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Impact of Whey pH at Drainage on the Physicochemical, Sensory, and Functional Properties of Mozzarella Cheese Made from Buffalo Milk

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In this study, the effects of whey pH at drainage on the physicochemical, sensory, and functional properties of mozzarella cheese made from buffalo milk during storage were investigated. Four cheese samples were manufactured using starter culture at different whey pH values [(A) 6.2, (B) 5.9, (C) 5.6, and (D) 5.3] and analyzed on the 1st, 28th, and 56th day. Ash, calcium, and phosphorus concentrations decreased as the whey pH at drainage was lowered. Cheese yield and calcium recovery were the highest in D cheeses. During storage, expressible serum levels decreased and nonexpressible serum levels increased, indicating an increase in the water holding capacity of the cheeses. Reducing the calcium content of cheeses increased meltability values, but an overly low calcium level (D cheeses) had an adverse effect on the meltability. The melting properties of cheese samples, except D cheeses, were improved with aging. A cheeses were the hardest and D cheeses the softest throughout storage. The 1st day sensory evaluations revealed that C and D cheeses were preferred and that A cheeses were not. All sensory properties of A cheeses were improved with storage. D cheeses were rated inferior to the others at the end of the storage time.

KEYWORDS: Mozzarella cheese; calcium; whey draining pH; storage

INTRODUCTION

Mozzarella cheese is one of the most common types of pasta filata cheeses in Italy, mainly used on pizza, and is customarily made from high-fat water buffalo milk, which provides its specific characteristics. Pasta filata cheeses are well-known for an exceptional plasticizing and kneading treatment of the fresh curd in hot water which passes on to the finished product its characteristic fibrous structure and melting and stretching properties (*1*, *2*).

Mozzarella cheese should form a continuous melt without individual cheese particles during cooking when used as a pizza ingredient (*3*, *4*). Mozzarella cheese is characterized by its shredability, meltability, and shredability with little pronounced flavor. Several factors such as milk pretreatments, cheese making procedures, composition, pH, and storage influence the functional properties of mozzarella cheese (*3*, *5*). The unique functional properties of mozzarella cheese are supposed to develop in two codependent phases. The first phase occurs during manufacture when the basic curd structure is established. The second one takes place during storage when functionality and curd structure are altered (*4*). pH is one of the major manufacturing parameters that plays an important role in the stretching and kneading ability of curd in hot water during cheese making, affecting the functional properties of cheese. Reducing the pH during cheese making is reported to increase the level of loss of calcium from the curd and increases the extent of fusion of para casein particles (*3*). Calcium plays an integral role in cheese texture by cross-linking protein. If the total calcium content of cheese is reduced, then the amount of cross-linking between casein polymers is reduced and the cheese becomes softer (*6*). Decreasing the pH of the curd at drainage increases the level of nonmicellar calcium and lowers the calcium content of cheeses (*6*). The curd drained at pH 5.9 had 17% less calcium than the curd drained at pH 6.4 even though both were cheddared to pH 5.2 (*4*).

There are several studies about the effects of different pH's and calcium concentrations on the functional properties of mozzarella cheeses made from cow's milk (*3, 5–18*). pH adjustment can be achieved by a natural or conventional way (starter culture addition) or direct acidification via one acid or a combination of acids such as citric, acetic, and lactic acid, and glucono δ-lactone (*3, 5–13, 19*). Direct acidification is preferred due to the straightforward control of acid development and time saving. In contrast, starter culture addition for acid development does not need any external food additive for this purpose. There are a limited number of studies on water buffalo milk mozzarella cheeses (*20–25*), and there has been no research on the effect of acid development by starter culture on the

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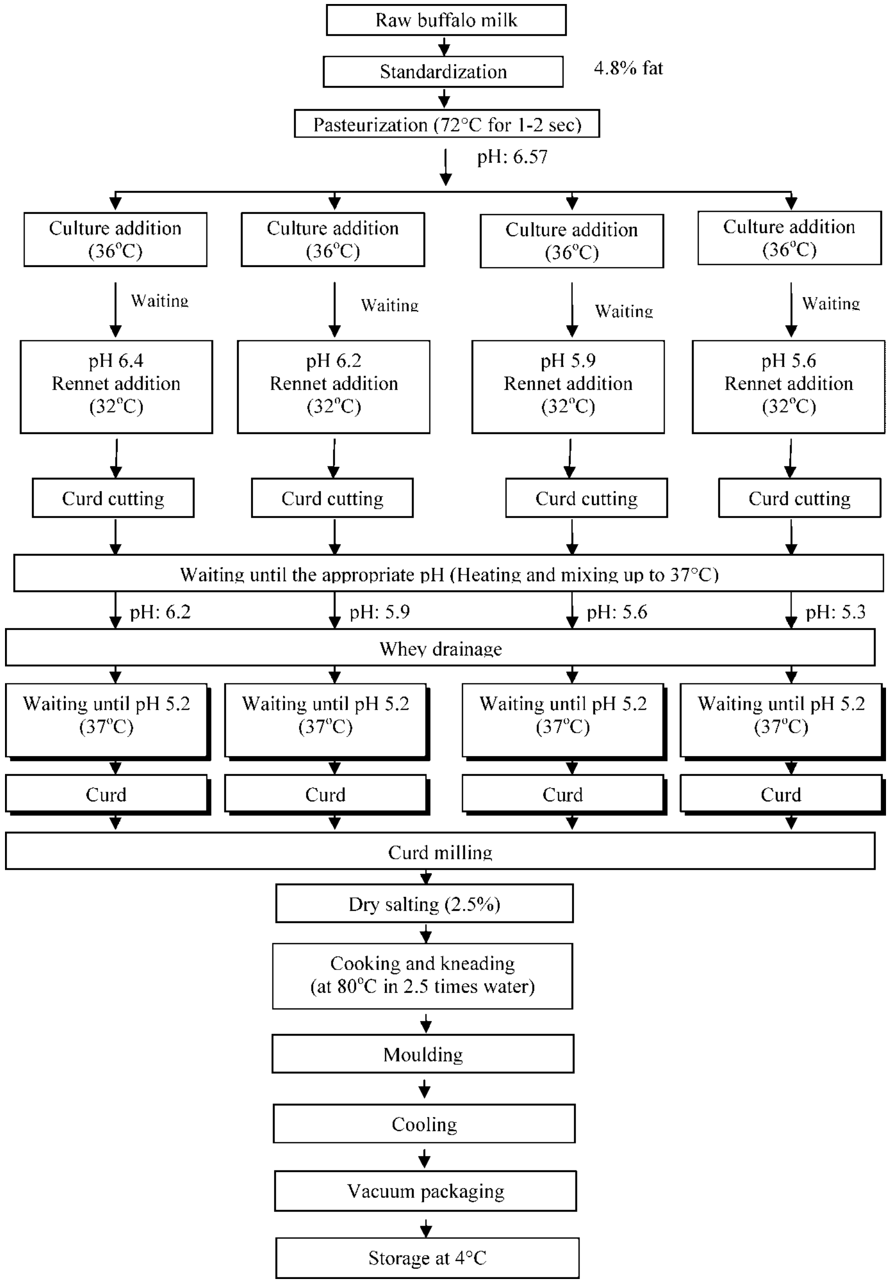

Figure 1. Process for manufacturing mozzarella cheeses.

functional properties of buffalo mozzarella cheeses. The objective of this study was to investigate the effect of pH and calcium content and their interaction on the physicochemical and functional properties of water buffalo mozzarella cheese during storage.

MATERIALS AND METHODS

Materials. Mozzarella cheeses were manufactured at the Dairy Pilot Plant of the Food Engineering Department, Faculty of Engineering, Ondokuz Mayıs University. 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.

Cheese Manufacture. Four vats of mozzarella cheese were made on the same day using water buffalo milk. The manufacturing procedures for the four 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**). The buffalo milk was standardized to a 4.8% fat ratio, HTST pasteurized at 72 °C for 2–3 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, 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 for each one. 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% (w/w) and hand stretched in 82 °C water (2.5 kg of water/kg of curd) until forming a smooth mass 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, then vacuum packed, and stored at 4 °C. Cheesemaking was performed in triplicate.

Sample Preparation. Cheese samples were grated to give <1 mm particles for chemical analyses and color tests, packed in glass jars, and stored at 4 °C until analysis was carried out (*8*).

Chemical and Functional Analyses. Milk, whey, stretchwater, and cheese samples were analyzed for pH, titratable acidity, fat, protein, moisture, and ash according to the methods described by Bradley et al. (*26*). Salt contents of cheeses and strechwater were determined according to the method of Metin and Ozturk (*27*). Calcium and phosphorus contents were analyzed according to the AOAC Official Method 2000 (*28*). The percentages of fat, protein, and calcium recoveries were calculated by dividing the weight of fat, protein, or calcium present in each cheese by the total weight of fat, protein, or calcium in the milk and then multiplying by 100. The actual cheese yield was determined by dividing the weight of cheese by the weight of milk used to make cheese and multiplied by 100. Cheese samples during storage were analyzed for the following. The pH was measured on cheese slurry prepared from 10 g of cheese and 10 g of distilled water (*20*). Titratable acidity was determined by the method of AOAC (*28*).

The amount of expressible serum was determined by centrifugation of grated cheese at 12500*g* for 75 min at 25 °C. The expressed serum and fat mixture were held at 4 °C until the surface layer of fat solidified. Serum phase was separated and weighed. The nonexpressible serum was then calculated from the data on moisture content and expressible serum (*29*).

Cheese meltability was measured by the modified Schreiber test (*2*). Cheese samples (30 mm diameter and 10 mm height) in triplicate were placed in Petri dishes and heated at 130 °C for 10 min. After the melted cheese had cooled to room temperature, its area (square centimeters) was measured using a planimeter (Sokkisa model KP-90 planimeter).

The Zwick Texture Analyzer was used for hardness analysis of cheeses. Samples were cut as 25 g (30 mm × 30 mm × 20 mm) samples for each measurement. The probe was a Kramer shear head, and test results (three per sample) are expressed in newtons.

The color of cheese cross section and cook color were analyzed by Hunter *L*, *a*, and *b* values with a Konica Minolta (Osaka, Japan) sensing chroma-meter (CR-400). Before measurements, the instrument was calibrated with its white reference tile. Cook color was measured in the grated cheeses (15 g) within the test tubes (27 mm diameter, 250 mm length) before and after the cheeses were cooked in a boiling water bath for 60 min (*30*). Eight to ten readings were taken from each test tube, and the average value was used. The *L* (whiteness) and *b* (yellow to blue) values were used to evaluate color changes for the samples.

Sensory Analyses. Cheese samples were organoleptically examined by a group of 8–12 panelists at the University of Ondokuz Mayis, Food Engineering Department, according to the method modified from Coppola et al. (*23*) with maximum scores of 5, 5, and 10 for appearance and color, body texture, and flavor, respectively. Panelists were asked to note any of the following: unnatural color, dull color, and uneven surface for appearance; rough, lack of stretching, pasty, stick, 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, fat, protein, ash, calcium, phosphorus, salt, expressible serum, meltability, hardness, color, and sensory properties was performed by one-way and two-factor randomized complete block design using Minitab statistical software (*24*). The factors were cheese type (A, B, C, and D cheeses) and storage time (1, 28, and 56 days). The mean differences were analyzed using Duncan's multiple-range test at $P < 0.05$.

RESULTS AND DISCUSSION

In this study, the raw water buffalo milk (pH 6.66, 6.6% fat, and 16.50% TS) was standardized to the 4.8% fat ratio and pasteurized by using a plate heat exchanger (Kromel). The pasteurized milk (pH 6.57, 14.7% TS, 4.8% fat, 4.3% protein, 0.8% ash, 193.4 mg/100 g of calcium, and 116.6 mg/100 g of phosphorus) was used for mozzarella cheese production. The ranges for total solids, calcium, and phosphorus contents of buffalo milk were reported to be 16.8–20.8%, 179–241, and 110–139 mg/100 g, respectively (*31*). Mozzarella cheeses made from buffalo milk were standardized to a 4–5% fat ratio (*20*–*22*).

Cheesemaking Details. The proposed pH values for A, B, C, and D cheeses were 6.4, 6.2, 5.9, and 5.6, respectively, at setting and 6.2, 5.9, 5.6, and 5.3, respectively, at whey drainage, and the achieved results are listed in **Table 1**. The total cheesemaking time from inoculation to milling was affected by the changes in the pH of inoculation, setting, and draining. It took 2 h and 7 min to bring the pH to 6.4 in A cheeses and 3 h and 16 min for the pH of 5.6 in D cheeses after the addition of starter culture at pH 6.57. However, setting was significantly faster in D cheeses (19 min) than in A cheeses (43 min). In addition, the samples with high setting pH values required significantly more time to reach the pH of 5.2 compared with the samples with lower setting pH values. The total manufacturing time of A cheeses (5 h and 37 min) was almost 1 h longer than those of the other cheeses (4 h and 45 min).

Table 1. Treatments and Details of the Procedures Used for the Manufacturing of Mozzarella Cheeses

	cheese A	cheese B	cheese C	cheese D
pH at different stages of manufacture				
at starter culture addition	6.57	6.57	6.56	6.56
at set	6.40	6.14	5.88	5.60
at whey drainage	6.20	5.87	5.60	5.30
at curd milling	5.20	5.20	5.20	5.20
details of cheesemaking steps				
protein-to-fat ratio of milk	0.9	0.9	0.9	0.9
temperature of milk on addition of starter culture (°C)	37	37	37	37
set temperature (°C)	32	32	32	32
strech-water temperature (°C)	80–85	80–85	80–85	80–85
curd temperature at the end of the stretching (°C)	58–60	58–60	58–60	58–60
times for cheesemaking stages (min)				
starter addition to set (ripening period)	127	146	176	196
set (rennet addition) to cut	43	30	15	19
cut to whey drainage	6	5	4	13
end of drain to dry salting	136	82	52	35
stretching	10	10	10	10
total time	322	273	257	273

Whey Composition. All whey from the production lines was collected, mixed, and analyzed for gross composition. The chemical composition of whey samples from A, B, C, and D cheeses is presented in **Table 2**. The pH and titratable acidity values of whey samples were statistically different depending on the whey draining pH of each cheese ($P < 0.05$). The fat, protein, and total solid contents of whey samples were similar ($P > 0.05$). The ash, calcium, and phosphorus contents of whey samples were significantly affected by the pH at drainage ($P < 0.05$). Whey samples collected from D cheeses (low pH) had significantly higher ash, calcium, and phosphorus levels, while the samples from A cheeses (high pH) had the opposite ($P < 0.05$). The average calcium contents in our study are similar to those found in cow's milk mozzarella cheese by Metzger et al. (*6*).

Stretchwater Composition. Cheese curds after whey drainage were dry salted in a ratio of 2.5% on the basis of the curd weight, stretched, and kneaded in hot water at 80 °C, and at the end of the stretching process, three samples were taken from the stretching water and analyzed with respect to their chemical composition (**Table 2**). The pH value of the strechwater from A cheeses was the highest of the values for the four cheeses ($P < 0.05$) and parallel to the pH at drainage during the manufacturing process. As the draining pH decreased, the degree of migration of total solids and protein from the curd to the strechwater significantly increased ($P < 0.05$), but the differences among the B–D cheeses were not significant ($P > 0.05$).

Table 2. Mean Composition of Whey and Stretchwater Samples[a]

	cheese A	cheese B	cheese C	cheese D
whey				
pH	6.20 ± 0.005 a	5.86 ± 0.010 b	5.51 ± 0.011 c	5.05 ± 0.005 d
lactic acid (%)	0.173 ± 0.003 c	0.226 ± 0.001 bc	0.348 ± 0.088 ab	0.500 ± 0.109 a
total solids (%)	6.75 ± 0.067 a	6.20 ± 0.653 a	6.45 ± 0.056 a	6.73 ± 0.042 a
fat (%)	0.20 ± 0.000 a	0.20 ± 0.000 a	0.15 ± 0.000 a	0.20 ± 0.000 a
protein (%)	0.86 ± 0.025 a	0.79 ± 0.058 a	0.77 ± 0.051 a	0.82 ± 0.025 a
ash (%)	0.42 ± 0.005 c	0.45 ± 0.024 bc	0.47 ± 0.008 b	0.55 ± 0.0210 a
calcium (mg/100 g)	41.64 ± 0.530 d	62.78 ± 4.661 c	75.23 ± 0.897 b	105.75 ± 0.72 a
phosphorus (mg/100 g)	42.26 ± 0.390 d	46.44 ± 3.249 c	50.54 ± 0.196 b	62.53 ± 0.502 a
stretchwater				
pH	6.08 ± 0.020 a	5.78 ± 0.020 d	5.88 ± 0.005 c	5.96 ± 0.005 b
lactic acid (%)	0.059 ± 0.003 a	0.067 ± 0.025 a	0.078 ± 0.002 a	0.071 ± 0.002 a
total solids (%)	2.43 ± 0.137 b	3.02 ± 0.066 a	3.07 ± 0.045 a	3.12 ± 0.056 a
fat (%)	0.80 ± 0.200 b	1.07 ± 0.057 a	0.83 ± 0.115 b	1.10 ± 0.000 a
protein (%)	0.08 ± 0.006 b	0.15 ± 0.021 a	0.16 ± 0.001 a	0.16 ± 0.007 a
ash (%)	1.27 ± 0.050 a	1.20 ± 0.139 a	0.95 ± 0.071 b	1.04 ± 0.070 b
salt (%)	0.95 ± 0.007 a	0.89 ± 0.018 b	0.89 ± 0.024 b	0.86 ± 0.006 c
calcium (mg/100 g)	21.49 ± 2.106 b	27.55 ± 2.522 a	26.35 ± 0.467 a	24.43 ± 2.700 ab
phosphorus (mg/100 g)	10.64 ± 0.727 a	13.47 ± 2.652 a	12.81 ± 0.330 a	13.43 ± 0.747 a

[a] Results are means ± the standard deviation of triplicate analysis, and different letters indicate that one-way ANOVA mean values of A, B, C, and D cheeses within the same category are significantly different ($P \leq 0.05$).

Table 3. Mean Composition of Buffalo Milk Mozzarella Cheeses[a]

	cheese A	cheese B	cheese C	cheese D
moisture (%)	45.36 ± 0.390 a	50.67 ± 0.834 b	51.14 ± 1.040 b	51.62 ± 0.860 b
fat (%)	25.13 ± 0.851 a	20.67 ± 0.763 b	21.43 ± 1.401 b	20.17 ± 1.041 b
fat in dry matter (%)	45.99 ± 1.718 a	41.91 ± 1.957 a	43.85 ± 2.477 a	41.71 ± 2.757 a
protein (%)	24.82 ± 0.305 a	24.41 ± 0.270 a	22.95 ± 0.459 b	22.17 ± 0.391 c
salt (%)	0.31 ± 0.096 b	0.38 ± 0.026 b	0.40 ± 0.000 ab	0.49 ± 0.028 a
ash (%)	2.70 ± 0.056 a	2.27 ± 0.025 b	2.10 ± 0.026 c	1.93 ± 0.020 d
calcium (mg/100 g)	886.5 ± 37.55 a	763.8 ± 31.56 b	706.6 ± 65.80 b	587.7 ± 4.16 c
calcium (mg/g of protein)	35.71 ± 1.290 a	31.28 ± 0.968 b	30.76 ± 2.277 b	26.52 ± 0.640 c
phosphorus (mg/100 g)	452.5 ± 7.509 a	357.8 ± 7.210 b	347.1 ± 14.29 b	325.3 ± 8.454 c

[a] Results are means ± the standard deviation of triplicate analysis, and different letters indicate that one-way ANOVA values of means of A, B, C, and D cheeses within the same category are significantly different ($P \leq 0.05$).

The highest salt content was found in the strech-water samples from A cheeses ($P < 0.05$). Migration of calcium and phosphorus to the strechwater is similar for all cheeses in contrast to the whey values (**Table 2**).

Gross Composition, Yields, and Recoveries of Cheese. The chemical composition of mozzarella cheeses is presented in **Table 3**. A cheeses had a significantly lower level of moisture and a higher level of fat and ash than the other cheeses ($P < 0.05$). The difference among B–D cheeses for moisture and fat was not significant ($P > 0.05$). The higher moisture content of D cheeses may be attributed to its relatively low calcium-to-protein ratio, which would be conducive to a greater degree of casein hydration (*8*). The average fat value in our study is similar to that determined in buffalo milk mozzarella cheese by Jana and Upadhay (*22*). The protein ratio significantly decreased as the pH at drainage declined from A to D cheeses ($P > 0.05$). This decline is parallel to the reduction of the calcium-to-casein ratio in cheeses. Our results are similar to those obtained by Guinee at al. (*8*), Joshi at al. (*3*), and Sheehan and Guinee (*13*). Although the same amount of salt was used for all cheeses, salt content was significantly different and D cheeses had the highest concentration ($P < 0.05$). This may be explained by the fact that the migration of salt from the curd to the stretchwater was the slowest in D samples (**Table 2**). A significant decline in ash content was observed in cheeses from A to D ($P < 0.05$). These values are comparable to the values of cow's milk mozzarella cheeses determined by Yun at al. (*14*), Metzger at al. (*12, 19*), Guinee at al. (*8*), Joshi at al. (*3, 5, 10*), and Sheehan at al. (*13*).

Table 4. Mean Cheese Yield and Recoveries for Fat, Protein, and Calcium

	cheese A	cheese B	cheese C	cheese D
cheese yield (%)	13.51	14.13	14.79	15.79
fat recovery (%)	71.05	60.90	66.08	66.39
protein recovery (%)	78.55	80.85	79.55	82.03
calcium recovery (%)	61.95	55.85	54.07	47.83

The calcium and phosphorus levels of cheeses (886.5–587.7 and 452.6–325.3 mg/100 g) were significantly decreased ($P < 0.05$) by the decrease in setting and whey drainage pH as reported in other studies (*5–7, 9, 10, 12–14, 19*). Reducing the pH at setting and at whey drainage results in an increased level of solubilization of micellar calcium phosphate and a related increase in the concentration of soluble calcium in the whey while in contact with the curds. The whey acts a vehicle in which the soluble calcium is removed from the curds at whey drainage (*8*). The increased calcium and phosphorus levels of cheese whey and stretchwater (**Table 2**) support the literature and our results.

Table 4 shows yields and fat, protein, and calcium recoveries of cheese samples. The highest cheese yield was observed in D cheeses, while those of A cheeses were the lowest even though the total loss of solids to the stretchwater from A cheeses was the lowest (**Table 2**). This may be explained by the fact that D cheeses had the highest moisture level (**Table 3**) and lowest calcium recovery (**Table 4**). It was postulated that the decrease in pH values at setting and whey drainage increases the moisture

Table 5. Effect of Whey pH at Drainage on the pH and Titratable Acidity Values of Mozzarella Cheeses during Storage[a]

property	cheese type	storage day 1	storage day 28	storage day 56
pH	A	5.26 ± 0.040 aAB	5.28 ± 0.030 aA	5.21 ± 0.032 aB
	B	5.15 ± 0.021 bA	4.99 ± 0.015 cB	4.97 ± 0.049 bB
	C	5.11 ± 0.006 bA	5.05 ± 0.011 bB	4.89 ± 0.035 cC
	D	5.11 ± 0.021 bA	5.03 ± 0.011 bB	4.83 ± 0.036 cC
titratable acidity (%)	A	0.716 ± 0.067 aA	0.503 ± 0.002 cB	0.480 ± 0.047 bB
	B	0.770 ± 0.065 aA	0.856 ± 0.184 abA	0.691 ± 0.178 baA
	C	0.781 ± 0.028 aA	0.714 ± 0.038 bA	0.730 ± 0.053 aA
	D	0.768 ± 0.005 aA	0.921 ± 0.070 aA	0.877 ± 0.120 aA

[a] Results are means ± the standard deviation of triplicate analysis, and different lowercase letters indicate that one-way ANOVA values of means of A, B, C, and D cheeses within a storage time are significantly different ($P \leq 0.05$). Different uppercase letters indicate that one-way ANOVA values of means of A, B, C, and D cheeses between storage times are significantly different ($P \leq 0.05$).

level as a consequence of mineral present in the curd (*8*, *11*, *13*, *14*). The recoveries for protein and calcium in A cheeses are similar to those obtained in the control samples by Metzger et al. (*6*). The acidification to pH 5.3 by the starter culture in our study retained the same amount of calcium in the samples preacidified to pH 6.0 by citric acid (*6*). It was found that mozzarella cheeses made from preacidified milks had lower levels of calcium compared to control samples (*19*).

Effect of Whey pH at Drainage on the Physicochemical, Functional, and Sensory Properties during Storage. Changes in pH and acidity values of mozzarella cheeses during storage are listed in **Table 5**. The pH values of all cheeses at day 1 were close to the milling pH of cheeses (5.2), but the pH values of A cheeses, 5.26, were found to be statistically higher than the others ($P < 0.05$). A slight decrease in the pH values of cheeses was observed as the storage time increased. The pH changes between cheese types were profound at the 56th day due to the moisture contents of the cheeses. The reduced pH was mentioned in the literature by Metzger et al. (*19*). In contrast, some researchers observed an opposite trend in pH after manufacturing (*8*, *13*). The differences between pH values in various studies may be dependent upon cheese manufacturing procedures, buffering capacity of curd, lactate-to-protein ratio, thermal inactivation of starter culture, and soluble and colloidal calcium phosphate ratio (*8*, *13*).

The titratable acidity values of cheese samples (**Table 5**) were similar at day 1 and significantly different the rest of the time, and storage time did not have a significant effect on the acidity values of the samples ($P > 0.05$).

The level of expressible serum (ES) has been used as an indirect measure of water holding capacity of cheese, with a low level representing a high water holding capacity (*32*, *33*). The level of nonexpressible serum (NES) may be used as a more direct index of the water holding capacity of the cheese matrix, with a high level indicating a high water holding capacity. Changes in ES and NES levels of mozzarella cheeses during storage are listed in **Table 6**. In agreement with the results of previous studies, which showed a decrease in the level of ES or an increase in the level of NES over time for mozzarella (*8, 13, 19, 29, 32*), ES levels decreased and NES levels increased during storage, indicating an age-related increase in the water holding capacity of the casein. An increase in water holding capacity during storage has a desired effect on the melt, flow, and stretching properties of mozzarella cheeses (*13, 33*). The average levels of NES and NESP (grams per gram of protein) were the lowest at the 1st day and the highest at the 56th day, but the difference between the 28th and 56th day was not significant ($P < 0.05$). It might be expected that D cheeses with the lowest calcium concentration would have the highest NES, but NES and NESP values for all cheeses are not significantly different ($P > 0.05$). Therefore, the protein content is the main factor responsible for water holding capacity (*34*).

Changes in the meltability of mozzarella cheeses during the storage period are listed in **Table 6**. The melt areas of cheese samples were significantly increased by the decrease in setting and whey drainage pH ($P < 0.05$). The highest meltability was observed in C cheeses and the lowest in A cheeses on day 1 ($P < 0.05$). In contrast, at the end of the storage period, A cheeses had the highest while D cheeses the lowest values. In general, A cheeses had the highest and D cheeses the lowest, and there was no difference between the meltability values of B and C cheeses ($P > 0.0\%$). For a good meltability, a strong interaction between protein and moisture in the cheese structure is required. Lowering of the calcium content causes an increased level of interaction between proteins and surrounding water. The protein matrix expands and becomes more hydrated, resulting in greater melting (*10*). D cheeses have almost lost all meltability at the end of the storage period, while A cheeses underwent a significant improvement during storage compared with the very low meltability values on day 1; the maximum value exceeded the values of other cheeses on day 56 ($P < 0.05$). The improvements in meltability of B and C cheeses are limited to the lower degrees throughout storage ($P < 0.05$). This result supports the hypothesis that mozzarella cheeses should be ripened for a maximum of 1 month. For cheese manufacturers, reducing the calcium content to a constant level by controlling the pH especially at whey drainage is clearly an advantage, because they can obtain increased melt on day 1 (C cheeses) without waiting for 15–30 days of refrigerated storage (A cheeses). Lowering of the calcium content to 25, 35, and 45% increased the meltability values of part skim mozzarella cheeses made from cow's milk by 1.4, 2.1, and 2.6 times, respectively (*10*). In our study, reduction of 15% calcium (B cheeses vs A cheeses) caused an increase in melt area by 1.4 times and an 20% reduction in calcium content (C cheeses vs A cheeses) led to a 1.5 times increase, but a further reduction in calcium content (35% in D cheeses vs A cheeses) did not improve the melt area (1.3 times) as much as in B cheeses. This result supports the suggestion that reduction of the level of calcium beyond 35% might not be as advantageous with respect to increasing the melt area of cheese (*10*).

The average hardness values of cheese samples throughout storage are presented in **Table 6**. Hardness values of all cheese samples were decreased by the increase in setting and whey draining pH. In general, A samples were the hardest, and then B samples ($P < 0.05$), but there was no significant difference between C and D samples even though D cheeses had lower hardness values ($P > 0.05$). Storage time significantly affected hardness values, and all cheeses became softer as the storage time increased; however, the average hardness values for 28 and 56 days were not significant ($P < 0.05$). This result as in the meltability values suggests that it is unnecessary to ripen mozzarella cheeses more than 1 month.

Changes in *L* values of mozzarella cheeses during storage are listed in **Table 7**. Although the highest cross-section *L* value was observed in D cheeses and the lowest in A cheeses, the mean *L* values for the B, C, and D cheeses were not significant ($P < 0.05$). *L* values significantly increased until the 28th day of storage and declined more then those of the first day at the end of the storage period ($P < 0.05$). *L* values taken just before cooking were significantly lower than the *L* values taken from

Table 6. Effect of Whey pH at Drainage on the Expressible and Nonexpressible Serum, Meltability, and Hardness Values of Mozzarella Cheeses during Storage[a]

property	cheese type	storage day 1	storage day 28	storage day 56
expressible serum (g/100 g of cheese)	A	8.91 ± 2.41 cA	4.07 ± 2.16 bB	1.72 ± 1.56 cB
	B	11.01 ± 0.59 bcA	9.43 ± 1.41 aA	5.87 ± 2.52 bA
	C	13.25 ± 1.18 bA	10.16 ± 1.71 aB	12.46 ± 1.52 aAB
	D	16.80 ± 1.52 aA	12.60 ± 2.79 aAB	10.83 ± 2.04 aB
nonexpressible serum (g/100 g of cheese)	A	37.61 ± 3.13 aB	40.33 ± 1.05 aAB	43.64 ± 1.24 aA
	B	36.63 ± 3.77 aB	40.66 ± 1.56 aAB	44.80 ± 3.35 aA
	C	36.48 ± 1.41 aB	42.26 ± 0.99 aA	38.68 ± 1.99 bB
	D	38.07 ± 1.20 aA	39.26 ± 2.45 aA	40.79 ± 2.90 abA
nonexpressible serum (g/g of protein)	A	1.58 ± 0.16 aA	1.69 ± 0.09 aA	1.76 ± 0.03 aA
	B	1.53 ± 0.07 aB	1.70 ± 0.07 aAB	1.84 ± 0.14 aA
	C	1.57 ± 0.05 aB	1.83 ± 0.09 aA	1.69 ± 0.12 aAB
	D	1.62 ± 0.01 aB	1.67 ± 0.04 aB	1.84 ± 0.09 aA
melt area (cm^2)	A	15.79 ± 1.9 bB	38.19 ± 4.6 aA	39.35 ± 3.01 aA
	B	21.94 ± 0.3 abB	25.60 ± 1.7 bAB	30.72 ± 6.50 bA
	C	23.90 ± 2.1 aA	26.87 ± 0.8 bA	27.77 ± 3.50 bA
	D	20.27 ± 5.6 abB	11.15 ± 0.7 cB	12.33 ± 0.15 cA
hardness (N)	A	338.85 ± 4.6 aA	300.24 ± 32.2 aB	296.67 ± 5.7 aB
	B	294.75 ± 59.1 aA	221.66 ± 60.6 bA	229.20 ± 19.7 bA
	C	245.50 ± 35.0 abA	169.18 ± 20.7 bA	189.05 ± 47.3 bcB
	D	201.00 ± 35.3 bB	153.49 ± 29.5 bA	170.56 ± 18.2 cA

[a] Results are means ± the standard deviation of nine analyses, and different lowercase letters indicate that one-way ANOVA values of means of A, B, C, and D cheeses within a storage time are significantly different ($P \leq 0.05$). Different uppercase letters indicate that one-way ANOVA values of means of A, B, C, and D cheeses between storage times are significantly different ($P \leq 0.05$).

Table 7. Effect of Whey pH at Drainage on the Color (*L* and *b*) Values of Mozzarella Cheeses during Storage[a]

property	cheese type	storage day 1	storage day 28	storage day 56
L value cross section	A	78.06 ± 0.30 aA	80.12 ± 1.44 aA	73.98 ± 1.63 cB
	B	77.67 ± 1.16 aB	81.52 ± 1.88 aA	76.38 ± 0.54 bB
	C	80.17 ± 0.76 aA	81.14 ± 2.35 aA	76.38 ± 0.78 bB
	D	80.06 ± 2.57 aA	80.51 ± 3.74 aA	78.37 ± 0.45 aA
L value ground cheese before cooking	A	73.64 ± 0.13 bB	75.45 ± 1.04 bB	80.80 ± 2.66 aA
	B	74.50 ± 0.44 abB	73.76 ± 0.67 bB	78.92 ± 1.46 aA
	C	73.64 ± 0.27 bC	77.47 ± 1.20 aB	81.90 ± 1.22 aA
	D	74.68 ± 0.75 aC	77.97 ± 0.60 aB	82.37 ± 1.37 aA
L value ground cheese after cooking	A	49.37 ± 1.21 bB	52.85 ± 0.26 bAB	55.09 ± 3.17 aA
	B	50.55 ± 1.71 bA	55.02 ± 2.33 bA	55.22 ± 4.51 aA
	C	55.05 ± 1.54 aAB	58.74 ± 1.23 aA	53.42 ± 2.65 aB
	D	56.98 ± 0.95 aB	61.30 ± 1.02 aA	57.60 ± 2.05 aB
b value cross section	A	14.86 ± 0.15 aA	17.42 ± 1.28 aC	12.34 ± 0.28 aB
	B	14.57 ± 0.2 abA	17.61 ± 0.19 aC	12.48 ± 0.17 aB
	C	14.44 ± 0.29 bA	17.00 ± 0.96 aC	12.58 ± 0.54 aB
	D	14.04 ± 0.11 cA	17.64 ± 0.82 aC	12.88 ± 0.14 aB
b value ground cheese before cooking	A	10.49 ± 0.44 aB	10.31 ± 0.62 aB	17.75 ± 0.10 aA
	B	10.46 ± 0.44 aB	10.15 ± 0.48 abB	16.90 ± 0.32 aA
	C	9.74 ± 0.36 aB	9.44 ± 0.66 abB	16.38 ± 1.49 aA
	D	9.98 ± 0.26 aB	9.85 ± 0.25 bB	15.97 ± 0.72 aA
b value ground cheese after cooking	A	18.64 ± 0.48 bB	29.38 ± 0.45 aA	21.54 ± 4.81 aB
	B	18.56 ± 0.85 bC	24.54 ± 1.57 bA	21.84 ± 1.41 aB
	C	20.43 ± 0.58 aA	23.52 ± 2.22 bA	18.95 ± 5.95 aA
	D	19.44 ± 0.71 abB	28.08 ± 0.67 aA	24.80 ± 5.96 aAB

[a] Results are means ± the standard deviation of nine analyses, and different lowercase letters indicate that one-way ANOVA values of means of A, B, C, and D cheeses within a storage time are significantly different ($P \leq 0.05$). Different uppercase letters indicate that one-way ANOVA values of means of A, B, C, and D cheeses between storage times are significantly different ($P \leq 0.05$).

cross sections and showed a different profile. A and B cheeses and C and D cheeses had similar *L* values, but these two groups were significantly different from each other ($P < 0.05$). Storage time significantly increased *L* values of all cheeses throughout storage ($P < 0.05$). Cooking significantly decreased the *L* values of all samples. D cheeses had the highest and A cheeses the lowest values ($P < 0.05$). Storage time increased *L* values significantly, but the difference between day 28 and 56 was not significant ($P > 0.05$).

The effects of pH at whey drainage and storage time on *b* values (yellow to blue) of mozzarella cheeses are shown in **Table 7**. In general, decreasing the pH value at whey drainage (lowering calcium content) did not have any significant effect on the *b* values of the samples taken from cross section and after cooking ($P < 0.05$). *b* values of A and B samples before cooking were significantly higher than those of C and D samples. *b* values significantly increased until day 28 and then declined for the samples taken from cross section and after cooking, However, *b* values before cooking did not significantly change until the 28th day ($P > 0.05$) and then increased ($P < 0.05$).

Changes in sensorial properties of mozzarella cheeses during storage are given in **Table 8**. The average appearance and color

Table 8. Effect of Whey pH at Drainage on the Sensory Properties of Mozzarella Cheeses during Storage[a]

property	cheese type	storage day 1	storage day 28	storage day 56
appearance	A	2.36 ± 0.165 bA	2.49 ± 0.083 aA	2.55 ± 0.19 aA
	B	2.56 ± 0.186 abA	2.36 ± 0.028 aAB	2.19 ± 0.11 bB
	C	2.59 ± 0.000 abA	2.44 ± 0.105 aA	2.15 ± 0.18 bB
	D	2.77 ± 0.080 aA	2.39 ± 0.172 aB	2.04 ± 0.22 bA
texture	A	3.51 ± 0.168 bA	3.63 ± 0.449 aA	3.69 ± 0.22 aA
	B	3.47 ± 0.226 bB	4.08 ± 0.217 aA	3.59 ± 0.08 aB
	C	3.92 ± 0.231 aA	3.76 ± 0.306 aA	3.59 ± 0.41 aB
	D	4.06 ± 0.051 aA	3.57 ± 0.624 aAB	3.15 ± 0.39 aC
flavor	A	7.04 ± 0.197 bA	7.26 ± 1.002 aA	6.72 ± 0.38 aA
	B	7.32 ± 0.200 bA	7.58 ± 0.299 aA	6.31 ± 0.25 aB
	C	7.71 ± 0.143 aA	7.28 ± 0.388 aA	6.31 ± 0.21 aB
	D	7.86 ± 0.045 aA	6.79 ± 0.402 aB	5.39 ± 0.44 bC
general acceptability	A	7.00 ± 0.209 cA	7.29 ± 0.679 aA	6.94 ± 0.33 aA
	B	7.23 ± 0.135 cA	7.58 ± 0.233 aA	6.70 ± 0.23 aB
	C	7.68 ± 0.117 bA	7.00 ± 0.272 aB	6.65 ± 0,08 aC
	D	7.98 ± 0.071 aA	6.75 ± 0.597 aB	5.59 ± 0.52 bC

[a] Results are means ± the standard deviation of triplicate analysis, and different lowercase letters indicate that one-way ANOVA values of means of A, B, C, and D cheeses within a storage time are significantly different ($P \leq 0.05$). Different uppercase letters indicate that one-way ANOVA values of means of A, B, C, and D cheeses between storage times are significantly different ($P \leq 0.05$).

scores of cheeses were not significant ($P > 0.05$) and significantly declined throughout storage; however, appearance scores of A cheeses increased throughout storage in contrast to the decline for the others. Although the mean differences between cheeses are not significant ($P > 0.05$), the highest body and texture score was given to C cheeses, the lowest was given to A cheeses on day 1, D cheeses received the lowest scores, and the others were not significantly different from each other at the end of the storage time ($P > 0.05$). Texture scores significantly declined during storage (except A cheeses), but the values on days 1 and 28 and on days 28 and 56 were similar ($P > 0.05$). Only A cheeses had increased texture scores throughout the storage period. Forty percent of the panelists criticized A samples for being too hard on day 1, and 60% of the panelists noted that D cheeses were too soft at the end of the storage time. This result is also supported by the hardness values of A and D cheeses (**Table 6**). The average flavor values of A and B cheeses were significantly lower than those of C and D cheeses on day 1, and the highest score was given to D cheeses and the lowest to A cheeses. In contrast, the highest flavor score was given to A cheeses and the lowest to D cheeses on day 56 ($P < 0.05$). Flavor scores of all cheeses did not significantly change until the 28th day of storage ($P > 0.05$) and then significantly declined ($P < 0.05$). This implies that mozzarella cheeses should not be ripened for more than 1 month. On the basis of sensory properties, it can be concluded that A cheeses were not preferred and C and D cheeses preferred on day 1. At the end of the storage time, all the sensory properties of A cheeses improved while D cheeses were rated inferior to the rest of the samples.

In conclusion, mozzarella cheese made from buffalo milk should be ripened for a maximum of 1 month and A or B cheeses should be preferred as ripened cheeses. In cases of cheeses going to market just after manufacture, C cheeses are suggested.

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