Storage changes in low sodium-processed Mozzarella cheese prepared using potassium-based emulsifying salts

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Abstract Dietary sodium has been considered to cause hypertension and other health ailments. Processed Mozzarella cheese (PMC) is a type of processed cheese that contains high sodium in the form of sodium chloride and sodium emulsifying salts. Henceforth, the aim of this study was to investigate the effect of partial substitution of sodium chloride (NaCl) with potassium chloride (KCl) and total replacement of sodium-based emulsifying salts with potassium-based emulsifying salts in the production of experimental PMC. A blend of potassium citrate and dipotassium phosphate (1:1) was used as an emulsifying salt. Changes in sensory attributes, physico-chemical characteristics, and functional properties of PMC were analyzed and compared with the corresponding changes in a control PMC made with NaCl and trisodium citrate. Products were evaluated after regular intervals of 7 days up to 56 days. Significant differences in flavor (28–56 days), sensory body and texture, acid degree value (0–42 days), soluble protein, titratable acidity (7–56 days), pH (7–56 days), meltability (0–56 days), and stretchability (4–49) were evident between the control and experimental PMC over storage. It appears that potassium replacement of sodium resulted in a low decrease in casein hydration and an increase in pH and bitterness. This subsequently resulted in a lower rate of proteolysis, lower flavor scores, lower meltability, lower titratable acidity, lower acid degree values, and greater stretchability and sensory body and texture scores.

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1 Introduction

Sodium chloride (NaCl) is added to cheese with the primary purpose to provide flavor and to control the growth of non-starter lactic acid bacteria. Besides these, it also plays major role in controlling the body and texture of cheese. Thus, NaCl is considered a vital ingredient in cheese (Guinee and Fox 2004). However, excess dietary sodium has been known to cause hypertension which may lead to cardiovascular diseases. As per WHO (2012), the recommended daily intake for sodium is 2.0 g, equivalent to 5.0 g of NaCl. However, daily sodium intake in most parts of the world is well in excess of this recommendation. Processed cheese contains 1,320 mg Na per 100 g cheese (Liem et al. 2011) and thus significantly contributes to the percentage of total daily sodium intake in part of a daily diet.

Processed Mozzarella cheese (PMC) is a type of processed cheese known for its meltability, stretchability, and shredability in pizza making and is very popular in India due to the large preferences for processed forms of cheese (Chavhan et al. 2014). It is prepared by heating and shearing of Mozzarella cheese in the presence of emulsifying salts. Monovalent cations and polyvalent anions make the basic structure of emulsifying salts wherein cations are mostly sodium based and therefore contribute to increased sodium content of PMC.

Several researchers have attempted to reduce the amount of salt addition in processed cheese, but such approaches have increased proteolysis and acidity resulting in a soft, pasty, and/or bitter cheese (Johnson et al. 2009). It also effects protein hydration which has major influence on the structure, physico-chemical stability, and physical properties of cheese. Addition of potassium salts to cheeses may result in higher pH, decreased hardness and cohesiveness, a slight increase in fat globule size, and increased flowability (El-Bakry et al. 2011). Such an influence on cheese properties could be related to differences in hydration of the cations with direct influence on casein interactions and fat emulsification.

Partial substitution of NaCl has been successfully attempted by using KCl and NaCl in equal proportion to produce acceptable cheeses. Efforts have been made to assess the physico-chemical, biochemical, textural, and functional changes occurring during storage in cheeses containing potassium as a partial or full replacement of sodium. Studies have monitored lipolysis in Feta cheese (Katsiari et al. 2000a), proteolysis in Feta cheese (Katsiari et al. 2000b) and Kefalograviera cheese (Katsiari et al. 2001), and chemical and functional changes in low-moisture Mozzarella cheese (Ayyash and Shah 2011) prepared with partial replacement of NaCl with KCl. Efforts have been made to produce processed cheese based on Cheddar using potassium-based emulsifying salts. Karahadian and Lindsay (1984) reported that the production of an acceptable processed cheese using potassium citrates and phosphates. Hoffmann et al. (2012) manufactured pasteurized block-type processed cheese with partial substitution of sodium by potassium in emulsifying salts with no significant loss of functional properties or sensory quality was observed as the bitter taste of potassium-containing salts was barely perceptible in the processed cheeses. Chavhan et al. (2014) reported manufacture of low sodium-processed cheese from Mozzarella employing combined approach of

partial substitution of NaCl with KCl and using potassium based emulsifying salts. The study reported that 70% reduction in overall sodium content through substitution of NaCl with KCl was possible without compromising sensory, textural, and functional attributes.

The objective of the present study was to determine and compare sensory, physico-chemical, and functional changes in PMC prepared with potassium-based emulsifying salts and mixtures of NaCl and KCl already optimized in the previous study of Chavhan et al. (2014) to that of control PMC (processed cheese made with NaCl and sodium-based emulsifying salts) during storage.

2 Materials and methods

2.1 Raw material

Buffalo's fresh milk was procured from Experimental Dairy, National Dairy Research Institute, Karnal (Haryana), India. Microbial rennet (Meito) obtained from *Mucor pusillus* var. *Lindt* was procured from Meito Sangyo and Co. Ltd., Tokyo, Japan. Sodium chloride was procured from M/S Tata chemicals, Mumbai, India. Commercial food grade KCl was purchased from Polypharma Pvt. Ltd. Mumbai, India. Potassium citrate and di-potassium phosphate were procured from J K Brothers Ltd. and Arti Phosphates Pvt Ltd., Mumbai, India, respectively. All other chemicals used were of analytical reagent grade.

2.2 Preparation of PMC

Processed cheese used in this study was manufactured using Mozzarella cheese as base. Mozzarella cheese was manufactured from buffalo milk by direct acidification method (Jensen et al. 1987) with certain modifications (Chavhan et al. 2014). Standardized milk was acidified at 7 °C, followed by rennet addition at 25 °C. Set curd was cut, cooked, plasticized, and molded as per the method used by Chavhan et al. (2014). This unsalted Mozzarella cheese was used as a base for manufacturing processed cheese.

For manufacturing PMC, a mixture of NaCl and KCl was used in the ratio of 70:30 on weight basis. Total salt content was kept at 1%. Emulsifying salt used in the study comprised a blend of potassium citrate and dipotassium phosphate (hereafter, referred to as emulsifying salt) in the ratio of 1:1. Level of emulsifying salt was kept at 2.0%. These levels were optimized in a similar study by Chavhan et al. (2014). Finely grated Mozzarella cheese was heated in a heating kettle, and as soon as the temperature reached 50 °C, salt mixture (NaCl and KCl) and emulsifying salt were added to it. The mixture was processed at 75 °C for 5 min followed by transfer to moulds. PMC was allowed to cool to ambient temperature (25 °C) and cut into blocks of 250 g and packed in KPA-Cryovac® packages. The packed product was kept at 5 °C for storage and further analyses. Storage changes in low sodium PMC were compared to a control (full sodium PMC) which was prepared by using 1% NaCl and 2% tri-sodium citrate and stored under same conditions. Moisture, fat, protein, and sodium content of control and experimental PMC were 46.95, 20.72, and 26.02% and 905 mg per 100 g and 46.18, 20.27, and 27.74% and 260 mg per 100 g, respectively.

2.3 Physico-chemical analysis

2.3.1 Titratable acidity and pH

The titratable acidity of the cheese was determined according to AOAC (1984). For pH estimation, 10 g of cheese was macerated with 10 mL of distilled water and the pH of resultant slurry was measured using microprocessor controlled pH Analyser (LabIndia, New Delhi, Version I) with combined glass electrode.

2.3.2 Acid degree value

Acid degree value (ADV) was carried out by the method suggested by Deeth and Fitzgerald (1976). Samples were prepared by mixing 5 g of grated cheese with 37.5 mL of 2% sodium citrate at 50 °C. Exactly 35 mL of this extract was used for ADV estimation.

2.3.3 Soluble protein

The soluble protein of cheese samples was determined following the procedure delineated by Kosikowski (1982) with some modifications. Approximately 3.0 g of cheese sample was accurately weighed and mixed with a small amount of Sharp's extraction solution at 40 °C. The volume was made up with Sharp's extraction solution (glacial acetic acid and sodium chloride) to 50 mL. After keeping for 1 h in water bath at 50–55 °C, solution was filtered through Whatman Filter Paper No. 42. Twenty-five milliliters of this filtrate was further used for soluble nitrogen estimation using Kjeldahl method (IDF, 1986).

2.4 Functionality

2.4.1 Meltability

Meltability was determined by the method of Oberg et al. (1993). Ten grams shredded cheese was added into a Pyrex glass tube (32 mm×250 mm) and gently tapped with a spatula until each of the samples occupied a length of about 3.5 cm. The one end of the tubes were sealed with stoppers (silicon rubber) and the other with aluminum foil having a small hole to allow gas to escape. The tubes were tempered at 4 °C for 30 min in a vertical position and then placed horizontally in an oven at 100 °C for 60 min. After cooling to room temperature, the length of melted cheese was recorded.

2.4.2 Stretchability

Stretchability of PMC was measured on pizza by following the procedure described by Walsh et al. (1998). Shredded cheese (0.25 g.cm²) was evenly distributed on the pizza base that was pre-cut in half, and it was placed in an oven at 280 °C for 4 min and then evaluated for stretchability. One half of the pizza was clamped, and the unclamped section was manually pulled until the extended cheese string joining both section of pizza base broke. The stretch was recorded by measuring the distance between the pizza halves.

2.5 Sensory analysis

The control and experimental PMC were presented for sensory evaluation to a panel of eight trained judges from Dairy Technology Division of National Dairy Research Institute, Karnal. All the judges were trained sensory analyst with a minimum 5 years' experience. A descriptive sensory score card with 90-point scale was used. Scorecard was developed based on American Dairy Science Association (ADSA) scorecard. However, maximum scores for sensory attributes were modified on the basis of recommendation of sensory panel. Maximum scores for flavor, body and texture, and color and appearance were 50, 30, and 10, respectively. The samples were presented in random order to eliminate carry over effects. The tests were carried out in individual booths under white light. Judges received approximately 20 g of each sample, served at the refrigeration temperature (7 °C) in disposable plastic cups coded with random three-digit numbers.

2.6 Statistical analysis

Product manufacturing trials were conducted in triplicate. However, reported results pertain to only one trial as there was non-significant difference among the replicate trials. The presented values are mean of three replicate analysis. The data were analyzed with SAS ver. 5.3 (SAS Institute Inc., Cary, NC). One-way analysis of variance was performed to investigate significant difference at 5% level of significance between control and experimental cheeses at same storage period and to examine the significance of storage period within a salt treatment. Post hoc analysis for finding out significantly different treatment was carried out using Tukey HSD technique.

3 Results and discussion

3.1 Sensorial changes during storage of PMC

Organoleptic scoring of control and experimental PMC are demonstrated in Fig. 1(a). A statistically significant ($p < 0.05$) effect of storage on flavor scores of control and experimental PMC was found. There was no significant difference in the flavor scores of control and experimental PMC up to 28 days of storage, but scores were statistically higher for the control after day 28. Up to 28 days, these results are in agreement with the findings of Katsiari et al. (1998) who reported no significant difference in the flavor between different Kefalograviera cheeses made with varying NaCl to KCl mixture (1:1) as compared to full sodium counterpart. After 28 days, experimental PMC obtained significantly lower ($p < 0.05$) scores for flavor due to the development of bitterness which has often been described with the use of potassium salts in cheese (Chavhan et al. 2014). Ayyash et al. (2012) studied the sensory attributes of Akawi cheese prepared with different proportions of NaCl and KCl and found significantly more bitterness for the samples containing NaCl to KCl (1:3) mixture during storage of 30 days.

Body and texture scores of both control and experimental PMC showed a significant ($p < 0.05$) decreasing trend during storage as represented in Fig. 1(b), but remained above the minimum acceptable limits, usually 65% score (Londhe et al. 2012) of the

total score during storage. Body and texture score of control and experimental PMC differed significantly ($p < 0.05$) throughout storage with a higher score for experimental PMC which was reported to be firmer. The difference was apparent from day zero. Experimental PMC had higher score possibly due to more extensive casein–casein interactions (Sheehan and Guinee 2004) as it is likely that to have more colloidal calcium phosphate because the cation exchange between calcium and potassium is less than sodium and calcium resulting in less soluble calcium (Carr et al. 2002).

The color and appearance of cheese is also important for consumer acceptance. Figure 1(c) shows a significant ($p < 0.05$) decrease in color and appearance scores of both the PMC with storage; however, there was no significant ($p > 0.05$) difference in both treatments.

3.2 Physico-chemical changes during storage of PMC

The physico-chemical changes in PMC during storage are shown in Fig. 2(a). The pH of the control and experimental PMC significantly ($p < 0.05$) decreased during storage. Experimental PMC had consistently significant higher pH values than the control, similar to results by Katsiari et al. (1998) and Ayyash and Shah (2011) for Kefalograviera and low-moisture Mozzarella cheese, respectively. This may be due

Fig. 1 Evolution in sensory scores of control and experimental PMC during storage **a** change in flavor, **b** change in body and texture, and **c** change in color and appearance (values represented are means of three replicates with their standard errors)

to the fact that KCl solution has 0.3 values higher for pH as compared to an equivalent solution of NaCl. This might be a possible reason for higher pH of experimental cheese. In agreement with pH, the titratable acidity of both the control and experimental PMC increased significantly ($p < 0.05$) with storage, in which the control PMC had consistently higher value (Fig. 2(b)). Control and experimental PMC had no significant ($p > 0.05$) differences in titratable acidity until after 7 days.

Enzymatic hydrolysis of triglycerides to fatty acids and glycerol, mono-, or diglycerides is considered an essential factor for flavor development in cheese (McSweeney and Sousa 2000). Lipolysis is a critical biochemical event during cheese ripening which has been studied extensively in different varieties. The ADVs of control and experimental PMC are given in Fig. 2(c). The ADV increased in both types of cheeses during storage similar to titratable acidity. The control was significantly higher up to 42 days of storage, however at the end of storage the difference was not significant ($p > 0.05$; Fig. 2(c)) and in agreement with studies by Katsiari et al. (2000a) and Reddy and Marth (1994) for Feta cheese and Cheddar cheese, respectively.

Proteolysis is the most complex and, in most varieties, the most important of the three primary biochemical events which occur in cheese during ripening. It promotes texture development and flavor development in cheese. It is catalysed by proteinases and peptidases from different sources such as milk coagulant, milk plasmin, starter

Fig. 2 Evolution in physico-chemical characteristics of control and experimental PMC during storage **a** change in pH, **b** change in titratable acidity, **c** change in ADV, and **d** change in % soluble protein (values represented are means of three replicates with their standard errors)

lactic acid bacteria, non-starter lactic acid bacteria, and secondary starters. It is evident from Fig. 2(d) that the soluble protein content of control and experimental PMC increased significantly ($p<0.05$) during storage of 56 days and was higher ($p<0.05$) in control PMC. Increased proteolysis in low-sodium Minas fresh cheese (Gomes et al. 2011), Akawi cheese containing NaCl substituted KCl (Ayyash et al. 2012), and reduced sodium Feta cheese (Katsiari et al. 2000b) during storage corroborates these findings. Sheehan and Guinee (2004) studied the effect of calcium content and pH on proteolysis in low moisture Mozzarella cheese during 70 days storage. They reported that at similar pH values, higher proteolysis in cheese having lower calcium to casein ratios may occur. Higher cation exchange capacity of sodium may result in larger conversion of colloidal calcium to soluble calcium (Carr et al. 2002) which exposes the casein–casein interactions for action by proteinases; perhaps due to this, control PMC had higher proteolysis (Feeney et al. 2002). Sheehan and Guinee (2004) also observed lower proteolysis in cheese having a higher pH, low moisture in non-fat substances, and lower rennet retention in curd. In our study, higher pH values due to KCl addition to experimental PMC might have resulted in less favorable conditions for the activity of residual rennet in proteolysis of cheese during storage (Mulvihill and Fox 1980). Also as evident from proximate composition of control and experimental PMC, the moisture content of latter was lower which may have retarded microbial growth. Less proteolysis of experimental PMC could also be possible due to the variable proteolytic activity of non-starter lactic acid bacteria.

3.3 Functional changes during storage of PMC

Meltability and stretchability are important functional properties of Mozzarella cheese which determine its suitability as an ingredient in pizza making. Meltability is defined as the ease with which cheese flows or spreads upon heating (Muthukumaran et al. 1999). Interactions between casein molecules govern melting and stretching properties of cheese (Lucey et al. 2003). Sodium promotes casein hydration and thus facilitates interactions between casein and entrapped fat globules which decreases meltability due to casein emulsification (Kinsella and Melachouris 1976). Fat in milk plays an important role in attributing functionality to cheese, as it has been reported that homogenized cheese milk leads to cross-linking of casein on the fat globule surface with the casein in the matrix, preventing the cheese from melting (Tunick et al. 1995). However, with decreasing casein–casein interaction due to solubilization of colloidal calcium phosphate to soluble calcium due to the exchange with sodium, meltability of cheese may increase. Meltability of control and experimental PMC during storage increased significantly ($p<0.05$) and differed significantly in both treatments ($p<0.05$) during storage with more pronounced results for control PMC. Figure 3(a, b) elaborates the effect of storage on meltability and stretchability of control and experimental PMC. Low-moisture Mozzarella cheese prepared with substitution of NaCl with KCl was observed to have increased meltability with storage (Ayyash and Shah 2011). This increase may be attributed to two factors: (1) an increase in proteolysis during storage, (Kindstedt et al. 2004) and (2) an increase in the conversion of insoluble calcium to soluble calcium, which in turn reduced the strength between casein networks (Feeney et al. 2002). Higher meltability in control cheese was possibly due to less protein–protein interaction owing to more calcium sequestration by trisodium citrate (Swenson

Fig. 3 Evolution in **a** meltability and **b** stretchability of control and experimental PMC during storage (values represented are means of three replicates with their standard errors)

et al. 2000). Also in a study by Carr et al. (2002), it was suggested that according to Hofmeister series, sodium ions have more hydration tendency as compared to potassium which means sodium competes more effectively with protein for existing water and causes higher solubilization of colloidal calcium and in turn higher casein hydration. This enhances the heat-induced displacement of adjacent layers of the para-casein matrix on heating which increases meltability (Sheehan and Guinee 2004). Less casein hydration in case of experimental cheese due to lower ratio of soluble to colloidal calcium thus leads to less soluble casein having comparatively stronger protein–protein interaction which in turn may reduce meltability of experimental PMC (El-Bakry et al. 2011).

Stretch is the ability of the casein network to continuously maintain its integrity without breaking when uninterrupted stress is applied to the cheese. Casein molecules have the capability to interact with each other for good stretch; consequently, they should release stress which is applied and become pliable, i.e. should not break (Lucey et al. 2003). It is known that for good stretching properties, relatively high concentrations of intact casein and critical concentrations of Ca and P are required (Lucey and Fox 1993). The stretchability of control and experimental PMC showed a significant ($p < 0.05$) decreasing trend with storage. The stretchability of the control was significantly ($p < 0.05$) lower than the experimental PMC from 0 to 49 days. Kindstedt et al. (2004) and Kuo et al. (2003) also reported a decrease in the stretchability of Mozzarella cheese during ripening, which may be due to plasmin activity, especially if the curd has not been subjected to a high heat treatment in the cooker or stretcher or cheese has been stored at very low temperatures (Lucey et al. 2003). Lower stretchability values of control PMC from 0 to 49 days could be the reason of weaker protein–protein interaction between casein particles attributed to the extensive solubilization of colloidal calcium phosphate in the presence of sodium ions during brining (El-Bakry et al. 2011) and possibly calcium sequestration by trisodium citrate salt which lead to formation of solubilized calcium and exposed casein which might have weaker strength between protein–protein linkages and thus resulted in lower stretchability.

4 Conclusion

It can be concluded from the present investigation that some sensory, physico-chemical, and functional properties of full sodium (control) and low sodium (experimental) PMC varied significantly during storage. Experimental PMC had lower proteolysis and thus lower flavor score and firmer body. It appears that calcium content and final pH of PMC may be playing an important role in governing these changes. Lower meltability and inversely higher stretchability for experimental PMC could be the reason of altered protein–protein interaction as a consequence of potassium based emulsifying salts and lower cation exchange and hydration tendency of potassium ion according to Hofmeister series.

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