

Preparation and Quality Evaluation of Mozzarella Cheese from Different Milk Sources

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Three mozzarella cheeses were prepared from three different milk sources i.e. cow (C), buffalo (B) and their mix (A) milks using DL (Streptococcus lactis and cremoris; S. Diacetalactic, Leuconostoc cremoris) and yoghurt cultures. Milk was standardized to C/F: 0.90/0.92 and process optimized for using all milk sources. The samples were analyzed for physico-chemical and functional properties, sensory attributes and protein and fat losses. Except ash and yield at 50% moisture; fat, protein, actual yield, pH, acidity and moisture were significantly influenced by milk sources. From sensory result, cow and mix cheeses were significantly superior, whereas functional properties were superior for cow cheese but buffalo and mix cheeses had higher nutritive value. From overall comparison, cow mozzarella cheese was ranked most suitable for pizza topping.

Keywords: Mozzarella cheese, Process optimization, Yield, Chemical and functional properties, Sensory quality

Introduction

Cheese making is simply a separation of curd from whey and kneading into a mouldable consistency. Cheese is a concentrated form of milk consisting of fat, protein, salt, minor milk components and moisture (Lincourt *et al.*, 2009). The important varieties of cheese produced in Nepal are yak cheese, Kanchan cheese, mozzarella like cheese and processed cheese (Colavito, 1994).

Mozzarella cheese is an unripened, soft and white cheese whose melting and stretching properties are highly suitable for pizza making (Kindstedt, 1995). It belongs to the family, classified as 'Pasta-filata' which involves the principles of skillfully stretching the curd in hot water to get smooth texture, and lively surface in cheese (Kosikowski, 1982). World cheese production has doubled in less than 25 years from 1961-1994 (Farkye, 2004). Industrial cheese production is continuously growing. World cheese production in 2004, 2005, and 2006 were 28.4, 29 and 29.8 million MT respectively (IDF, 2007). Mozzarella cheese production in 2007/08 in Nepal was 22000 kg/year. The projected production for the year 2008/09 was 66000 kg/year (Bajracharya, 2009) (by Personal Communication). The demand for mozzarella cheese all over the world is increasing due to expansion of pizza parlours and fast food chains. In Nepal, cheese is produced mainly from cow milk. The use of pure buffalo milk for cheese production in Nepal is not successful in commercial scale. Therefore, to optimize a process using different milk sources and improve quality of local cheese is the dire need of time. Buffalo's milk is ranked second in the world after cow's milk being more than 12% of the world's milk production (Ahmed *et al.*, 2008). In Nepal, buffalo milk is 70% of the total milk produced. Due to high vitamin A, protein and low cholesterol in buffalo milk, it can be more preferred species in cheese production (Zicarelli, 2004). The fat and protein content in cheese made of buffalo

milk is higher (Sameen *et al.*, 2008) due to higher total solids in buffalo milk than cow milk (Ganguli, 1992). Mozzarella cheese produced from buffalo milk is highly priced in most of the world (Sameen *et al.*, 2008). No researches have been conducted in Nepal in the field of mozzarella cheese making using cow, buffalo and their mix milk. Due to various reasons, the quality of mozzarella cheese made locally in Nepal does not remain consistent (Acharya, 2010). Some commercially produced cheeses have poor functional quality and yield. The best milk source for mozzarella cheese making for use in pizza topping is not known. Also no standard process has been developed to manufacture mozzarella cheese of good quality from cow, buffalo and milk of both.

Preparation and quality evaluation of mozzarella cheese from different milk sources can be a boost to the dairy farmers and industries. This can provide answer to the problem which milk source is best for manufacturing pizza topping mozzarella cheese. The study also helps the farmers to prepare mozzarella cheese traditionally at farm level. A process optimization in Nepalese context will be a boon to Nepal for producing consistent mozzarella cheese. The process standardization for buffalo milk will solve the problem related to cheese production and a good competent quality cheese can be launched commercially in market.

Materials and Methods

Milk samples: Cow (hybrid i.e. cross of Jersey and local cow) and buffalo, (local i.e. buffalo from Terai region of Nepal) milk samples were obtained from local area of Dharan, Sunsari, Nepal.

Starter culture and rennet: The starter culture, DL culture was obtained from DDC, Kathmandu and the yoghurt culture from DDC, Biratnagar. Rennet was collected from local cheese makers of Pashupatinagar, Illam.

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Methodology

The basic cheese making procedure was followed according to Lincourt *et al.*, (2009) with slight modifications (Barbano *et al.*, 1994; FAO 1995; Innovations in dairy 1998, 1999; Adhikari and Bhandari, 2004 and Basnet, 2009) for different milk sources which is given in Figure 1, 2 and 3.

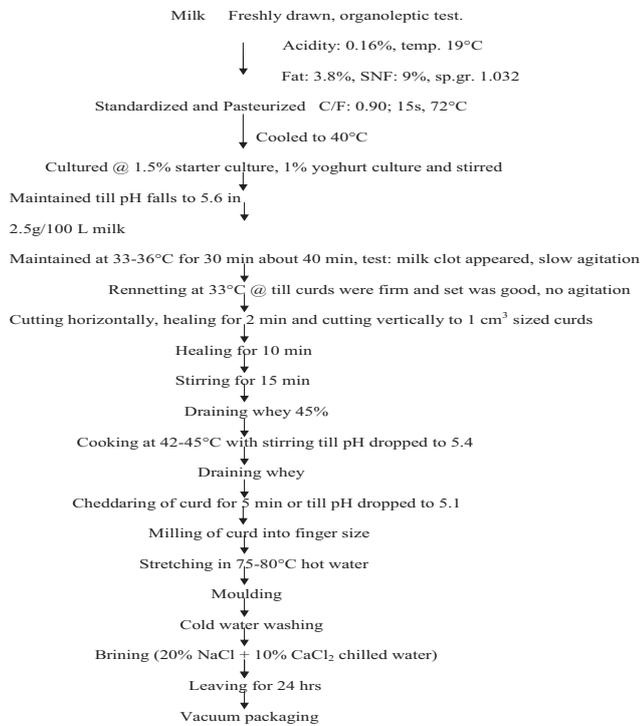


Figure 1. Preparation of Mozzarella cheese from cow milk

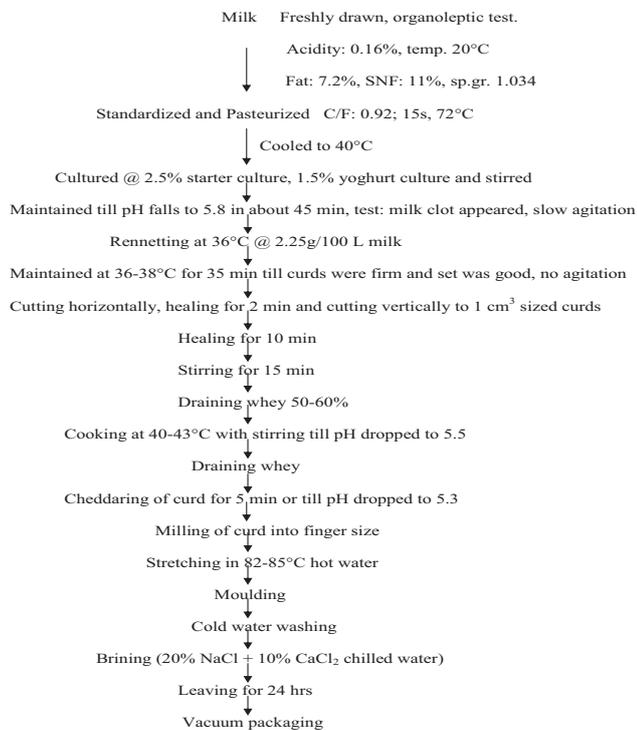


Figure 2. Preparation of mozzarella cheese from buffalo milk

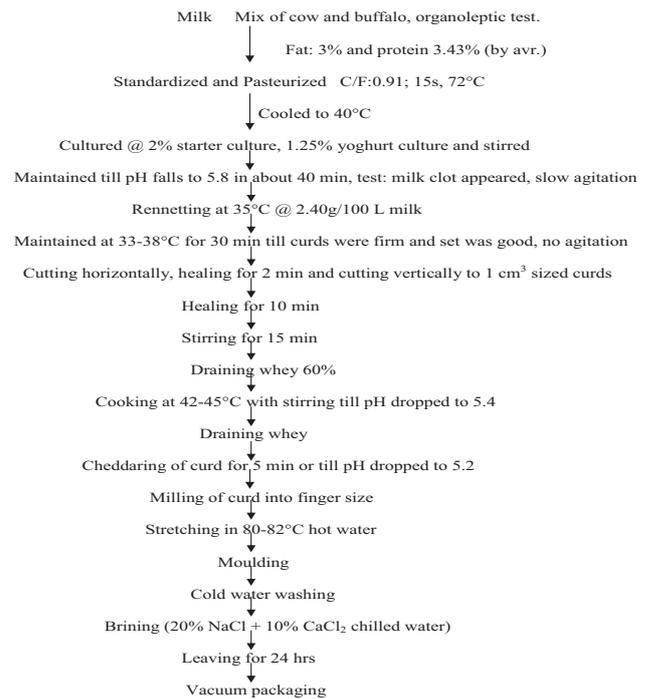


Figure 3. Preparation of mozzarella cheese from mix milk

In this study, milk was standardized to 3% fat and casein 2.72 - 2.76% having C/F 0.90 - 0.92. It was pasteurized to 72°C for 15 seconds and cooled to 40°C. It was cultured by 1.5-2.5% of starter culture and 1-1.5% of yoghurt culture with continuous agitation (1.5, 1% for cow milk, 2.5, 1.5% for buffalo milk and 2, 1.25% for mix milk respectively) to pH 5.6-5.8 (5.6 for cow, 5.8 for buffalo and 5.8 for mix milks respectively). Then the coagulum was cut first horizontally and then vertically. After 5-10 min of healing, stirring was done for 10-15 min. About 45-60% of whey was drained. The temperature of curd was slowly raised from 38°C to 42-45°C with stirring to expel whey until pH reaches 5.4-5.3. Whey was then drained. The curds were left for matting or cheddaring till pH drops 5.3-5.0. The matted curds were milled by the knife into a finger sized cut. Hot water @ 75-85°C (82-85°C for buffalo curd, 80-82°C for mix curd and 75-80°C for cow curd) was added to curds. The mass was then stretched and molded to shape. The hot, molded cheeses were cooled in chilled water and brined in saturated salt solution (20% NaCl) with 10% CaCl₂ for 24 hrs or till desired salt level. Then it was vacuum packed in the sterile plastic (LDPE, 200µm) pouch, weighed and cooled to 5°C in freezer.

Sampling: The sampling method of analysis was according to NDDDB, 2001. Cheese samples were stored at 5°C±2°C until analyses were completed.

Chemical analysis: Fat content was determined by Gerber method (NDDDB, 2001) in milk and by Van Gulik method (NDDDB, 2001) in cheese; total protein by Formal titration in milk and by Kjeldahl method in cheese; pH by a pH meter both in milk and cheese (NDDDB, 2001); acidity in milk as per NDDDB, (2001) and in cheese as per AOAC, (2005); calcium contents by volumetric

method (K.C. and Rai, 2007); ash content as per Ranganna, (2000); salt content as per NDDDB, (2001); moisture contents by oven drying method, DDC (1979) and yield as per Van Slyke and Price, (1979).

Functional properties

Meltability: A suitable modification on Schrieber test was made for the testing of the mozzarella cheese meltability.

Stretchability: Stretchability test was carried out based on the principle of 'stretch test' (Kosikowski, 1982) modified by Ghosh and Singh, (1990) due to its simplicity.

Sensory evaluation of cheese: Mozzarella cheese samples were evaluated by a selected panel of 9 judges on a 9-point hedonic rating scale for appearance, texture, flavor, taste and overall acceptability according to Ranganna, (2000).

Statistical data analysis: The data were analyzed by Genstat programming (Genstat Discovery version) at 5% level of significance. The means were compared using LSD and the best treatment was selected.

Results and Discussion

Preliminary trials: During the preliminary trials for different milk sources used in the experiment. Different amounts of

culture (range 1-1.5%) and rennet (2-2.5g/100L of milk) were used to prepare cow, buffalo and mix cheeses. The renneting temperature, stretching pH and temperatures for different milks used were varied in some trials. The stretchability, fat losses in whey and stretched water, protein retention and losses, flavor and taste profile (i.e. bitterness) and texture (i.e. smooth, fibrous and shiny or coarse, rubbery and tough) were taken as the basis for optimization of the process.

The key conclusion from the preliminary trials has been shown in flow diagrams in (Table 1 and Figure1, 2 and 3) for different milk sources which were the optimum conditions for mozzarella cheese preparation using different milk sources. In general, it can be concluded that culture of 1.5-2.5% starter culture and 1-1.5% yoghurt culture, rennet 2-2.5 g/100L milk, renneting temperature of 33-36°C, initial whey drainage of 45-60%, cooking temperature of 40-45°C, stretching pH of 5.1-5.3 and stretching hot water of 75-85°C were optimum conditions for preparation of mozzarella cheese from cow, buffalo and mix milk sources.

Cheese yields: The average actual yield of mozzarella cheese was 11.02% whereas the average yield for the cheese adjusted to 50% moisture was 10.75% (Table 2).

Table 1. Key conclusion for preparation of cow, buffalo and mix mozzarella cheeses

Parameters	Cow cheese	Buffalo cheese	Mix cheese
Milk (C/F)	0.90	0.92	0.91
Culture (Starter + Yoghurt)	1.5% + 1%	2.5% + 1.5%	2.0% + 1.25%
pH falls	5.6 in 40 Min	5.8 in 45 Min	5.8 in 40 Min
Renneting (T°C @ g/100L milk)	33°C @ 2.5g	36°C @ 2.25g	35°C @ 2.40g
Keep	33-36°C/ 30 Min	36-38°C/ 35 Min	33-38°C/ 30 Min
Draining whey	45%	50-60%	60%
Cooking	42-45°C / pH 5.4	40-43°C / pH5.4	42-45°C / pH 5.4
Cheddaring of curd	5 Min/ pH 5.1	5 Min/ pH 5.3	5 Min/ pH 5.2
Stretching water	75-80°C	82-85°C	80-82°C

Table 2. Yield of mozzarella cheese from different milk sources

Parameters*	A	B	C	LSD
Yield (%)	10.94 ^a (0.29)	10.71 ^a (0.38)	11.45 ^c (0.23)	0.302
Yield at 50% MC	10.63 ^a (0.07)	10.84 ^a (0.33)	10.77 ^a (0.02)	0.1912

*Mean having different superscripts letters are significantly different (p<0.05), Standard deviations are in parentheses. A-Sample of cow and buffalo milk, B- Sample of buffalo milk C- Sample of cow milk, MC, Moisture content.

Significant difference ($p < 0.05$) was found in the actual yields between A and C and B and C but non significant in yield between A and B. Significant difference in the actual yield of the cheese might be due to the increase in the moisture content of sample-C. Non significant difference was found in yield at moisture adjusted to 50% for all samples. Despite non-significant differences in the yield between the samples, sample-C can be regarded as better in terms of economic aspects. Cow milk having lower solids is easier to manufacture cheese requiring less culture and manufacturing time. This saves great deal of cost and energy.

Chemical composition: The chemical composition of mozzarella cheese prepared from different milk sources has been shown in Table 3. The prepared cheeses were in accordance with the Codex Standards 262-2007, (2009) for mozzarella cheese. From the time period of addition of coagulant to stretching was 109, 120 and 107min, respectively, for mix, buffalo and cow milk cheeses (Table 3). The moisture retained was decreased significantly ($p < 0.05$) as time period longer.

One of the approaches to increase cheese moisture was to shorten the total time for cheese-making process, thus decreasing the time for syneresis and increasing the moisture content of the cheese. To shorten total cheese-making time, the amount of culture was increased for faster acid development (Barbano *et al.*, 1994).

Moisture and fat contents were within the legal requirements for mozzarella cheese. The pH, acidity and salt concentration

of these cheeses were within the range for commercial cheeses. There was significant difference ($p < 0.05$) between samples in salt content. Similarly, there was significant difference ($p < 0.05$) between the samples for calcium content. Cow cheese with highest moisture content had low calcium content (485 mg/100g) compared to others. The moisture content was found inversely related to calcium content of the cheese. This complies with Keller *et al.*, 1973; Barbano *et al.*, 1994; Joshi *et al.*, 2004 and Sameen *et al.*, 2008.

Similarly, no significant difference ($p < 0.05$) was found in fat content between sample A and B on dry basis whereas significant difference was found between samples A and C and B and C. Similarly, significant difference was found in protein content between sample A and C on dry basis. This would be due to higher total solids in buffalo milk than cow milk (Ganguli, 1992).

Fat and protein retention in cheeses: The samples differ significantly ($p < 0.05$) in terms of fat and protein retention (Table 4). The average fat retention in this study was somewhat lower (80%). It might be due to lack of homogenization (Demott, 1983).

Significant difference ($p < 0.05$) was found in fat lost in whey and stretching water (Table 5) between samples A, B and C. Similarly, significant differences ($p < 0.05$) in protein losses was found for samples A and B with C; the losses being highest for cow cheese. The lower protein retention and higher losses might be due to soft curd in cheese making.

Table 3. Chemical composition of mozzarella cheese from different milk sources

Parameters*	A	B	C	LSD
MC (%)	51.41 ^a (1.30)	49.41 ^b (1.77)	53.43 ^c (0.95)	1.348
Fat (% db)	45.62 ^a (0.72)	45.85 ^a (0.09)	42.41 ^c (1.34)	0.859
Protein(% db)	49.59 ^a (2.57)	48.30 ^{ac} (1.18)	46.76 ^c (1.57)	1.823
M:P	2.17 ^a (0.09)	1.93 ^b (0.30)	2.45 ^c (0.06)	0.183
Ash	3.86 ^a (0.11)	3.92 ^a (0.28)	3.79 ^a (0.09)	0.181
Salt (%)	1.76 ^a (0.05)	1.80 ^b (0.06)	1.71 ^c (0.07)	0.002
Ca(mg/100g)	503 ^a (5.65)	512 ^b (8.63)	485 ^c (10.87)	8.42

*The values with different superscripts letters in same row are significantly different ($p < 0.05$). Figures in the parentheses are the standard deviation. A-Sample of cow and buffalo milk, B- Sample of buffalo milk, C- Sample of cow milk, MC, Moisture content

Table 4. Fat and protein retention of mozzarella cheese from different milk sources

Parameters*	A	B	C	LSD
Fat retention (%)	81.07 ^a (1.84)	83.83 ^b (1.460)	75.25 ^c (1.81)	1.67
Protein retention (%)	77.22 ^a (2.67)	76.53 ^a (2.57)	72.96 ^c (1.73)	2.302

*The values with different superscripts letters in same row are significantly different ($p < 0.05$). Figures in the parentheses are standard deviation. A-Sample of cow and buffalo milk, B- Sample of buffalo milk, C- Sample of cow milk

Table 5. Fat and protein losses in mozzarella cheese in different milk sources

Parameters*	A	B	C	LSD
Fat lost in whey (%)	12.1 ^a (0.35)	9.84 ^b (0.55)	13.9 ^c (0.69)	0.539
Fat lost in stretched water (%)	5.88 ^a (0.27)	6.2 ^b (0.31)	8.7 ^c (0.38)	0.318
Protein lost (Overall %)	22.77 ^a (2.67)	23.46 ^a (2.57)	27.04 ^c (1.73)	2.302

*The values with different superscripts letters in same row are significantly different ($p < 0.05$). Figures in the parentheses are standard deviation. A-Sample of cow and buffalo milk, B- Sample of buffalo milk, C-Sample of cow milk

Acidity and pH: The pH, acidity and calcium content of cheese are the important parameters in cheese structure, texture, functionality and flavor quality (Table 6). Significant differences were found in acidity and pH of all cheese samples at $p < 0.05$. Statistically, pH of the sample B was significantly higher than sample A and C. The higher pH in sample B could be the reason that it contains higher calcium content than others.

Similarly, acidity of the sample B was significantly lower than A and C which have non-significant difference ($p < 0.05$). High acid (or low pH) cheeses A and C have less calcium in finished cheese. Hence, they are fewer firms and have high functional properties. The flavor, taste and overall acceptance for high acid cheeses A and C were significantly higher than low acid cheese B.

Cheese firmness is associated with its calcium content, and its firmness decreased as calcium content decreases. During the cheese making process, the decrease in curd pH plays an important role in solubilizing the colloidal calcium phosphate

from the casein matrix, thus freeing a large proportion of calcium from the curd, losing it to the whey (Joshi *et al.*, 2003). Because firmness is a highly influential attribute on shredding quality, it is important to retain sufficient calcium in the cheese so it is not too soft and gummy when shredded.

The acidity on the other hand prevents the growth of spoilage organisms, affects the activity of coagulant during manufacturing and ripening, solubilizes the colloidal calcium phosphate, promotes syneresis and influences the activity of enzymes. Thus it affects cheese texture and flavor quality (Sameen *et al.*, 2008).

Functional properties: The functional properties of the mozzarella cheese prepared by using different milk sources have been presented in Table 7. Significant difference ($p < 0.05$) was found in meltability for samples A, B and C. Significant difference was found in stretchability for samples B and C only.

Table 6. Acidity and pH in mozzarella cheese in different milk sources

Parameters*	A	B	C	LSD
Acidity	0.76 ^a (0.02)	0.73 ^b (0.02)	0.78 ^a (0.01)	0.0232
pH	5.15 ^a (0.07)	5.21 ^b (0.06)	5.07 ^c (0.04)	0.058

*The values with different superscripts letters in same row are significantly different ($p < 0.05$). Figures in the parentheses are standard deviation. A-Sample of cow and buffalo milk, B- Sample of buffalo milk, C-Sample of cow milk

Table 7. Functional properties of mozzarella cheese from different milk sources

Parameters*	A	B	C	LSD
Meltability	4.05 ^a (0.11)	3.06 ^b (.027)	4.33 ^c (0.26)	0.2442
Stretchability	3.50 ^a (0.83)	3.00 ^a (0.81)	4.50 ^{ac} (0.83)	1.054

*The values with different superscripts letters in same row are significantly different ($p < 0.05$). Figures in the parentheses are standard deviation. A-Sample of cow and buffalo milk, B- Sample of buffalo milk, C-Sample of cow milk

Meltability and fat leakage: Meltability of different cheeses is presented in Table 7. On 5th day of storage, sample C had the maximum meltability followed by A and B which had 4.33, 4.05 and 3.06 respectively. The cheese sample C had highest meltability ratio due to the decreased calcium content and due to higher amount of moisture present (Mc Mahon and Oberg, 1990; Fife *et al.*, 1996) compared to sample B and A.

The fresh cheese was typically firm and has poor melting properties and although it was stretchable. However as the cheese matures, the texture softens and there is an increase in melt (Rowney *et al.*, 1999). The improvement in meltability is due to dislodgement of para-casein matrix (Sheehan and Guinee, 2004). As the time period increased the meltability of all cheese samples increased significantly but the trend of increase remained same (Sameen *et al.*, 2008).

There was significant difference ($p < 0.05$) between samples in salt content. Melting profile can be correlated to fat leakage i.e. (free oil formation) and salt content. In this study, meltability was highest with cow cheese having low salt content (hence increased fat leakage) compared to mix and buffalo cheese (Kindstedt and Kailey, 1992). The present study was performed with unhomogenized milk. Unhomogenized state of milk fat also increases fat leakage (Tunick, 1994; Rudan *et al.*, 1999).

Stretchability: The statistical analysis shows that the samples were significantly different ($p < 0.05$). The LSD values indicate that the sample C was significantly different from sample B but there was no significant difference between samples A and B and A and C. Stretchability of sample C was found to be highest as shown in Table 7. According to Joshi *et al.*, (2004), decrease in the calcium content would lead to the decreased structural rigidity of the cheese matrix consequently increasing the stretchability. The obtained result complies with the Gunasekaran and Kuo, (2002), who concluded cheese with greater meltability had the higher stretchability.

There was significant difference ($p < 0.05$) between samples for moisture to protein ratio. The ratio was found to be highest for sample C followed by A and B. With the increase of M: P ratio, the functionality of mozzarella cheese is found to be increased. This complies well with Merrill *et al.*, 1994 and Fife *et al.*, 1996.

Sensory evaluation of cheese: The sensory scores of the mozzarella cheese are Graphically in Table 8. The statistical analysis shows that the flavor, texture, taste and overall characteristics are significantly different ($p < 0.05$). Due to variation in milk sources, appearance was not significantly affected ($p < 0.05$). Appearance of the cheese samples was found to be white and shiny. The texture of the cheese sample was soft. However, sample B was slightly hard and rubbery. This might be due to the higher calcium content in the cheese.

Among sensory attributes the flavor is considered to be the most important factor in determining consumer's response. Flavor of all samples on 5th days of storage improved because during ripening the metabolic processes are responsible for the basic flavor and texture changes (Smith *et al.*, 2005). In cheeses when biochemical reactions continued for breakdown of fat and protein by activity of microbial and residual rennet more flavoring compound were produced and casein was hydrolyzed which give smooth texture (Barbano *et al.*, 1994). Most of the panelists liked the flavor of sample C followed by A and B. Similarly, proportionately higher amount of lactic acid produced by the natural flora of mix milk sample A could be the reason for the highest score for taste. Slight bitter taste was observed with the sample B. This might be due to higher amount of calcium content as compared to others. The sample C had the second higher score for taste. The results revealed higher overall acceptance, flavor, texture and taste for cow and mix cheese than buffalo cheese (Table 8).

Table 8. Mean score of sensory attributes of mozzarella cheese

Parameters*	A	B	C	LSD
Flavor	7.67 ^a (1.00)	6.77 ^b (0.83)	7.77 ^a (0.83)	0.703
Appearance	7.66 ^a (1.11)	7.00 ^a (0.86)	7.88 ^a (0.78)	0.983
Taste	8.33 ^a (0.71)	7.00 ^b (0.86)	7.66 ^{ab} (1.00)	0.79
Texture	7.55 ^a (0.52)	7.00 ^a (0.71)	7.33 ^a (0.71)	0.732
Overall	8.00 ^a (0.86)	6.67 ^b (1.00)	8.00 ^a (0.71)	0.865

*The values with different superscripts letters in same row are significantly different ($p < 0.05$). Figures in the parentheses are standard deviation. A-Sample of cow and buffalo milk, B- Sample of buffalo milk, C-Sample of cow milk

Conclusion

Cheese made from buffalo and mix milk had higher nutritive value i.e. higher fat and protein content than cow cheese. Cheese made from cow milk had higher functional scores. Hence it is more suitable for pizza topping than cheese from buffalo and mix milk. On sensory evaluation, cheese made from cow and mix milk had higher overall scores. Hence mix cheese can alternately used to pizza topping. The fat and protein losses in all cheese samples were minimized by bringing variation in process, technology and ingredients for mozzarella cheese making which were better than commercial process employed. A process has been optimized to produce mozzarella cheese from different milk sources of uniform composition.

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