Technical paper

The Effect of Preservative Liquid Composition on Physicochemical Properties of Mozzarella Cheese

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The effect of preservative liquid on the physicochemical properties of Mozzarella cheese during storage was examined. Mozzarella cheese was analyzed 2 weeks after storing in liquid composed of 0–0.2% citric acid, 0–2% sodium chloride, and 0–1% calcium chloride dihydrate. The addition of citric acid and sodium chloride to the liquid increased the moisture content and decreased the hardness of cheese. On the other hand, cheese became harder as the calcium chloride concentration increased. Additionally, citric acid was the main cause of skin formation on the surface of cheese. These results indicate that changes in the combination and concentrations of compounds dissolved in the preservative liquid significantly influence the physicochemical properties of Mozzarella cheese. Thus, the selection of preservative liquid could be one of the important factors determining the product quality.

Keywords: mozzarella cheese, preservative liquid, storage

Introduction

Mozzarella cheese is categorized as a pasta filata cheese originating in Italy, but the production and consumption of this cheese have spread worldwide. Mozzarella cheese has an elastic body and fibrous structure, and its flavor is creamy and mild. The ability to melt well and to stretch when heated is characteristic of the cheese. Mozzarella cheese can be generally divided into 2 categories based on moisture content: low moisture and high moisture. The former typically has a moisture content of 45–52%, longer shelf life, and is vacuum-packed. The latter typically has a moisture content of 52–60%, shorter shelf life, and is packaged in a plastic bag along with preservative liquid (i). The preservative liquid is mainly used to maintain freshness and to prevent deformation during distribution, and is traditionally prepared from “stretching process water” that consists of salt and diluted whey (Paonessa, 2004). In some cases, salted water with citric acid and lactic acid, or sterilized water, is also used. Mozzarella cheese is often sold immersed in this preservative liquid and is exposed to it

until reaching the consumer. Therefore, the quality of the cheese can be changed by prolonged exposure to such a solution. Some components in the cheese could migrate between the cheese and preservative liquid to reach equilibrium, resulting in changes in the quality of cheese with storage time. A previous study by Laurienzo *et al.* (2008) on the effects of preservative liquid on the quality of cheese showed that migration of components between the cheese and preservative liquid was prevented by adding a polysaccharide thickener to the preservative liquid. In addition, Luo *et al.* (2013) demonstrated that calcium in a preservative liquid affected salt diffusion and the distribution of water in Mozzarella cheese. However, a detailed study on the liquid type and composition of the preservative liquid has not yet been undertaken.

We examined the effect of preservative liquid composition on the physicochemical properties of Mozzarella cheese during storage. To accomplish this aim, preservative liquid varying in concentrations of citric acid, sodium chloride, and calcium chloride dihydrate was prepared, and the composition and functional

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properties of cheese were determined after the cheese was immersed and stored in the liquid.

Materials and Methods

Cheese making Raw cow's milk, which has 4.2% fat and 3.2% protein, was collected by the Betsukai factory of Morinaga Milk Industry Co., Ltd. The milk was poured into the cheese vat after it was pasteurized at 75°C for 15 s and cooled to 39°C. Calcium chloride dihydrate (60 mg/kg) and frozen *Streptococcus thermophilus* starter culture (50 mg/kg, Sacco S.r.l) were added to the milk. After addition of the starter, 110 mg/kg calf rennet (Chr. Hansen) was added; 30 min after the rennet addition, the coagulum was cut into approximately 40 mm cubes, and then the curd-whey mixture was cooked for 90 min. After 90 min, the whey was drained. When the pH of the curd reached 5.3, the curd was stretched in hot water (85°C). After organization of the curd by stretching, the curd was molded into a 105-g spherical shape and cooled in cold water (14°C) for 30 min. After the curd was cooled, 120 g of preservative liquid, composed of 0–0.2% citric acid, 0–2% sodium chloride, and 0–1% calcium chloride dihydrate (5°C) was added. Analyses were performed after storage at 5°C for 2 weeks.

Compositional analysis The moisture content of the cheese and serum was measured according to a standard method (IDF, 1982). Protein content of the samples was measured using the modified Dumas method (IDF, 2000). The pH of cheese was measured by direct insertion of a D-51 pH probe (Horiba, Japan) into cheese, which was ground whole. The calcium content of cheese and serum was measured using inductivity-coupled plasma emission spectrometry (ICPE-9000, Shimadzu, Japan) at a wavelength of 422.7 nm after samples were dried, ashed, and re-dispersed using hydrochloric acid. Insoluble calcium content per protein was calculated in accordance with the method developed by Hassan *et al.* (2004) using the equation below:

Insoluble Ca (mg / g protein) =

$$\left(\frac{\text{Ca (mg / 100 g) in cheese}}{\text{moisture (\%) in cheese}} - \frac{\text{Ca (mg / 100 g) in serum}}{\text{moisture (\%) in serum}} \right) \frac{\text{moisture (\%) in cheese}}{\text{protein (\%) in cheese}} \quad \dots\dots\text{Eq. 1}$$

Serum was extracted from the cheese by a method similar to that by Guo and Kindstedt (1995). A whole cheese sample was centrifuged in an automatic high-speed refrigerated centrifuge (SCR20B, Hitachi Koki, Japan) at 12,500 × g for 75 min at 25°C and the supernatant was carefully removed. After the sample was cooled to 5°C, it was filtered through Whatman No. 1 filter paper and the filtrate was extracted.

Hardness measurement The breaking force of the cheese was measured using a texture analyzer (RE2-33005SI, Yamaden, Japan). The sample was cut into 20 mm thick sections, leaving the center of the cheese, which were tempered to 10°C for more than 2 h prior to measurements. A cylindrical plunger 8 mm in diameter

was inserted into the center of each sample to 75% of the height of the sample (15 mm) at a speed of 1 mm/s. The breaking force was referred to as the hardness of the cheese. Measurements were performed in triplicate.

Sensory evaluation of skin formation Five trained panelists evaluated the skin formation on the surface of cheese by appearance and texture, and rated the skin formation on a scale of 1 – 3 as follows:

1: No skin formed

2: Soft skin formed

3: Hard skin formed

All tests were performed in triplicate.

Experimental design and statistical analysis The 3³ full factorial design was chosen in this study. The three independent variables were the citric acid, sodium chloride, and calcium chloride dihydrate concentrations in the preservative solution. Concentration ranges of citric acid, sodium chloride, and calcium chloride dihydrate in this study were set at 0–0.2%, 0–2%, and 0–1%, respectively, by considering the acceptable taste of Mozzarella cheese. The complete design consisted of 81 runs including three replicates at each point. The response variables under observation were moisture content, cheese pH, insoluble calcium content per protein, and hardness. Additionally, surface condition was analyzed by treating the ordinal scale as an interval scale for the convenience of analysis. The data fit the second-order equation for all response variables. Statistical analyses were performed by multiple (step-wise) regression analysis. All analyses including calculation of correlation coefficients between each of the response variables were performed using JMP software (SAS Institute, Cary, NC).

Results

The effect of preservative liquid on composition The moisture content, pH, and insoluble calcium per protein of the cheese after 2 weeks of storage at different liquid compositions are presented in Table 1. Table 2 shows the adjusted coefficient of determination and a partial regression coefficient for the moisture content, pH, and insoluble calcium per protein of the cheese after storage at 5°C for 2 weeks.

The adjusted coefficient of determination (R^2 adjusted) for moisture content was high at 0.727, indicating a good fit with the second-order equation. The concentrations of citric acid, sodium chloride, and calcium chloride significantly affected the moisture content of cheese. The interaction of sodium chloride and calcium chloride was also significant. This result indicated that moisture content increased as the concentration of citric acid and/or sodium chloride increased, and a higher calcium chloride concentration lowered the moisture content. Additionally, the observed interaction between sodium chloride and calcium chloride suggested that the degree of increased moisture content by sodium chloride addition became smaller at high calcium chloride

Table 1. Values of the independent variables and the response variables for the 3³ full factorial design.

Independent variables			Response variables (± SE)				
CitA (%)	NaCl (%)	CaCl ₂ (%)	Moisture content (%)	Cheese pH	Insoluble calcium (mg/g)	Hardness (N)	Skin formation
0	0	0	53.81 ± 0.96	5.70 ± 0.03	18.31 ± 1.11	5.76 ± 0.32	1.0 ± 0
0	0	0.5	54.67 ± 0.72	5.54 ± 0.02	20.40 ± 1.01	7.02 ± 0.41	1.0 ± 0
0	0	1.0	55.08 ± 0.67	5.48 ± 0.03	20.04 ± 1.04	7.12 ± 0.54	1.0 ± 0
0	1.0	0	58.37 ± 1.07	5.74 ± 0.02	15.39 ± 1.02	4.42 ± 0.45	1.0 ± 0
0	1.0	0.5	56.26 ± 0.78	5.62 ± 0.04	17.07 ± 1.03	5.25 ± 0.25	1.0 ± 0
0	1.0	1.0	56.43 ± 0.77	5.51 ± 0.02	20.24 ± 0.65	7.24 ± 0.53	1.0 ± 0
0	2.0	0	60.04 ± 0.85	5.75 ± 0.04	17.41 ± 1.27	3.72 ± 0.50	1.0 ± 0
0	2.0	0.5	57.46 ± 0.49	5.65 ± 0.02	18.24 ± 1.00	4.17 ± 0.29	1.0 ± 0
0	2.0	1.0	57.83 ± 0.92	5.51 ± 0.03	19.11 ± 0.70	5.58 ± 0.64	1.0 ± 0
0.1	0	0	57.72 ± 0.39	5.52 ± 0.03	16.17 ± 0.91	5.24 ± 0.35	2.0 ± 0
0.1	0	0.5	57.04 ± 0.55	5.45 ± 0.02	15.94 ± 0.91	5.52 ± 0.37	2.2 ± 0.1
0.1	0	1.0	57.69 ± 0.45	5.36 ± 0.02	18.04 ± 1.00	5.12 ± 0.42	2.0 ± 0
0.1	1.0	0	59.46 ± 0.70	5.55 ± 0.02	13.56 ± 0.98	3.70 ± 0.06	2.0 ± 0
0.1	1.0	0.5	59.08 ± 0.55	5.46 ± 0.01	16.18 ± 0.97	4.36 ± 0.21	2.0 ± 0
0.1	1.0	1.0	58.57 ± 0.56	5.40 ± 0.01	17.65 ± 1.04	4.45 ± 0.58	2.0 ± 0
0.1	2.0	0	60.39 ± 0.30	5.59 ± 0.04	14.47 ± 0.96	2.86 ± 0.26	2.0 ± 0
0.1	2.0	0.5	60.08 ± 0.42	5.49 ± 0.02	12.98 ± 0.71	3.96 ± 0.11	2.0 ± 0
0.1	2.0	1.0	58.81 ± 0.32	5.40 ± 0.05	14.05 ± 0.90	4.44 ± 0.59	2.0 ± 0
0.2	0	0	56.89 ± 0.47	5.43 ± 0.03	15.26 ± 1.03	4.51 ± 0.24	2.9 ± 0.1
0.2	0	0.5	57.37 ± 0.38	5.36 ± 0.02	15.48 ± 1.03	4.15 ± 0.19	2.6 ± 0.2
0.2	0	1.0	58.24 ± 0.38	5.25 ± 0.01	17.83 ± 0.90	3.82 ± 0.25	2.7 ± 0.2
0.2	1.0	0	60.08 ± 0.68	5.45 ± 0.02	14.25 ± 0.95	3.47 ± 0.22	2.2 ± 0.1
0.2	1.0	0.5	59.34 ± 0.24	5.35 ± 0.02	13.69 ± 0.89	3.58 ± 0.39	2.0 ± 0
0.2	1.0	1.0	58.64 ± 0.48	5.30 ± 0.03	14.19 ± 1.65	3.71 ± 0.22	2.3 ± 0.2
0.2	2.0	0	61.74 ± 0.51	5.45 ± 0.03	11.97 ± 1.03	3.12 ± 0.17	2.0 ± 0
0.2	2.0	0.5	61.60 ± 0.44	5.40 ± 0.02	10.93 ± 0.98	2.94 ± 0.11	2.0 ± 0
0.2	2.0	1.0	61.57 ± 0.41	5.28 ± 0.05	15.54 ± 0.90	2.77 ± 0.14	2.0 ± 0

CitA = citric acid; NaCl = sodium chloride; CaCl₂ = calcium chloride dihydrate**Table 2.** Second-order polynomial models obtained for moisture content, pH, and insoluble calcium content of cheese.

Response variables	Independent variables	Coefficient	R ² adjusted	p-value
Moisture content	Constant***	55.479	0.727	<0.001
	CitA***	14.172		
	NaCl***	1.723		
	CaCl ₂ *	-0.627		
	(NaCl - 1.0 × CaCl ₂ - 0.5)**	-1.092		
Cheese pH	Constant***	5.673	0.898	<0.001
	CitA ***	-1.237		
	NaCl***	0.025		
	CaCl ₂ ***	-0.189		
	(CitA - 0.1 × CaCl ₂ - 0.5)*	0.317		
Insoluble Calcium	Constant***	18.307	0.571	<0.001
	CitA ***	-20.597		
	NaCl***	-1.264		
	CaCl ₂ ***	2.210		

CitA = citric acid; NaCl = sodium chloride; CaCl₂ = calcium chloride dihydrate*, **, ***, significantly different at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively

Table 3. Second-order polynomial models obtained for hardness and skin formation

Response variables	Independent variables	Coefficient	R^2 adjusted	p -value
Hardness	Constant***	5.934	0.789	<0.001
	CitA ***	-10.111		
	NaCl***	-0.818		
	CaCl ₂ ***	0.828		
	(CitA - 0.1 × NaCl - 1.0)*	2.317		
	(CitA - 0.1 × CaCl ₂ - 0.5)***	-11.417		
	(NaCl - 1.0 × CaCl ₂ - 0.5)*	0.422		
Skin formation	Constant***	1.255	0.781	<0.001
	CitA ***	6.481		
	NaCl***	-0.130		
	(CitA - 0.1 × NaCl - 1.0)***	-1.759		

CitA = citric acid; NaCl = sodium chloride; CaCl₂ = calcium chloride dihydrate

*, **, ***, significantly different at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively

Table 4. Correlation coefficients between response variables

	Cheese pH	Insoluble calcium	Hardness	Skin formation
Moisture	-0.252*	-0.721***	-0.840***	0.368***
Cheese pH		0.204	0.215	-0.637***
Insoluble calcium			0.705***	-0.450***
Hardness				-0.454***

*, **, ***, significantly different at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively

concentrations.

Adjusted coefficient of determination for cheese pH also showed a high level of fit (R^2 adjusted = 0.898). All three independent variables were significant in regards to cheese pH. The partial regression coefficient indicated that pH was most strongly influenced by the concentration of citric acid, as expected. A significant interaction of citric acid concentration and calcium chloride was observed.

Insoluble calcium per protein of the cheese showed a low level of fit, based on the adjusted coefficient of determination comparison with other response variables (R^2 adjusted = 0.571). All three independent variables were significant to insoluble calcium content. Insoluble calcium was strongly influenced by the concentration of citric acid and was reduced by an increase in citric acid concentration. Insoluble calcium decreased with increasing concentrations of sodium chloride and increased with increasing concentrations of calcium chloride. A significant interaction between independent variables was not observed.

The effect of preservative liquid on functional properties The hardness and surface condition of cheese were measured in triplicate under 27 different conditions (Table 1). As indicated in Table 3, the adjusted coefficient of determination (R^2 adjusted) of hardness was 0.789, indicating good agreement between observed and fitted values. This result demonstrated that the cheese is softened as citric acid and/or sodium chloride concentration increases. In contrast, hardness increased with increasing calcium

chloride. Interactions between citric acid and sodium chloride, citric acid and calcium chloride, and sodium chloride and calcium chloride were all significant. The concentration of citric acid was more effective at low concentrations of sodium chloride, the effect of citric acid was higher in the presence of calcium chloride, and the effect of sodium chloride was higher at low concentrations of calcium chloride.

For the surface condition of cheese, the adjusted coefficient of determination (R^2 adjusted) was 0.781, indicating a good fit to the second-order polynomial. Citric acid and sodium chloride concentrations significantly influenced the texture of cheese skin. Increased citric acid concentration resulted in harder skin. On the other hand, increased sodium chloride concentration resulted in softer skin. Additionally, the interaction of citric acid and sodium chloride concentrations was significant. The effect of citric acid on skin condition was high when the concentration of sodium chloride was low.

Relationship between response variables Table 4 shows the results of correlations between response variables. A strong negative correlation was seen between cheese moisture and hardness when changing the composition of the preservative solution ($r = -0.840$). Insoluble calcium per protein was positively and negatively correlated with hardness and moisture content, respectively ($r = 0.705$ and -0.721). Skin formation was negatively correlated with cheese pH ($r = -0.637$). The pH value of cheese did not show any strong correlation with moisture content, insoluble

calcium, or hardness of cheese.

Discussion

This study revealed that changes in the combination and concentration of compounds dissolved in a preservative liquid have a measurable impact on the physicochemical properties of Mozzarella cheese. Addition of citric acid resulted in an increase in cheese moisture content and a decrease in insoluble calcium content and pH. The addition of citric acid also influenced the hardness of the cheese; increasing the amount of citric acid added resulted in softer cheese.

The effects of adding acid on the physicochemical properties of cheese have been well studied. Some types of Mozzarella cheese are produced by directly adding acid to cheese milk (Breene *et al.*, 1964). According to this method, lowering of the cheese milk pH leads to decreased insoluble calcium bound to casein micelles, resulting in decreased calcium content in cheese and producing softer cheese that melts well. In another experiment where cheese was directly acidified by acetate vapor, the viscosity of melted cheese decreased and soluble calcium in cheese increased when the cheese pH decreased from 6.5 to 5.2 (Ge *et al.*, 2002).

The dissolution of insoluble calcium in cheese has a major impact on the physical and chemical properties of cheese (Lucey *et al.*, 2003). According to previous studies, 77.9% of total calcium in Mozzarella cheese exists in an insoluble state and the amount of insoluble calcium per protein is 20.4 mg/g the day after production (Mizuno *et al.*, 2009). As insoluble calcium gradually dissolves during storage, the functional properties of the cheese change (Guo and Kindstead, 1995). The results of these previous experiments were concordant with our results, in that changes in the physicochemical properties of cheese were caused by the action of the acidifier. Thus, these changes in the properties of Mozzarella cheese in our study could be explained by the following mechanism: insoluble calcium, a component of the cross-linking between the casein matrix of cheese, is partially lost as the pH decreases by addition of acid, and negatively charged groups of casein are exposed by the dissolution of insoluble calcium. When the pH of the system is still relatively high (i.e., >5.0) the local increase in negative charge causes greater electrostatic repulsion between casein molecules. This results in the casein aggregates becoming more open and porous and facilitates casein hydration, resulting in an increase in moisture and a decrease in hardness. This phenomenon could occur on the surface of cheese at the start, and then proceeds to the center as the acid penetrates into the cheese.

The addition of sodium chloride also had a great impact on the hardness of cheese. Gaucheron *et al.* (2000) showed that the calcium bound to casein micelles dissolves after the addition of sodium chloride to the milk. They suggested that a decrease in the hardness of cheese, due to the addition of sodium chloride, could also be caused by the loss of cross-linking between casein

molecules due to solubilization of the insoluble calcium. When producing brine-salted cheese, it is known that the outside of the cheese becomes slimy and sticky. A factor influencing these defects is thought to be the usage of fresh brine lacking calcium. This phenomenon is the result of protein hydration due to the exchange of insoluble calcium with sodium ions from the added sodium chloride. A similar phenomenon could have occurred in our study, and may have manifested as a decrease in the hardness of cheese.

To prevent the slimy and sticky surface of brine-salted cheese, a small amount of calcium chloride is often added to brine (McSweeney, 2007). In our study, calcium chloride was selected as an independent variable to verify this effect of calcium chloride on Mozzarella cheese during storage. With increasing addition of calcium chloride, the hardness was increased in this study. Increased soluble calcium (calcium ions) by addition of calcium chloride may inhibit dissociation of insoluble calcium from the surface of cheese, which is in agreement with Luo *et al.* (2013).

During this experiment, the surface of some cheese samples became hard and skin was formed, which is often seen with Mozzarella cheese made from water buffalo milk. This skin on the cheese surface became hard as the concentration of citric acid was increased. The formation of skin is assumed to be relevant to the isoelectric point of casein, because the pH of the preservative liquid of skin-formed samples was 4.7–4.9, which was lower than the pH of ground cheese and close to the isoelectric point of casein. This skin tended to weaken due to protein hydration when the concentration of sodium chloride increased. Hardness of skin by sensory evaluation was not positively correlated with the hardness of cheese (Table 4).

pH levels in this experiment were not strongly correlated with hardness, moisture content, or insoluble calcium of cheese. This may be because the pH of the cheese was measured after grinding of the whole cheese, as there is a pH difference between the outer and inner sections even after 2 weeks of storage.

Previous studies on various cheeses have shown that calcium content, pH, and degree of protein degradation are important parameters for the texture and functionality of cheese (Lucey and Fox, 1993, Lucey *et al.*, 2003). The composition of cheese, including the moisture content, also influences the properties of cheese. These parameters are likely related to each other and one could affect the other. In our study, the changes in physicochemical properties proceeded slowly from the outside of cheese during storage, as the components migrated between the cheese and preservative liquid.

Conclusion

This study demonstrated that variable concentrations of citric acid, sodium chloride, and calcium chloride in preservative liquid significantly influence the physicochemical properties of Mozzarella cheese. The addition of citric acid and sodium chloride

increased the moisture content and decreased the hardness, likely owing to relaxation of the casein network and hydration of casein, followed by solubilization of the insoluble calcium. On the other hand, calcium chloride inhibited solubilization of the insoluble calcium, leading to harder texture and less moisture reduction. Additionally, citric acid was the main cause of skin formation on the surface of cheese, likely because citric acid results in changes to the cheese surface pH to reach the isoelectric point of casein. Since Mozzarella cheese is usually stored in preservative liquid until used, the effect of preservative liquid on the properties of cheese should be considered when the liquid is prepared.

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