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Effect of storage on the physico-chemical, color and microbiological properties of cheese prepared from stored pre-cheese

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Abstract
The investigation was carried out to evaluate the physico-chemical properties of Mozzarella cheese made from pre-cheese stored under atmosphere and vacuum conditions for 9 days. A significant difference (p<0.05) in the values for acidity, free fatty acid and soluble nitrogen content was observed. Acidity values increases from 0.51 to 0.54 % in atmospheric and 0.51 to 0.53% in vacuum stored pre-cheese, free-fatty acid value also increases i.e from 0.04- 0.24% in atmosphere and 0.04- 0.22% in vacuum packed stored pre-cheese, increase in soluble nitrogen content for the cheese made from atmosphere packed stored pre-cheese was 0.09-0.13% and 0.09-0.12% in vacuum stored. Meltability of cheese made from atmospheric stored pre-cheese increases form 3.98-4.70 cm and 3.98-4.40 cm in vacuum stored pre-cheese. Stretchability score was more for cheese made from pre-cheese stored in vacuum packed i.e 5 than atmosphere stored i.e 3 at the end of storage day. No significant difference in the values for color i.e a*, b* and l* was observed during storage period. It was concluded that increase in acidity, free-fatty acid and soluble nitrogen, stretchability value was more in case of cheese made from atmospheric stored pre-cheese. Meltability increases in case of atmospheric stored sample than vacuum stored. Overall acceptability was more in case of cheese made from vacuum stored pre-cheese than atmospheric stored. It was observed that mozzarella cheese prepared from stored pre-cheese was acceptable after 9 days of storage.

Keywords: mozzarella cheese, physico-chemical characteristics, pre-cheese microbiological quality and sensory quality

Introduction
Mozzarella cheese is the one of the most popular varieties of cheese in the world because of its primary use on the pizza topping (Kindstedt 2004) [15]. It occupies third place in Indian cheese market placed after processed cheese and cheese spreads. Mozzarella is a soft, unripened cheese variety of pasta filata family. It has moist, soft, elastic texture and lively surface sheen and when fresh posses a slightly salty taste and pleasant aroma. It also possesses a unique property called stretchability i.e. ability to form fibres or strings when hot. It is ideal for preparing a variety of food items especially as a topping on Pizza. Mozzarella cheese is made in many countries from cow milk, buffalo milk and even milk powder. The demand of Mozzarella cheeses is growing as the global demand for pizza and other foods has increasing manifold.

Milk production pattern faces great fluctuation/variation during the course of year. Production is abundant during winters and falls dramatically during summer months thereby lowering the fluid milk supply to population and widening the gap between supply and demand. During flush/glut season milk solids are conserved by processing them into a number of concentrated and dried products so that these can be put into use in lean period. A large number of such sources of milk solids i.e. concentrated milks, dried milks, casein powder and caseinates, whey powder and whey protein concentrates, dried cream, butter and butter oil/ghee are manufactured. These milk products have added advantages of longer shelf life than milk, guaranteed supply, uniform quality and improved microbiological quality.

In this work it is aimed to determine the important properties of mozzarella cheese made from fresh and stored pre-cheese stored under vacuum packaging (VP) and atmospheric packaging

(AP), and also to compare these two packaging techniques to be able to determine the more suitable packaging condition.

Materials and methods

Polyethylene bags used as packaging material was from local market, Ludhiana. Skim milk powder and cream used for the preparation of mozzarella cheese was purchased from local market and normal milk was obtained from dairy plant, Guru Angad Dev University, Ludhiana. Preparation of mozzarella pre-cheese was carried out in the Dairy plant Laboratory, department of food science and technology, P.A.U campus, Ludhiana.

Preparation of Mozzarella cheese: For preparation of Mozzarella cheese the milk was first filtered/clarified and then standardised with 14% total solids adjusting casein and fat ratio 0.7. The standardized milk was homogenized at 1000 Mpa, then pasteurized at 72^0 C for 15 sec and cooled to 25°C. The chilled milk was acidified with 5% citric acid to get pH of 5.7.

Renneting and cutting of curd: The temperature of acidified milk was raised to 30 °C. Then diluted microbial rennet was added @ 1g/100 litres of milk at 30^0 C milk. The renneted milk was left undisturbed for 30 min for setting of curd. The properly set curd was cut with sterilized cheese knives and then allowed to stand in whey for 5-10 min.

Cooking, stirring and draining: The temperature of curd was raised @1 °C for every 5 min till 41°C and maintained for 10 min with continuous stirring to achieve uniform cooking and to avoid lump formation. Then the whey was drained to aggregate the curd particles.

Stretching, moulding, brining: For proper stretching and elasticity, Mozzarella cheese was stretched in hot water 75 °C for 10 min and moulded, which were then immersed in pasteurised chilled (8–10 °C) brine solution (18% w/v) for 24 h.

Packaging materials: High barrier bags namely polyethylene bags were used for packaging.

Acidity: The titarble acidity was determined by titrating 10ml of sample against standard alkali (0.1N NaOH) using phenolphthalein as an indicator. The results were expressed as percent lactic acid.

Free fatty acids: The fat breakdown/ lipolysis in mozzarella cheese was determined by estimating free fatty acids (FFA) (per cent Oleic acid) adopting the procedure of (AOAC 2000) [3]. 5 g of sample was accurately weighed, ground and transferred into conical flask and 50 ml Benzene was added. Sample was shaken for 30 min on the rotary shaker. It was filtered and 5 ml of the filtrate was taken, to it 5 ml of ethyl alcohol was added. Then it was titrated against 0.02N KOH till light pink end point reached. Phenolphthalein (0.5%) was added as an indicator. The FFA value was calculated using the formula:

$$\text{FFA (\%Oleic acid)} = \frac{282 \text{ X vol of alkali used X N of alkali X 100 X 1000}}{\text{Weight of sample}}$$

Where, V= ml of 0.02 N KOH used
W= weight of the sample

Soluble nitrogen: Nitrogen soluble in 12% TCA-SN is indication of proteolysis. To estimate the degree of proteolysis in the mozzarella cheese, the method reported by Dave *et al.* (2003) [4] was followed. Mozzarella processed cheese was grated and 1.5 g sample was blended with 25 ml of TCA for 45sec and then transferred to a beaker. The blender jar was then washed with an additional 20 ml of 12% TCA to collect all nitrogen and the mixture (45 ml of 12% TCA and 1.5 g cheese) was left for a further 10 min for complete extraction of TCA soluble nitrogen. Each sample was filtered through Whatman No. 42 filter paper and the filterate was used to estimate nitrogen by Kjeldhal method.

Meltability: The Schreiber melt test was performed on mozzarella cheese samples using the modified method from Muthukumarappan *et al.* (1999) [13]. Samples were cut in the form of discs with 32mm diameter and 7 mm height, discs of equal weights were randomly selected and placed on aluminium plates, covered with a glass petri plates and tempered at room temperature for 30 min. The average weight of the discs was approx 8g. Tempered samples were then heated in oven at 100^0 C for 5 min. The meltability of cheese was determined by measuring the final diameter of the cheese discs at 4 different locations after they were cooled to room temperature and the average value was reported as meltability of cheese.

Stretch ability: Grated cheese (10g) was placed on a slice of bread (1x 10 x 10 cm) and heated for 1min in microwave oven and cooled for 1 min. The melted cheese was assessed subjectively for stretching by placing a fork into the cheese layer lifting and measuring the distance the cheese stretched without breaking (Lemay *et al.* 1994) [9] and ranking as followed over 30cm -5, 20-30cm – 4,10-20 cm –3, 10cm -2 and below 5 cm- 1.

Color measurement: Color analyses were carried out using Hunter Lab by assessing L*, a*, b* values. The instrument was calibrated with black and white standard plates before analysis. The reported data are the mean of four determinations.

Microbiological studies: Mozzarella cheese was analysed for TPC, *Escherichia Coliforms* by the method of Spencer and Spencer (2001).

Sensory evaluation: Mozzarella cheese made from stored pre-cheese was subjected to sensory evaluation by semi-trained panel of 10 judges on a 9-point Hedonic scale (Amerine *et al.* 1965).

Statistical analysis: The experiments were conducted with 3 replicates and completely andomized design (CRD) was adopted to calculate the stastical significance (Snedecor and Cochran 1968).

Results and discussion

Mozzarella cheese made from AP (atmospheric packed), VP (vacuum packed) pre-cheese were analysed on 3rd, 6th and 9th day of storage.

Physico-Chemical composition of cheese made from stored pre-cheese: Table 1 shows the physico-chemical composition of cheese made from stored pre-cheese. It was observed that at first day of storage acidity values for both the cheese samples from atmospheric and vacuum stored were same i.e. 0.51 per cent. A significant difference in the values of acidity was observed. On the last day i.e. the ninth day of storage there was significant increase in acidity value in both atmospheric and vacuum packed stored samples was observed. For atmospheric packed stored samples the value increased from 0.51 to 0.54 per cent and vacuum packed stored samples it increases from 0.51 to 0.53 per cent. It was concluded that increase in acidity was more in the stored samples packed under atmosphere than vacuum stored samples. The increase in acidity may be due to growth of microorganisms that fermented lactose to lactic acid.

Table 1: Effect of storage and packaging in the chemical properties of mozzarella cheese made from pre-cheese

Storage Days	Acidity (%)			Free fatty acids (% oleic acid)			Soluble nitrogen content (%)		
	Control	Atm. packed Cheese	Vac. packed Cheese	Control	Atm. packed cheese	Vac. packed cheese	Control	Atm. packed cheese	Vac. packed cheese
0	0.510	0.510	0.510	0.100	0.040	0.040	0.120	0.090	0.090
3	0.524	0.520	0.520	0.180	0.130	0.110	0.124	0.092	0.091
6	0.530	0.538	0.532	0.200	0.170	0.150	0.126	0.110	0.10
9	0.510	0.545	0.538	0.250	0.240	0.220	0.125	0.130	0.12
CD (5%)	A = 0.36 B = NS AB = NS			A = 0.15 B = 0.10 AB = 0.21			A = 0.17 B = 0.20 AB = 0.10		

A = Storage days; B = Type of packaging s; AB = Storage days x Type of packaging

Free fatty acids: There was significant increase in free fatty acid content of stored pre cheese (figure 2). At first day of storage the values for control was 0.10 per cent, pre-cheese packed under atmosphere was 0.04 per cent and pre cheese packed under vacuum was 0.04 per cent. During the storage period there was significant increase in the free fatty acid content of stored cheeses. At the end of storage day of 9 day the free fatty acid value increased from 0.10 to 0.25 per cent in cheese made from control pre-cheese, 0.04 to 0.24 per cent in cheese made from pre-cheese packed under atmosphere 0.04 to 0.22 per cent in pre cheese packed under vacuum sample. Free fatty acid contents of stored samples packed under atmospheric conditions were significantly greater than there for vacuum packed stored samples. The results were in line with those recorded by Alam and Goyal (2006) [1] during refrigerated storage of Mozzarella pre cheese. The increase in FFA value was due to enzymatic/bacterial action in cheese samples causing lipolysis during storage (Alam and Goyal 2006) [1]. Lower lipolytic breakdown occurred in vacuum packaged pouches because all the air was sucked out and an anaerobic environment was created which minimized microbial growth and enzymatic activities.

Soluble nitrogen: With increase in storage period, there was significant increase in the values from 0.12 per cent packed under atmospheric conditions at day 0 to 0.13 per cent at the end of 9 days in atmospheric control cheese made from stored pre-cheese, similarly soluble nitrogen value increased values increases from 0.09 to 0.13 per cent after a storage period of 9 days in atmospheric packed stored cheese sample and 0.09 to 0.12 per cent in vacuum packed stored cheese made from pre-cheese sample. The samples packed under atmospheric conditions has high soluble nitrogen values than those vacuum packed. The degradation of protein increased during storage there by the soluble nitrogen was increased. Alam and Goyal (2006) [1] reported that proteolysis was less in vacuum packaged cheese than atmospheric packaged cheese during storage at deep freeze conditions.

The increase in proteolysis could be due to the residual coagulatory enzymes produced by microorganisms (White and Marshall 1972, Micketts and Olson 1974) [19, 12].

Physical properties

Meltability and stretch ability: Table 2 shows effect of storage and packaging conditions on meltability and stretch ability values of cheese made from stored pre-cheese. The meltability increased significantly along with storage period and it was observed that meltability was more in cheese made from stored pre-cheese packed under atmospheric packaging. A significant ($p \leq 0.05$) difference was obsereved in the meltability values between the atmospheric and vacuum stored pre-cheese. Meltability values increased from 3.98 cm to 4.70 cm in cheese made from atmospheric stored pre-cheese and to 4.40 in vacuum stored pre-cheese. The increase in meltability of cheese during storage is in line with Tunick *et al*. (1997) [18] and Rudan *et al*. (1999) [15]. The increase in meltability was due to degradation of protein matrix of proteolysis. Tunick *et al*. (1997) [18] also reported that protein breakdown in mozzarella cheese was a result of residual coagulant and milk plasmin breakdown of αs_1-casein and αs_1-1 reduced cohesiveness and softened the body, thus increasing the meltability. Data regarding the stretch ability of Mozzarella cheese made from stored pre-cheese is given in the Table 2. The stretch ability score at 0 day for control cheese which was packaged under atmospheric condition and sample was 4 and the score was same for stored sample packed under vacuum. After storage of 9 days the stretch ability score for cheese sample made from stored pre-cheese under atmosphere decreased to score of 3 but remained 4 for atmospheric packed stored control. There was no significant change in the stretch ability score for cheese made from stored pre-cheese packed under atmospheric and vacuum conditions.

Table 2 Effect of storage and packaging on physical properties of mozzarella cheese made from pre-cheese

Storage days	Meltability (cm)			Stretchability (score)		
	Control	Atm. packed	Vac. packed	Control	Atm. packed	Vac. packed
0	4.25	3.98	3.98	4.00	4.00	4.00
3	4.28	4.25	4.10	4.00	4.00	5.00
6	4.32	4.30	4.20	4.00	4.00	5.00
9	4.36	4.70	4.40	4.00	3.00	5.00
CD (5%)	A = 0.16; B = NS; AB = 0.23			A = NS; B = NS; AB = NS		

A = Storage days; B = Packaging conditions; AB = Storage days x Packaging conditions

Color: The results for colour measurement are presented in the Table 3. The packaging type and storage did not significantly influenced the colour of cheese. At day 0 of storage the L* value of the cheese stored under atmosphere was 89.00 and vacuum packaged was 89.26. After the storage time of 9 days the L* value decreases from 89.00 to 88.50 in cheese made from atmospheric stored pre cheese form 89.26 to 88.68 in cheese made from vacuum packed pre cheese. It was observed that there was non-significant drop in L* value with the storage. a' value indicates the redness. In case of cheese made from atmosphere stored pre cheese it was observed that at 0 day value of atmospheric stored sample for was 1.27 and vacuum stored was 1.27; there was no such significant drop in the a values of color on sixth day of storage. As on sixth day the values for atmospheric stored sample dropped from 1.27 to 1.26 and vacuum stored from 1.27 to 1.23. At the end of storage day the values dropped to 1.25 in atmospheric stored samples and 1.23 in vacuum stored samples. But drop in values in case of vacuum packed were more than that of atmospheric stored. b* values of the stored samples changed during the storage period. At day 0 the value was 13.80 for atmospheric sample and 13.82 for vacuum sample. The b value of cheese made from stored pre cheese decreased from 13.80 to 13.79 in atmospheric storage on 3rd day of storage, but there was no change in values for vacuum packed cheese. At the end of storage day a significant result was observed the values changed from 13.80 to 13.76 in atmospheric storage and from 13.80 to 13.80 in vacuum stored cheese on 9th day of storage. Same results were reported by other researches like Favati *et al*. (2007) [6], who stated that during storage the colour was stabilized both in vacuum and atmospheric storage.

Table 3: Effect of storage and packaging on color values (L*, a* and b*) of mozzarella cheese prepared from pre-cheese

Storage days	L*		a*		b*	
	Atmosphere packed	Vacuum packed	Atmosphere packed	Vacuum packed	Atmosphere packed	Vacuum packed
0	89	89.26	1.27	1.27	13.80	13.82
3	88.97	89.23	1.26	1.25	13.79	13.82
6	89.64	88.72	1.26	1.23	13.78	13.81
9	88.50	88.68	1.25	1.23	13.76	13.80
CD (5%)	A = NS; B = NS		A = NS; B = 0.13		A = 0.16; B = 0.11	
	AB = NS		AB =NS		AB = NS	

A = Storage days; B = Packaging conditions; AB = Storage days x Packaging conditions

Microbiological analysis: The microbiological results are shown in Table 4. The storage period and packaging technique had significant ($p<0.05$) effect on the total plate count of cheese made from stored pre-cheese. There was significant increase in the total plate count (TPC) of cheese made from stored pre-cheese.

Total plate count of control sample at (Figure 14) Zero as day was $35x10^2$cfu/g and it was observed that total plate count of control increases upto $78x10^2$cfu/g after 9 days of storage period. In atmospheric storage samples the increases in total plate count was from $32x10^2$cfu/g to $76x10^2$cfu/g, where as in case of cheese made from vacuum stored pre-cheese the increase was from $31x10^2$cfu/g to $74x10^2$cfu/g. A significant increase in the TPC was observed in all the cheese sample made from stored pre-cheese. But it was noted that increase in TPC was more in case of control cheese than cheese made from atmospheric stored cheese and vacuum stored cheese. The increase in microbial count in cheese may be due to its equipment handling, air or on humans that had direct contact with the fluid milk or cheese (Marth and Steel 2005) [10]. According to Donnelly (2006) [5] the intrinsic factors that dictate the potential for microbes to grow, persists or decline in the cheese were moisture content, pH and acidity, nutrient potential, presence of antimicrobial compounds and competitive microflora. Extrinsic factors included such as type of packaging, time and temperature of storage and holding conditions, processing steps and product history. The interaction of these factors dictated the potential for microbial growth in the cheese. Table shows that the pathogen namely *coliforms* was absent in the first and subsequent days of storage in all the cheese samples. Even at the end of storage day ninth day there was no growth of *coliforms* was observed. This may be because of high temperature (75°C) processing, low pH and sterilized packaging of cheese (Ryser and Marth 1987) [16] (Marth and Steel 2005) [10].

Table 4 Effect of storage and packaging on microbiological quality (*E. coli* and TPC) of mozzarella cheese made from pre-cheese

Storage Days	*E. coli*			TPC (10^2cfu/g)		
	Control	Atmosphere packed cheese	Vacuum packed cheese	Control	Atmosphere packed cheese	Vacuum packed cheese
0	ND	ND	ND	35	32	31
3	ND	ND	ND	48	46	42
6	ND	ND	ND	59	55	51
9	ND	ND	ND	78	76	74
CD (5%)	A = NS; B = NS; AB = NS			A = 0.17; B = 0.24; AB = 0.34		

A = Storage days; B = Packaging conditions; AB = Storage days x Packaging conditions

Overall acceptability: It was observed from the table and figure that overall acceptability score for cheese made from stored pre-cheese decreased significantly ($p<0.05$) with the storage period (Fgure13). At zero day of storage the highest score for overall acceptability was for vacuum cheese sample 8.33, followed by control cheese 7.5 and then atmosphere cheese 7.17. As storage progresses at 3rd day the overall acceptability score for the samples decreases from 7.5 to 7.33 in control cheese, 8.33 to 8.17 in vacuum stored cheese and 7.5 to 6.33 in atmosphere stored cheese samples. The decrease in the overall acceptability of cheese made from stored pre-cheese may be due to proteolysis during storage and it was more in case of sample stored under atmospheric condition. At last ninth day of storage period it was noted that overall acceptability score decreases from 8.33 to 7.83 in vacuum stored sample and 7.5 to 6.33 in control followed by 7.17 to 7.00 in case of atmosphere stored sample. The more decrease in overall acceptability score was in case of cheese made from Atmospheric stored sample followed by control.

Table 5 Effect of storage and packaging on the overall acceptability of mozzarella cheese prepared from pre cheese

Storage days	Packaging		
	Control	Atmosphere packed cheese	Vacuum packed cheese
0	7.50	7.17	8.33
3	7.33	7.5	8.17
6	7.87	7.67	8.17
9	6.33	7.00	7.83
CD (5%)	A=0.21; B=0.12; AB=NS		

A = Storage days; B = Packaging conditions; AB = Storage days x Packaging conditions

Conclusion

Based on the results of physco-chemical, microbial and sensory analyses of cheese, ade from atmospheric and vacuum packed pre-cheese for 9 days, it is possible to express that shelf-life of mozzarella cheese can be extended. Based on the sensory scores and other parameters it was found that vacuum packed cheese was more acceptable than atmospheric packed cheese. The sensory scores for vacuum packed cheese samples were more than atmospheric stored samples because of formation of oxidized flavour and microbial load was low for vacuum packed cheese samples. And also, as a result the packaging type whether vacuum packed or atmospheric packed didn't significantly affected acidity, stretchability and color. This situation may be result of vacuum applied on the pre-cheese.

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