Electrophoretic behaviour of casein hydrolysis of modified atmosphere packaged Mozzarella cheese

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Abstract: An attempt was undertaken to investigate the electrophoretic behaviour of Mozzarella cheese (MC) packed under modified atmospheric packaging (MAP) conditions in two different packaging materials (Cryovac (70 microns) (P1) and LLD/BA*/Nylon-6/BA*/LDPE (110 microns) (P2). A definite quantity (approximately 250 g) of MC was stored in high barrier bags under five varying conditions viz., air (C1), vacuum (C2), 100 % CO₂ (C3), 100 % N₂ (C4) and a mixture of 50% N₂ and 50% CO₂ (C5) and stored at 7±1°C. Periodically, the product was evaluated for its electrophoretic behaviour in the form of casein hydrolysis using low molecular weight (LMW) SDS-PAGE. During refrigeration storage, the degradation of protein started after four weeks with two major bands. However, there was no difference in the pattern of casein fractions of MAP Mozzarella cheese during storage at 7±1°C under 5 atmospheres upto 4 weeks, regardless of package type. After 8 weeks of storage, the intensity of bands decreased and lower molecular weight peptides increased.

Keywords: Electrophoretic properties, MAP, Mozzarella cheese, Refrigeration storage, Shelf-life,

Modified Atmosphere Packaging (MAP) refers to the storage of any food product in high barrier packaging and flushing of gases inside the package which changes overtime due to activity of the product (Khoshgozaran et al. 2012). MAP possesses several advantages like shelf-life enhancement, quality preservation (Deshwal et al. 2018), quality degradation (Li et al. 2014), and reduced microbial activity (Alam and Goyal 2011). Mozzarella cheese (MC) is a 'pasta filata' type of cheese which refers to its stretching property. Under refrigeration storage without any MAP Mozzarella cheese possess a shelf life of only 15 days, as atmospheric air exaggerates the deteriorating reactions thus affecting sensory quality, texture and shelf-life adversely (Alam et al. 2016). Hydrolysis of cheese proteins is responsible for the development of texture and organoleptic features of most ripened varieties of cheese. The amount of intact casein had been found to affect even the stretching properties of Mozzarella cheese. Unlike most ripened varieties of cheese, protein hydrolysis profile of Mozzarella cheese are limited (Farkye et al. 1991). A very limited information is available on the influence of MAP on its electrophoretic properties during refrigeration. Therefore, a study was undertaken to investigate the influence of MAP on hydrolysis characteristics of Mozzarella cheese under refrigerated conditions.

The research work was carried out at National Dairy Research Institute (NDRI), India. A qualitative methodology was adopted for the evaluation of casein hydrolysis of modified atmosphere packaged Mozzarella cheese. Previously, Alam et al. (2016) prolonged the shelf-life of Mozzarella cheese by storing it under MAP and also studied variation in textural properties during storage. Another study on proteolysis of goat cheese (colourless and brined) was also carried out by Barac et al. (2013) using SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and a strong correlation between moisture content and proteolysis was reported.

For preparing Mozzarella cheese, mixture of buffalo (Murrah breed) and cow milk (crossbred cows) was procured from Experimental Dairy, NDRI, Karnal. The method described by Upadhyay, (2003) was used for preparing Mozzarella cheese with slight modifications. Two different high barrier packaging materials (Cryovac (70 microns) (P1) and LLD/BA*/Nylon-6/

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Fig. 1 Electrophoretic behavior of casein hydrolysis of MAP Mozzarella cheese (Plate I: Fresh; Plate II: after four weeks; Plate III: after 12 weeks; IV: after 6 months; Plate V: after 12 months)

BA*/LDPE (110 microns) (* with polymer additives) (P2) were used for the packaging of Mozzarella cheese samples. A definite quantity (approximately 250 g) of MC was stored at $7 \pm 1^\circ\text{C}$ in high barrier bags under five varying conditions viz., air (C1), vacuum (C2), 100% CO_2 (C3), 100% N_2 (C4) and a mixture of 50% N_2 and 50% CO_2 (C5).

The hydrolysis of casein of Mozzarella cheese was determined using the technique described by Creamer (1991). The samples were electrophoresed using a mini gel electrophoresis unit (Techware, M/s Sigma Chemical, USA) based on low molecular weight (LMW) SDS-PAGE with power supply set at 210 volt (maximum) and 70 mAmpere (maximum). Approximately 0.5 grams of grated Mozzarella cheese samples were freeze dried in petriplates at 10 Mbar (25°C). Freeze dried sample of Mozzarella cheese (0.5 g) was transferred to centrifuge tube containing 25 ml sample buffer. The tube was then centrifuged at 10,000 g for 10 minutes and 2 mL of the centre portion was taken, heated in a water bath at 100°C for 90 seconds and bromophenol blue solution was added. The resolving and stacking gel were prepared using acrylamide, bis- acrylamide, glycerol, Tris base, Tris HCl and SDS as per standard procedures. The gel solution was decanted into Mini-Slab apparatus and overlaid with about 0.5 ml water. After the gel had set, the water was removed using tissue paper.

The stacking gel was mixed with 24 μl of TEMED, adjusted to 20°C , and 30 μl of ammonium per sulphate solution was added. It was then poured into the former and the comb was inserted. After setting, excess gel and the comb were removed and 5 μl of the 2 % cheese solutions carefully loaded into each slot. The chamber buffer containing SDS, glycine and Tris base was then put into the apparatus. The gel was then stained with 50 ml Coomassie brilliant blue R solution for one hour and destained for an hour, with 100 ml change of destaining solution. Proper care was taken to prevent excess removal of too much dye at the very first change. The gel was then photographed. All the tests were performed twice with three runs ($n = 2 \times 3$) of each sample.

The proteolysis in cheese during storage was studied using electrophoresis. The degree of casein hydrolysis of stored and formation of smaller peptides in MAP Mozzarella cheese was examined using LMW SDS- PAGE. A perusal of Photoplate I (electrophorogram prepared by using fresh Mozzarella cheese sample) revealed two bands for acid casein sample (lane 1) and two bands for fresh MC samples (lane 2). During refrigeration storage the degradation of protein started after four weeks with two major bands (Plate II) (Figure 1). However, there was no difference in the pattern of casein fractions of MAP MC during storage at $7 \pm 1^\circ\text{C}$ under 5 atmospheres (C1, C2, C3, C4 and C5)

upto 4 weeks. After 8 weeks of storage the intensity of bands decreased and lower molecular weight peptides increased. After three months of storage the intensity of bands and lower molecular weight peptides increased as shown in electrophorogram (Plate III) (Figure 1). It is clear from the Photo plate III that degradation of β - casein was not much, whereas α - casein degraded faster (C1) upto 6 weeks of storage. Similar results had been reported by various studies related to Mozzarella cheese (Farkye et al. 1991; Ghosh and Singh, 1996). Proportion of α_{s1} casein degradation to α_{s2} fraction was more prominent on 8 weeks in C2 and C4. This additional α_s bands (α_{s1} and α_{s2}) would have appeared due to degradation of casein by microbial presence.

At deep freeze storage condition casein of MC samples did not degrade upto 6 months. The degradation of α_s -casein started after 6 months (Photo plate IV) (Figure 1) and increased with the storage time till 12 months (Photo plate V) (Figure 1), regardless of package type and atmospheric conditions. The casein in deep frozen sample (-10°C to -15°C) didn't degrade upto 4 months of storage irrespective of the type of packaging material. The slight degradation in α_s -casein started after 6 months of storage and thereafter increased with the storage time.

These results pertaining to proteolysis of cheese samples during refrigeration storage ($7\pm 1^{\circ}\text{C}$) also showed that the soluble N_2 content was lowest in case of product that was packed under C3 followed by C5, C4, C2 and C1 respectively. An indirect proportional relationship between degree of proteolysis and hardness of cheese had been reported by Fedrick and Dullely (1984) and Fathollahi et al. (2010). Correspondingly, the decrease in hardness with storage time had been reported by Lawrence et al. (1987). However, Tunick et al. (1991) stated increase in hardness with decrease in moisture content of Mozzarella cheese ripened for varying storage period.

Conclusions

Electrophoretic plates reveal that there was no difference in the pattern of casein fractions of MAP Mozzarella cheese packages in all modified atmospheres upto 4 weeks irrespective of packaging during refrigeration storage. A lower degradation of β - casein was observed, whereas α - casein degraded till 6 weeks of storage period (C1). Proportion of α_s -I casein degradation to α_s -II fraction was more prominent during 8th week of storage in vacuum and 100% N_2 . At deep freeze storage condition casein of MC samples did not degrade upto 6 months.

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