



Effect of Brine Calcium Concentration on the Surface Solubilization and Texture of Fresh Perline Mozzarella Cheese

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ABSTRACT

Softening of cheese surface is a common problem especially in brined cheeses. In this study, the effects of the brine calcium concentrations on the texture of fresh perline Mozzarella cheese were investigated. The compositions of cheeses were analyzed 2 weeks after production. Brine protein content were monitored at 2 and 4 week of storage. The effect of the brine calcium concentration on the texture and meltability of cheeses were monitored Texture Profile Analysis (TPA) and Schreiber meltability test at 2 and 4 weeks of storage. The decrease in brine calcium concentration increased the protein transfer from cheese to brine, leading to an increase in the moisture content of cheese. As the calcium concentration increased in brine, an increase in the hardness, and decrease in adhesiveness and meltability of the cheeses were observed during storage. In conclusion, softening/solubilization of the surface of fresh perline Mozzarella cheese can be prevented with increasing the brine calcium concentration.

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Introduction

Fresh Mozzarella is a pasta filata cheese manufactured in various size and shapes and sold in brine in retail stores. One of the most common problems in Mozzarella is softening on the surface of the cheese and losing the structural integrity and shape (Luo et al., 2013). Soft and slimy surface negatively affect the appearance and consumer preference. Composition, pH, rate of proteolysis, Ca and salt content and cheese manufacturing protocol are several factors that affect the final texture of cheeses (Lucey et al., 2003). For brined cheeses, brine composition influences the equilibrium between brine and cheese, and it is critical for controlling textural and rheological properties of cheeses (Geurts et al., 1972).

Fresh Mozzarella is a high moisture pasta filata cheese with milky buttery flavor originated in Italy (Luo et al., 2013). Although traditional Mozzarella cheese is manufactured from water buffalo, today large amount of Mozzarella cheese is produced from bovine milk due to its availability (Faccia et al., 2019). Generally, Mozzarella cheeses are produced as low moisture and high moisture Mozzarella. Low moisture Mozzarella is primarily

produced for ingredient purposes such as topping for pizza. High moisture Mozzarella cheese is consumed as table cheese (Faccia et al., 2019). High moisture Mozzarella cheeses are produced in varying sizes. Recently production and consumption of perline (pearl size) Mozzarella has been increasing in Turkey. Perline Mozzarella cheeses are known as various names in Turkish market such as Süt Damlası, Topi, Topik, and parents prefer these cheeses for their kids to get necessary cheese nutrients. As the size of perline Mozzarella is quite small considering bocconcini (bite-sized) and ovoline (egg-sized), migration of cheese solids into brine occurs much higher rate due to their larger surface area.

Fresh Mozzarella cheese is generally sold in preservative liquid (commonly water or brine) to preserve characteristics. Mass transfer between cheese and brine continue until reaching an equilibrium, thus product characteristics are prone to some changes during marketing (Mizuno et al., 2016). Laurenzio et al. (2008) reported that addition of polysaccharides into preservative liquid prevented the migration of cheese solid into the

preservative media. Luo et al. (2013) reported that calcium in brine hinders the migration of salt from brine to cheese. Utilization of improper preservative liquid may lead to slimy surface on the cheese, cloudy and blurry appearance in the liquid or even complete solubilization of the cheese into brine which negatively affect consumer preference.

In this study, we wanted to investigate the effect of Ca concentration on perline Mozzarella cheese texture during 4 weeks storage. To our knowledge, this will be the first study investigating the brine Ca concentration in perline Mozzarella cheese.

Materials and Methods

Cheese manufacture

Perline Mozzarella cheeses were manufactured in a local dairy plant (Gunesoglu Sut, Sakarya, Turkey). Full fat milk (3.6 ± 0.1 fat, 3.3 ± 0.3 protein, 12.8 ± 0.5 total solids) was pasteurized at 74°C for 30 s, cooled to 37°C . Direct vat set thermophilic culture (TM1; MicroMilk; Cremona, Italy) was added at the rate of 10 g for 450 kg of cheese milk. 30 minutes pre-ripening was applied to cheese milk to provide culture activity, then calf rennet (Naturen; Chr. Hansen, Istanbul, Turkey) was added to cheese milk at the rate of 63 g for 450 kg of cheese milk. The coagula were set around 50 min and cut after subjectively evaluated by the cheesemaker. Curd temperature in the vat was increased to 40°C in 30 min. As pH reached 6.2, whey was drained and curd was transferred into a separate vat for acid development. As the curd pH reached pH 5.1, the curd was milled and transferred to cooker strecher. Curd was stretched in brine solution containing 3% salt and the temperature of 80°C . Curd exited the cooker strecher at 66°C and entered ball making machine with diameter of 1 cm. After the shapes were formed cheeses were cooled at 12°C water bath for 15 min. The added CaCl_2 concentrations of retail preservative liquid is given in Table 1. After manufacture, cheeses were stored in 4°C .

Compositional Analyses

Cheese milk was analyzed for total solids (948.12; AOAC, 2007), protein (total percentage $\text{N} \times 6.38$, Kjeldahl method; 991.20; AOAC, 2007), fat (2000.18; AOAC 2007), and pH (Hanna pH211; Hanna Instruments, Woonsocket, RI, USA) at the day of cheese manufacture. Cheeses were analyzed for total solids (941.08; AOAC, 2007), ash (945.46; AOAC 2007) and salt (975.20; AOAC 2007) at 2 wk of storage. Retail brine was analyzed for protein (total percentage $\text{N} \times 6.38$, Kjeldahl method; 991.20; AOAC, 2007) content during storage. Cheese pH was monitored using a spear-tip pH electrode (Inlab solid pro; Mettler Toledo, Columbus, OH, USA) during storage.

Cheese Texture and Meltability

Texture profile analysis (TPA) was carried out using a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA). Perline Mozzarella samples were used as whole for textural analyses. Samples were compressed 30% in TPA test by a 75 mm aluminum cylinder probe with a cross-head speed of 0.8 mm s^{-1} . All tests were performed at 5°C and replicated at least 5 times.

Cheese meltability was measured by Schreiber meltability test as described at (Voigt et al., 2012). Whole perline Mozzarella cheeses were placed into oven at 232°C for 5 min and cooled for 30 min. Average distance values (mm) of four readings of diameter at different places on the melted circles were averaged and recorded.

Experimental Design and Statistical Analyses

Perline Mozzarella cheeses were placed into the same retail brine (pH 5.0, salt 5.2%) with 6 different added CaCl_2 concentrations (0, 0.2, 0.4 0.6, 0.8 and 1.0%). Analysis of variance (ANOVA) testing was carried out by using JMP (11.0 version; SAS Institute Inc., Cary, NC, ABD). Data obtained from analyses were compared with Tukey-HSD multiple comparisons test, and significance was determined at $P < 0.05$. Pearson's correlation coefficients were estimated between various responses.

Results and Discussion

Composition

The composition of perline Mozzarella cheeses at 2 wk of storage are given in Table 2. Increasing the Ca concentration decreased pH and moisture content of the cheeses. Increase in the ash content of the cheeses can be explained by the increase in total solid content of the cheeses with increased Ca concentration in brine. However, decrease in the cheese pH with increasing Ca concentrations of brine was unexpected. One way to explain the decrease in the cheese pH with increased brine Ca concentration is that cheeses lost structural integrity at low brine Ca concentration and solubilized (Figure 1). Similarly, Mizuno et al. (2016) reported that increase in brine Ca concentration in ovoline Mozzarella decreased cheese pH. The solubilization of cheeses opened the protein network to brine, which lead to increased solubilization of insoluble Ca (INSOL Ca) phosphate. It is a well-known phenomenon that as protein bound Ca solubilized from the caseins, phosphate buffers and increase pH (Lucey et al., 1993; Lucey et al., 2003; Salaun et al., 2005). Previous studies indicated that decrease in brine Ca concentration led to solubilization of INSOL Ca concentration in cheese (Faccia et al., 2019; Mizuno et al., 2016).

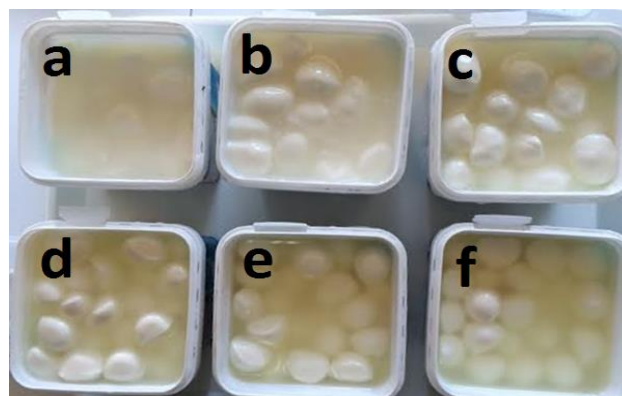


Figure 1. Images of perline Mozzarella cheeses in 0.0 (control) (a), 0.2 (b), 0.4 (c), 0.6 (d), 0.8 (e), and 1.0% (f) CaCl_2 containing brines at 2 wk of storage.

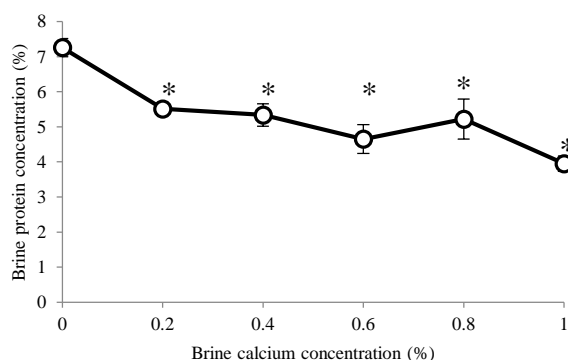


Figure 2. Protein contents of brines at various brine calcium concentrations at 2 wk of storage. (* denotes significantly (P<0.05) different from control)

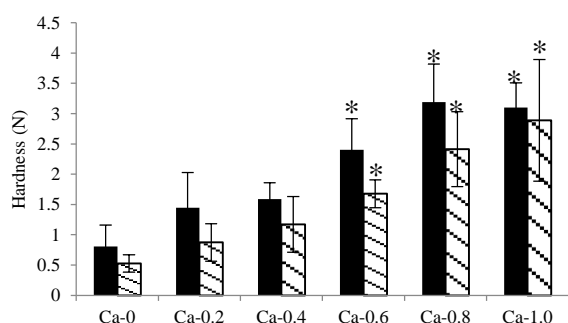


Figure 3. Hardness of perline Mozzarella cheeses at 0.0 (control) (Ca-0.0), 0.2 (Ca-0.2), 0.4 (Ca-0.4), 0.6 (Ca-0.6), 0.8 (Ca-0.8), and 1.0% (Ca-1.0) calcium containing brines at 2 (■) and 4 (▨) wk of storage. (* denotes significantly (P<0.05) different from control)

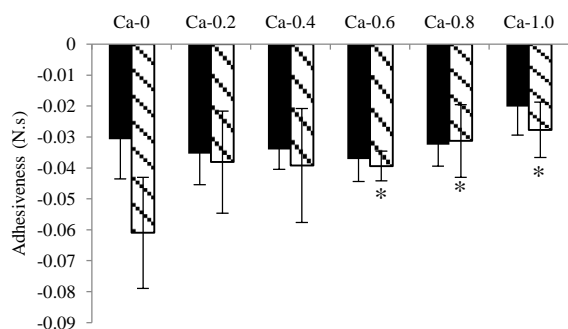


Figure 4. Adhesiveness of perline Mozzarella cheeses at 0.0 (control) (Ca-0.0), 0.2 (Ca-0.2), 0.4 (Ca-0.4), 0.6 (Ca-0.6), 0.8 (Ca-0.8), and 1.0% (Ca-1.0) calcium containing brines at 2 (■) and 4 (▨) wk of storage. (* denotes significantly (P<0.05) different from control)

There was a negative correlation ($R^2 = 0.92$, $P < 0.05$) between brine Ca concentration and the moisture content of the cheeses, in other words, increase in the brine Ca concentration decreased the cheese moisture content. Geurts et al. (1972) conducted one of the first studies on the effect of brine Ca concentration on cheese surface. They reported that utilization of Ca in brine used in Gouda cheese manufacture prevented soft rind defect and the moisture content of the Gouda cheeses manufactured in Ca containing brine was lower. Previous studies on Mozzarella cheese were also reported that increase in brine Ca concentration

decreased the moisture content of cheese (Luo et al., 2013; Mizuno et al., 2016). The changes between brine Ca concentration and brine protein concentration are given in Figure 2. As the structural integrity of the cheeses is lost and cheese protein diffuses into brine. The brine Ca concentration and brine protein concentration were negatively correlated ($R^2 = 0.87$, $P < 0.05$), i.e., as the brine Ca concentration increased the protein content diffused to the retail brine decreased. This result suggests that even small concentration of Ca in brine had a great impact on structural integrity of perline Mozzarella cheeses compared to control (without Ca addition) brine. Salt content of perline Mozzarella cheeses were unaffected by the brine Ca concentration ($P > 0.05$). Previous studies suggested that water channels between protein fibers that occurs during pasta-filata process were primarily responsible for salt diffusion and these channels were became less visible during storage due to protein hydration in Mozzarella cheese (Kuo et al., 2003; Sheehan et al., 2005; McMahon et al., 1999). Luo et al. (2013) reported that brine Ca interfered with salt diffusion from brine into Mozzarella cheeses. Salt diffusion and protein hydration occur in cheeses in the first 2 wk of storage, thus we believe that we did not observe differences in the salt content between the cheeses of storage and our experimental cheeses were analyzed for salt at 2 wk (Metzger et al., 2001; Guo and Kindstead, 1995).

Cheese Texture

Hardness of cheese samples increased with the increase of Ca concentration in brine (Figure 3.). Moisture content of cheese directly influences the cheese texture. Previous studies reported that increase in cheese moisture decreases cheese hardness (Gunasekaran and Ak, 2003). Our textural results are highly correlated with cheese moisture. Cheese hardness and cheese moisture results were negatively correlated at 2 wk ($R^2 = 0.91$, $P < 0.05$) and 4 wk of storage ($R^2 = 0.87$, $P < 0.05$), i.e., increase in Ca concentration of the brine decreased the moisture content of the cheeses, thus cheese hardness increased with brine Ca concentration. Cheeses manufactured with higher than 0.6% brine Ca concentration exhibited significantly ($P < 0.05$) higher hardness at 2 and 4 wk of storage. Cheese adhesiveness did not exhibit significant differences at 2 wk of storage (Figure 4.). However, adhesiveness of cheeses manufactured in brine higher than 0.6% Ca was significantly lower than control at 4 wk of storage. McMahon et al. (2005) reported that increase in Ca concentration in block type nonfat Mozzarella cheese did not influence cheese adhesiveness; however, similar to our results they also reported that increase in moisture content of cheese significantly increased cheese adhesiveness. Springiness and cohesiveness were not influenced with brine Ca concentration (results not shown).

Table 1. Composition of brines prepared for perline Mozzarella cheeses.

Sample	CaCl ₂ (%)	pH	Salt (%)
Ca-0 (Control)	0*	5.0	5.2
Ca-0.2	0.2	5.0	5.2
Ca-0.4	0.4	5.0	5.2
Ca-0.6	0.6	5.0	5.2
Ca-0.8	0.8	5.0	5.2
Ca-1.0	1.0	5.0	5.2

*Without calcium addition

Table 2. Moisture, ash, salt and pH values of perline Mozzarella cheeses at 2 wk of storage.

Sample	Moisture (%)	Ash (%)	Salt (%)	pH
Ca-0 (Control)	82.1 ± 2.4 ^a	4.0 ± 0.2	2.6 ± 0.2	5.8 ± 0.0 ^a
Ca-0.2	80.6 ± 1.7 ^a	4.2 ± 0.2	2.6 ± 0.2	5.8 ± 0.0 ^a
Ca-0.4	77.1 ± 1.3 ^b	4.2 ± 0.3	2.7 ± 0.4	5.8 ± 0.1 ^a
Ca-0.6	71.4 ± 1.3 ^c	4.2 ± 0.2	2.6 ± 0.3	5.8 ± 0.1 ^a
Ca-0.8	73.1 ± 3.1 ^c	4.2 ± 0.3	2.6 ± 0.1	5.7 ± 0.0 ^b
Ca-1.0	71.9 ± 1.1 ^c	4.3 ± 0.3	2.6 ± 0.2	5.5 ± 0.1 ^c

^{a-c}Means within the same column not sharing a common superscript differ (P<0.05).

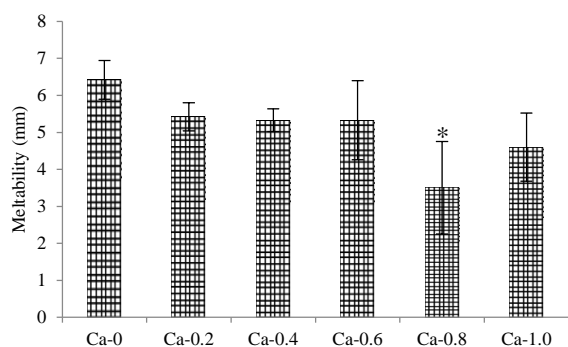


Figure 5. Meltability of perline Mozzarella cheeses at 0.0 (control) (Ca-0.0), 0.2 (Ca-0.2), 0.4 (Ca-0.4), 0.6 (Ca-0.6), 0.8 (Ca-0.8), and 1.0% (Ca-1.0) calcium containing brines at 2 wk of storage.

(* denotes significantly (P<0.05) different from control)

Cheese Meltability

Cheese meltability results obtained by Schreiber test is given in Figure 5. There was a negative correlation between brine Ca concentration and cheese meltability ($R^2 = -0.81$, $P < 0.05$), i.e., increase in brine Ca concentration decreased cheese meltability. Cheeses stored in 0.8% Ca brine exhibited significantly lower meltability than control ($P < 0.05$). Lucey et al. (2003) reported that moisture content directly influences the meltability of cheese. McMahon et al. (2005) reported that increase Ca concentration on block type nonfat Mozzarella decreased cheese meltability. Thus, the lower moisture content of cheeses at high Ca containing brine must be responsible for low melting observed. There are two main forces that affects casein micelles; hydrophobic interactions and electrostatic repulsion (Horne, 1998). As INSOL Ca in cheese solubilizes negatively charged phosphate groups in protein network weakens the structure, leading to higher meltability. Excessive solubilization of INSOL Ca at pH values closer to the isoelectric pH of caseins lead to increase in meltability due to increased hydrophobic interaction between caseins. Calcium in the soluble phase of the brine prevents the solubilization of INSOL Ca, leading to more integral protein matrix (Lucey et al., 2003). Higher brine Ca concentration may also impair solubilization of INSOL Ca in our cheeses, leading to less meltability.

Conclusion

Increase in the Ca concentration of the brine decreased the protein diffusion from cheese to brine, prevented blurry appearance of brine and improved textural properties. Even

small amounts of Ca in brine is very effective to prevent solids loss from cheese into brine. Informal sensory evaluation revealed that brines containing 1% Ca resulted in unwanted bitter and metallic flavor (results not shown). In conclusion, utilization of Ca higher than 0.6% in retail brines prevent solubilization/melting of the perline Mozzarella during 1 mo of storage. We believe that results of this study will be helpful for local and international perline Mozzarella producers.

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