^{a,A}	0.74 $\pm$ 0.07 ^{a,A}
	5.9	0.71 $\pm$ 0.03 ^{ab,A}	0.71 $\pm$ 0.08 ^{a,A}	0.8 $\pm$ 0.05 ^{a,A}
	5.6	0.8 $\pm$ 0.03 ^{a,A}	0.76 $\pm$ 0.03 ^{a,A}	0.84 $\pm$ 0.05 ^{a,A}
Total N (%)	6.4	3.99 $\pm$ 0.2 ^{a,A}	3.83 $\pm$ 0.28 ^{a,A}	4.18 $\pm$ 0.22 ^{a,A}
	6.2	3.77 $\pm$ 0.1 ^{ab,A}	3.87 $\pm$ 0.09 ^{a,AB}	4.1 $\pm$ 0.18 ^{ab,A}
	5.9	3.53 $\pm$ 0.16 ^{b,A}	3.64 $\pm$ 0.28 ^{a,A}	3.73 $\pm$ 0.11 ^{bc,A}
	5.6	3.52 $\pm$ 0.03 ^{b,A}	3.57 $\pm$ 0.1 ^{a,A}	3.48 $\pm$ 0.02 ^{c,A}

^{a-c}Means with same letters in a column within the category data are not significant at $P > 0.05$.

^{A,B}Means with same letters in a row within the category data are not significant at $P > 0.05$.

amino acids, ammonia, and other minor compounds. These were released mainly by bacterial enzymes (Kuchroo and Fox, 1982; Pavia et al., 2000). The 12% TCA-SN level is regarded as the ripening depth index (Sousa and Malcata, 1997). On the first day of storage, cheese samples did not differ significantly, but at the end of the storage, whey draining pH 5.6 samples showed significantly higher 12% TCA-SN and 12% TCA-SN/TN contents ($P < 0.05$). Yun et al. (1995) reported similar 12% TCA-SN and 12% TCA-SN/TN contents during storage. Cheese with lower draining pH showed more proteolysis (pH 4.4-SN and 12% TCA-SN), which may be due to a greater lactobacilli content of these cheeses. Residual coagulant activity retained in the curd is the major source of proteolytic activity in most cheeses except pasta filata varieties and those with a high cook temperature in which enzymes from this source (chymosin, pepsin, fungal acid proteinases, plant acid proteinases) are denatured extensively. The action of plasmin is of particular importance in pasta filata and high-cook cheeses (because it is a heat-stable enzyme) and in cheeses in which the pH increases during ripening (because the pH optimum of plasmin is approximately 7.5; Upadhyay et al., 2004). We did not measure the amounts of plasmin or any enzyme in the cheese samples. However, pH clearly influences plasmin dissociation from casein micelles, with most or all of the

plasmin activity dissociated at pH 4.6 to 4.7 (Nielsen, 2003). On the other hand, proteolysis in Mozzarella is caused mainly by residual coagulant (Farkye et al., 1991) and enzymes from culture microorganisms (Barbano et al., 1992). The major sources of proteolytic activity observed in Mozzarella cheeses may be residual coagulant, plasmin, or enzymes from the starter culture.

The 2.5% PTA-SN fraction contains very small peptides, amino acids, and smaller nitrogen compounds except dibasic amino acids and ammonia (Ardö, 1999). There was no significant difference among the 2.5% PTA-SN and 2.5% PTA-SN/TN contents of the cheese samples. Similar PTA-SN/TN levels were reported by Sheehan and Guinee (2004) for Mozzarella cheese.

SDS-PAGE

To quantify proteolysis in Mozzarella cheese, densitometric evaluation was done after SDS-PAGE. The mean relative proportions of the casein fractions of 3 trials are presented in Table 4.

Analysis by SDS-PAGE confirmed that α_S -casein decreased significantly for samples with whey draining pH 6.2, 5.9, and 5.6 during storage, but for whey draining pH 6.4 samples the degradation of α_S -casein was significant only on d 60. Electrophoretograms of

Table 2. Effect of whey pH at drainage on total solids, fat, fat in DM, and salt (means $\pm$ SD)

Whey draining pH	Total solids (%)	Fat (%)	Fat in DM (%)	Salt (%)
6.4	53.58 $\pm$ 0.60 ^a	24.17 $\pm$ 0.15 ^a	45.10 $\pm$ 0.34 ^b	0.48 $\pm$ 0.08 ^c
6.2	48.36 $\pm$ 0.93 ^b	21.90 $\pm$ 0.10 ^b	45.25 $\pm$ 1.09 ^b	0.56 $\pm$ 0.06 ^c
5.9	47.07 $\pm$ 0.15 ^{bc}	22.07 $\pm$ 0.06 ^b	46.89 $\pm$ 0.21 ^a	0.70 $\pm$ 0.02 ^b
5.6	45.90 $\pm$ 0.74 ^c	19.90 $\pm$ 0.10 ^c	43.25 $\pm$ 0.51 ^c	0.83 $\pm$ 0.02 ^a

^{a-c}Means with same letters in a column within the category data are not significant at $P > 0.05$.

Table 3. Effect of whey pH at drainage on soluble nitrogen fractions (means $\pm$ SD)

Fraction ¹ (%)	Whey draining pH	Storage time (d)		
		1	30	60
pH 4.4-SN	6.4	0.087 $\pm$ 0.006 ^{c,c,B}	0.207 $\pm$ 0.006 ^{b,A}	0.207 $\pm$ 0.006 ^{d,A}
	6.2	0.123 $\pm$ 0.006 ^{cb,C}	0.223 $\pm$ 0.012 ^{b,B}	0.257 $\pm$ 0.006 ^{c,A}
	5.9	0.207 $\pm$ 0.035 ^{a,B}	0.317 $\pm$ 0.021 ^{a,A}	0.297 $\pm$ 0.006 ^{b,A}
	5.6	0.143 $\pm$ 0.006 ^{b,B}	0.34 $\pm$ 0.036 ^{a,A}	0.373 $\pm$ 0.012 ^{a,A}
pH 4.4-SN/TN	6.4	2.21 $\pm$ 0.11 ^{c,B}	5.37 $\pm$ 0.35 ^{b,A}	4.98 $\pm$ 0.11 ^{d,A}
	6.2	3.2 $\pm$ 0.23 ^{bc,B}	5.79 $\pm$ 0.22 ^{b,A}	6.23 $\pm$ 0.42 ^{c,A}
	5.9	5.93 $\pm$ 1.33 ^{a,B}	8.78 $\pm$ 0.93 ^{a,A}	8.02 $\pm$ 0.08 ^{b,AB}
	5.6	4.08 $\pm$ 0.19 ^{b,B}	9.55 $\pm$ 0.83 ^{a,A}	10.68 $\pm$ 0.18 ^{a,A}
12% TCA-SN	6.4	0.06 $\pm$ 0.01 ^{a,B}	0.13 $\pm$ 0.01 ^{ab,A}	0.15 $\pm$ 0.0 ^{b,A}
	6.2	0.1 $\pm$ 0.02 ^{a,B}	0.14 $\pm$ 0.0 ^{ab,A}	0.15 $\pm$ 0.01 ^{b,A}
	5.9	0.06 $\pm$ 0.01 ^{a,B}	0.11 $\pm$ 0.01 ^{b,B}	0.14 $\pm$ 0.01 ^{b,A}
	5.6	0.08 $\pm$ 0.03 ^{a,B}	0.15 $\pm$ 0.02 ^{a,A}	0.19 $\pm$ 0.01 ^{a,A}
12% TCA-SN/TN	6.4	1.45 $\pm$ 0.19 ^{a,B}	3.34 $\pm$ 0.02 ^{ab,A}	3.56 $\pm$ 0.19 ^{b,A}
	6.2	2.78 $\pm$ 0.46 ^{a,B}	3.61 $\pm$ 0.08 ^{ab,A}	3.48 $\pm$ 0.27 ^{b,AB}
	5.9	1.66 $\pm$ 0.36 ^{a,B}	3.05 $\pm$ 0.35 ^{b,A}	3.66 $\pm$ 0.14 ^{b,A}
	5.6	2.48 $\pm$ 0.86 ^{a,B}	4.07 $\pm$ 0.6 ^{a,A}	5.48 $\pm$ 1.4 ^{a,A}
2.5% PTA-SN	6.4	0.01 $\pm$ 0.0 ^{a,C}	0.018 $\pm$ 0.001 ^{a,B}	0.045 $\pm$ 0.0 ^{b,A}
	6.2	0.01 $\pm$ 0.0 ^{a,C}	0.026 $\pm$ 0.009 ^{a,B}	0.058 $\pm$ 0.005 ^{a,A}
	5.9	0.01 $\pm$ 0.0 ^{a,B}	0.029 $\pm$ 0.009 ^{a,A}	0.036 $\pm$ 0.0 ^{b,A}
	5.6	0.01 $\pm$ 0.0 ^{a,C}	0.019 $\pm$ 0.001 ^{a,B}	0.039 $\pm$ 0.005 ^{b,A}
2.5% PTA-SN/TN	6.4	0.25 $\pm$ 0.02 ^{a,C}	0.47 $\pm$ 0.05 ^{a,B}	1.09 $\pm$ 0.06 ^{b,A}
	6.2	0.27 $\pm$ 0.01 ^{a,C}	0.67 $\pm$ 0.21 ^{a,B}	1.4 $\pm$ 0.09 ^{a,A}
	5.9	0.28 $\pm$ 0.02 ^{a,B}	0.82 $\pm$ 0.27 ^{a,A}	0.98 $\pm$ 0.03 ^{b,A}
	5.6	0.28 $\pm$ 0.01 ^{a,C}	0.52 $\pm$ 0.03 ^{a,B}	1.13 $\pm$ 0.15 ^{b,A}

^{a-d}Means with same letters in a column within the category data are not significant at $P > 0.05$.

^{A-C}Means with same letters in a row within the category data are not significant at $P > 0.05$.

¹SN = soluble nitrogen; TN = total nitrogen; PTA = phosphotungstic acid.

the cheeses at d 60 are shown in Figure 2. On d 1, decreasing whey drainage pH increased the degradation rates of α_S -casein of the samples during refrigerated storage. At the end of storage, samples did not differ significantly. Breakdown of β -casein in whey draining pH 6.2, 5.9, and 5.6 samples was significantly higher than that in whey draining pH 6.4 samples at d 30. At the end of storage, the rate of breakdown of β -casein for whey draining pH 6.4 samples was greatest. There was

no difference among the whey draining pH 6.2, 5.9, and 5.6 cheeses during storage. β -Casein decreased significantly for whey draining pH 6.2, 5.9, and 5.6 samples during the first 30 d but did not change significantly after d 30. β -Casein concentration of whey draining pH 6.4 samples did not change significantly during storage. γ -Caseins and degradation products (with intermediate to low molecular weight) increased significantly during storage. Because γ -caseins are degradation products of

Table 4. Effect of whey pH at drainage on caseins fractions (means $\pm$ SD)

Fraction	Whey draining pH	Storage time (d)		
		1	30	60
α_S -Casein	6.4	40.44 $\pm$ 1.92 ^{a,A}	37.421 $\pm$ 0.25 ^{a,A}	28.43 $\pm$ 1.71 ^{a,B}
	6.2	37.343 $\pm$ 0.14 ^{ab,A}	33.39 $\pm$ 1.18 ^{b,B}	30.31 $\pm$ 2.25 ^{a,B}
	5.9	38.078 $\pm$ 0.98 ^{ab,A}	32.978 $\pm$ 1.56 ^{b,B}	27.11 $\pm$ 0.34 ^{a,C}
	5.6	35.912 $\pm$ 1.08 ^{b,A}	32.38 $\pm$ 0.78 ^{b,B}	28.493 $\pm$ 1.52 ^{a,C}
β -Casein	6.4	39.06 $\pm$ 5.36 ^{a,A}	39.443 $\pm$ 1.34 ^{a,A}	33.2 $\pm$ 1.06 ^{b,A}
	6.2	36.175 $\pm$ 1.74 ^{a,A}	31.668 $\pm$ 1.73 ^{b,B}	29.15 $\pm$ 1.59 ^{b,B}
	5.9	37.74 $\pm$ 1.05 ^{a,A}	31.280 $\pm$ 0.96 ^{b,B}	30.985 $\pm$ 0.17 ^{ab,B}
	5.6	36.252 $\pm$ 1.15 ^{a,A}	30.637 $\pm$ 0.78 ^{b,B}	29.027 $\pm$ 1.62 ^{b,B}
γ -Caseins and other degradation products	6.4	20.505 $\pm$ 1.73 ^{b,B}	23.137 $\pm$ 1.14 ^{b,B}	38.37 $\pm$ 1.72 ^{a,A}
	6.2	26.48 $\pm$ 1.88 ^{a,B}	34.942 $\pm$ 2.9 ^{a,A}	40.438 $\pm$ 1.74 ^{a,A}
	5.9	24.183 $\pm$ 0.16 ^{ab,C}	35.743 $\pm$ 2.48 ^{a,B}	41.903 $\pm$ 0.18 ^{a,A}
	5.6	27.833 $\pm$ 1.62 ^{a,C}	36.987 $\pm$ 1.15 ^{a,B}	42.482 $\pm$ 2.7 ^{a,A}

^{a,b}Means with same letters in a column within each fraction are not significant at $P > 0.05$.

^{A-C}Means with same letters in a row within each fraction are not significant at $P > 0.05$.

Figure 2. Sodium dodecyl sulfate-PAGE electrophoretograms of the cheeses at d 60. Color version available in the online PDF.

β -casein (Hassan and El-Deeb, 1988; Marshall et al., 1988; Mayer, 1996, 1997), increases in γ -caseins during the storage period parallel the degradation of β -casein. Because α_s -casein and β -casein in whey draining pH 6.4 samples did not change in the first 30 d, γ -caseins and degradation products did not increase significantly during this period. γ -Caseins and degradation products in whey draining pH 6.2, 5.9, and 5.6 samples were significantly higher than whey draining pH 5.6 samples at first 30 d.

Microbiological Properties

Table 5 shows the results of the microbial counts for the samples. Numbers of psychrotrophic bacteria had a tendency to increase during d 1 to 30 but then decrease after 30 d. Yeast and mold counts showed a significant increase during the first 30 d of storage but no significant change ($P > 0.05$) occurred after this period. Our mean results for yeasts and molds were lower than other reported values (Coppola et al., 1988, 1990). On d 1 of storage, samples did not differ significantly.

Decreasing the whey pH value at drainage significantly increased counts of mesophilic lactobacilli in the samples ($P < 0.05$). Mesophilic lactobacilli counts range from 4.81 to 9.03 log cfu/g (De Angelis et al., 2008) and from 2 to 8.4 log cfu/g (De Candia et al., 2007) in traditional Mozzarella cheeses. Our results are similar to those found in these studies.

Numbers of thermophilic lactobacilli ranged from 3.33 to 7.2 log cfu/g in our cheeses. Decreasing whey drainage pH significantly increased thermophilic lactobacilli counts of the samples during refrigerated storage ($P < 0.05$). Similar results were reported by De Candia et al. (2007) and De Angelis et al. (2008). The mean

thermophilic lactobacilli counts of our samples were lower than that reported by Coppola et al. (1988).

In general, mesophilic and thermophilic lactococci counts of cheeses were significantly different on d 30 and d 60 of storage ($P < 0.05$). Counts of thermophilic lactococci decreased slightly throughout 60 d of storage probably because of the decrease in whey draining pH. Mesophilic and thermophilic lactococci counts in our study were similar to those found in Mozzarella cheese (Coppola et al., 1988) and higher than those reported by De Angelis et al. (2008) in Mozzarella cheeses made by using commercial starter.

Escherichia coli and total coliforms were not detected in any of the cheese samples during refrigerated storage. This proves that the necessary hygienic conditions were applied in the manufacturing process.

Sensory Evaluation

The results of sensory evaluation of Mozzarella cheese obtained using different whey draining pH are shown in Table 6. The average flavor scores of all cheese samples were not significantly different ($P > 0.05$). Storage time had a negative effect on flavor scores, and all scores significantly decreased during storage ($P < 0.05$), which was expected because Mozzarella cheese is a nonaged cheese.

The defect most frequently observed in all samples was lack of flavor. The average appearance and texture scores of cheeses were not significant ($P > 0.05$) and did not decline significantly throughout storage ($P > 0.05$). However, texture scores of whey draining pH 5.6 cheeses significantly decreased during the first 30-d period but were not significantly changed ($P > 0.05$) after this period. Based on sensory properties, it can be

Table 5. Effect of whey pH at drainage on microbiological properties (log cfu/g; means $\pm$ SD)

Property	Whey draining pH	Storage time (d)		
		1	30	60
Psychrotrophic bacteria	6.4	4.79 $\pm$ 0.21 ^{a,B}	6.08 $\pm$ 0.36 ^{a,A}	5 $\pm$ 0.4 ^{a,B}
	6.2	1.91 $\pm$ 1.65 ^{b,B}	5.12 $\pm$ 0.29 ^{b,A}	3.82 $\pm$ 0.71 ^{a,AB}
	5.9	4.83 $\pm$ 0.08 ^{a,A}	5.22 $\pm$ 0.47 ^{ab,A}	5.12 $\pm$ 1.07 ^{a,A}
	5.6	4.88 $\pm$ 0.13 ^{a,A}	5.16 $\pm$ 0.14 ^{b,A}	5.03 $\pm$ 0.47 ^{a,A}
Yeasts and molds	6.4	1.62 $\pm$ 1.32 ^{a,A}	3.92 $\pm$ 0.16 ^{a,A}	2.92 $\pm$ 1.45 ^{b,A}
	6.2	1.26 $\pm$ 1.14 ^{a,B}	3.97 $\pm$ 0.02 ^{a,A}	3.12 $\pm$ 0.39 ^{ab,A}
	5.9	<1 ^{a,B}	3.52 $\pm$ 0.36 ^{a,A}	2.93 $\pm$ 0.3 ^{b,A}
	5.6	<1 ^{a,B}	4.01 $\pm$ 0.39 ^{a,A}	4.39 $\pm$ 0.78 ^{a,A}
Mesophilic lactobacilli	6.4	5.13 $\pm$ 0.68 ^{b,B}	6.26 $\pm$ 0.36 ^{a,AB}	6.52 $\pm$ 0.25 ^{a,A}
	6.2	4.58 $\pm$ 0.11 ^{b,A}	5.27 $\pm$ 0.42 ^{ab,A}	4.58 $\pm$ 0.34 ^{b,A}
	5.9	7.09 $\pm$ 0.12 ^{a,A}	4.84 $\pm$ 0.83 ^{b,B}	6.13 $\pm$ 0.27 ^{a,A}
	5.6	7.53 $\pm$ 0.45 ^{a,A}	5.02 $\pm$ 0.11 ^{ab,B}	5.72 $\pm$ 0.42 ^{a,B}
Thermophilic lactobacilli	6.4	4.21 $\pm$ 0.26 ^{b,A}	4.04 $\pm$ 0.65 ^{b,A}	3.33 $\pm$ 0.2 ^{c,A}
	6.2	4.47 $\pm$ 0.0 ^{b,A}	3.9 $\pm$ 0.45 ^{b,A}	4.12 $\pm$ 0.13 ^{b,A}
	5.9	6.96 $\pm$ 0.11 ^{a,A}	6.76 $\pm$ 0.37 ^{a,A}	6.69 $\pm$ 0.08 ^{a,A}
	5.6	7.2 $\pm$ 0.14 ^{a,A}	7 $\pm$ 0.11 ^{a,AB}	6.79 $\pm$ 0.01 ^{a,B}
Mesophilic lactococci	6.4	8.68 $\pm$ 0.72 ^{a,A}	6.79 $\pm$ 0.95 ^{ab,B}	6.78 $\pm$ 0.24 ^{b,B}
	6.2	8.21 $\pm$ 0.18 ^{a,A}	5.76 $\pm$ 0.68 ^{b,B}	7.27 $\pm$ 0.63 ^{ab,A}
	5.9	8.24 $\pm$ 0.11 ^{a,A}	7.32 $\pm$ 0.37 ^{ab,B}	7.55 $\pm$ 0.13 ^{a,B}
	5.6	8.76 $\pm$ 0.12 ^{a,A}	7.44 $\pm$ 0.13 ^{a,B}	7.88 $\pm$ 0.13 ^{a,C}
Thermophilic lactococci	6.4	8.96 $\pm$ 0.17 ^{a,A}	8.13 $\pm$ 0.14 ^{a,B}	6.87 $\pm$ 0.38 ^{b,C}
	6.2	8.66 $\pm$ 0.5 ^{a,A}	7.71 $\pm$ 0.31 ^{a,B}	7.65 $\pm$ 0.1 ^{ab,B}
	5.9	7.75 $\pm$ 0.95 ^{a,A}	7.7 $\pm$ 0.54 ^{a,A}	7.38 $\pm$ 0.64 ^{ab,A}
	5.6	7.73 $\pm$ 0.41 ^{a,A}	7.67 $\pm$ 0.58 ^{a,A}	7.93 $\pm$ 0.24 ^{a,A}
Total coliforms	6.4	<1	<1	<1
	6.2	<1	<1	<1
	5.9	<1	<1	<1
	5.6	<1	<1	<1
<i>Escherichia coli</i>	6.4	<1	<1	<1
	6.2	<1	<1	<1
	5.9	<1	<1	<1
	5.6	<1	<1	<1

^{a-c}Means with same letters in a column within each property are not significant at $P > 0.05$.^{A-C}Means with same letters in a row within each property are not significant at $P > 0.05$.**Table 6.** Effect of whey pH at drainage on sensory properties (means $\pm$ SD)

Property ¹	Whey draining pH	Storage time (d)		
		1	30	60
Flavor	6.4	7.53 $\pm$ 0.57 ^{a,A}	6.66 $\pm$ 0.4 ^{a,A}	6.5 $\pm$ 0.78 ^{a,A}
	6.2	7.57 $\pm$ 0.35 ^{a,A}	6.43 $\pm$ 0.35 ^{a,B}	6.37 $\pm$ 0.29 ^{a,B}
	5.9	8.03 $\pm$ 0.21 ^{a,A}	6.83 $\pm$ 0.15 ^{a,B}	6.37 $\pm$ 0.29 ^{a,B}
	5.6	8.13 $\pm$ 0.35 ^{a,A}	7.40 $\pm$ 0.61 ^{a,A}	5.57 $\pm$ 0.61 ^{a,B}
Appearance	6.4	2.5 $\pm$ 2.23 ^{a,A}	2.27 $\pm$ 0.11 ^{a,A}	2.57 $\pm$ 0.15 ^{a,A}
	6.2	2.23 $\pm$ 0.06 ^{a,A}	2.07 $\pm$ 0.42 ^{a,A}	2.23 $\pm$ 0.06 ^{ab,A}
	5.9	2.5 $\pm$ 0.2 ^{a,A}	2 $\pm$ 0.36 ^{a,A}	2.13 $\pm$ 0.21 ^{ab,A}
	5.6	2.33 $\pm$ 0.15 ^{a,A}	1.97 $\pm$ 0.35 ^{a,A}	2.03 $\pm$ 0.21 ^{b,A}
Texture	6.4	4 $\pm$ 0.26 ^{a,A}	3.43 $\pm$ 0.4 ^{a,A}	3.63 $\pm$ 0.25 ^{a,A}
	6.2	4 $\pm$ 0.2 ^{a,A}	3.4 $\pm$ 0.36 ^{a,A}	3.63 $\pm$ 0.12 ^{a,A}
	5.9	4.1 $\pm$ 0.26 ^{a,A}	3.53 $\pm$ 0.46 ^{a,A}	3.5 $\pm$ 0.36 ^{a,A}
	5.6	4.13 $\pm$ 0.06 ^{a,A}	3.33 $\pm$ 0.25 ^{a,B}	3.2 $\pm$ 0.46 ^{a,B}
General acceptability	6.4	8 $\pm$ 0.82 ^{a,A}	7.17 $\pm$ 0.35 ^{a,A}	6.97 $\pm$ 0.81 ^{a,A}
	6.2	7.63 $\pm$ 0.12 ^{a,A}	7 $\pm$ 0.26 ^{a,B}	6.67 $\pm$ 0.25 ^{a,B}
	5.9	8.2 $\pm$ 0.7 ^{a,A}	7.43 $\pm$ 0.51 ^{a,AB}	6.67 $\pm$ 0.23 ^{a,B}
	5.6	8.2 $\pm$ 0.36 ^{a,A}	7.5 $\pm$ 0.3 ^{a,A}	5.57 $\pm$ 0.70 ^{a,B}

^{a,b}Means with same letters in a column within each property are not significant at $P > 0.05$.^{A,B}Means with same letters in a row within each property are not significant at $P > 0.05$.¹Maximum scores of 5, 5, and 10 for appearance and color, body and texture, and flavor, respectively.

concluded that whey draining pH 5.9 and 5.6 samples were preferred on d 1 and 30 of storage.

CONCLUSIONS

Drainage pH affected cheese proteolysis. Lower drainage pH caused higher pH 4.4-SN and pH 4.4-SN/TN. Degradation of caseins in whey draining pH 5.6 samples was greater than in other cheese samples. Draining pH caused increases in thermophilic and mesophilic lactobacilli counts of samples during refrigerated storage. Based on sensory properties, it can be concluded that whey draining pH 5.9 and 5.6 cheeses were preferred on d 1 and d 30.

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