



## Combined Effect of Milk Source and Acidification Method of Cheese Milk on Properties of Mozzarella Cheese

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### ABSTRACT

A 3×3 factorial arrangement of treatments in a completely randomized design was used to find out the combined effect of milk source {cow (CM), buffalo (BM) and mixed (CM:BM 1:1 ratio)} and the method of acidification {starter culture (SC): *Streptococcus thermophilus*, acetic acid (AA) and citric acid (CA)} of cheese milk on properties of Mozzarella cheese. Cheese made using BM and acidified with SC served as the control. Main effects of milk source and method of acidification of cheese milk showed a significant effect on yield of cheese whereas interaction effect was not observed. A significant interaction effect between milk source and method of acidification of cheese milk was observed for fat and protein percentages, meltability, *b*\* value and sensory properties of resultant cheese. Fat and protein content was significantly higher in cheese made from BM acidified using AA and in control, respectively. Meltability was superior in Mozzarella cheese manufactured from CM acidified using CA compared to the control. Sensorily, Mozzarella cheese manufactured from mixed milk (CM: BM 1:1 ratio) acidified using CA obtained the highest mean score for all the sensory attributes and was significantly superior compared to the control. Therefore, the milk source and the method of acidification of cheese milk are closely linked to the yield and the quality characteristics of Mozzarella cheese and hence, careful selection of raw materials and manipulation of processing conditions are required to get an optimum quality end product.

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### Introduction

Mozzarella is a soft, un-ripened variety of cheese originated in Battipaglia region in Italy and belongs to pasta-filata or stretched group of cheeses (Jana and Mandal, 2011). In pasta-filata cheeses, curd is first made according to the normal cheese production process and then placed in hot water or whey to make a solid mass and then it is converted into a uniform and elastic cheese mass by stretching. Among the different varieties of cheeses produced around the world, Mozzarella has shown a remarkable growth in production over the last century (Gwenole et al., 2010). The global Mozzarella cheese market was valued at US\$ 21.72 billion in 2018 and is projected to grow at a healthy Compound Annual Growth Rate (CAGR) of 4.4% over the forecasted period from 2019 to 2025 (Anon, 2020). Mozzarella cheese is used with different food products including veggies, meat products, beer, pizza and wine. It has been reported that increase in the demand for pizza, rapid changes in consumer behavior and changes in the normal lifestyle of people have contributed to the increase in Mozzarella cheese production (Abbas et al., 2014).

The properties of cheese including Mozzarella depend on a number of factors such as type of milk used for cheese making, manufacturing protocol including acidification method of cheese milk, type of starter culture (SC) and rennet enzyme used, plasticizing treatment, etc. (Rowney et al., 1999; Jana and Mandal, 2011). Mozzarella cheese is traditionally manufactured from buffalo milk because of its characteristics flavor (Ghosh et al., 1990). However, due to the lack of production of buffalo milk in many countries in the world, milk of other animals mainly cow has been successfully attempted its production. Further, Sameen et al. (2008) reported that Mozzarella cheese made from buffalo milk is expensive in many regions of the world. As a result, some dairy manufacturers mix buffalo milk with commonly available cow milk, for the production of Mozzarella cheese (Lopez-Calleja et al., 2005). Ahmad et al. (2008) reported that total cow milk production in the world in 2018 was about 683 million tons. It has been reported that, the estimated total cow and buffalo milk production was 385.7 and 85.9 million liters, respectively in Sri Lanka in 2018 (Central Bank Annual Report, 2018). Therefore, buffalo milk production in Sri Lanka is also

second to the cow milk production as in many other countries in the world.

Milk acidification is one of the most important steps in cheese manufacture which has a profound effect in assuring the desirable cheese curd characteristics. In here acids and/or SCs are added to the milk before processing it into cheese and it has an additional preservative effect as well, preventing the growth and activity of many pathogenic and spoilage bacteria (Sameen et al., 2010). Though, the cultures used to make fermented milk products are available in different forms (Zahoor et al., 2003), they are expensive and delay the production process and final product quality depends on the culture performance. Therefore, for manufacture of cheese, direct acidification method has gained considerable commercial interest as it does not rely on starter performance (Seth and Bajwa, 2015).

Many attempts have been made to produce Mozzarella cheese using direct acidification method. Keller et al. (1974) used phosphoric, acetic, hydrochloric, mallic and citric acids; Najafi et al. (2006) used lactic acid and citric acid; Seth and Bajwa (2015) used acetic, citric and lactic acids to obtain desired pH levels to make Mozzarella cheese. They have reported significant changes in the properties of Mozzarella cheese including changes in curd characteristics, rate of curd formation, rennet coagulation action, moisture content, mineral retention, rheological properties, yield and elasticity etc. due to the acidification method. However, studies related to combined effect of milk source and acidification method of cheese milk to investigate their effect on Mozzarella cheese yield and quality is lacking in the past literature.

Taking into consideration the above mentioned facts, the present investigation was undertaken to find out the combined effect of milk source and the method of acidification of cheese milk on yield, compositional, physicochemical, functional and sensory properties of Mozzarella cheese.

## Materials and Methods

### Materials

Fresh cow milk was obtained from the milking herd of the Faculty of Agriculture, University of Ruhuna, Sri Lanka and buffalo milk from a buffalo farm in the surrounding village. Thermophilic lactic culture (STI- 12) containing *Streptococcus thermophilus* and microbial rennet (CHY-MAX Extra) was obtained from Chr. Hansen, Denmark. Food grade acetic acid (AA), citric acid (CA) and sodium chloride (NaCl) were obtained from the local market.

### Manufacturing of Mozzarella Cheese

Mozzarella cheese was manufactured according to the method proposed by Arora et al. (2019) with slight modifications at the laboratory of the Department of Animal Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka. Raw milk from buffalo, cow and mixture thereof (1:1) were standardized separately into 3% fat after cream separation. The standardized raw milk samples were vat pasteurized (63°C/30 min) and were divided into three equal parts of 4 L. One part was cooled down to 31°C and other parts were cooled down to 4-8°C. Milk which was cooled down to 31°C was inoculated with thermophilic DVS culture (freeze dried form) at the rate of 0.006%. After an incubation period of 40 minutes, the

enzyme rennet (1.5 g/100 L of milk) was added into cheese milk. Approximately 40 minutes after the rennet addition, curd was cut into cubes, cooked at 38°C for 20 minutes, whey was drained off and the curd was kept for over-night until desired acidity was developed. The following day, whey from the curd was examined for titratable acidity and when it reached 0.70% lactic acid (LA), hot water (80-85°C) was added to the curd. Other milk parts were cooled down to 4-8°C and were acidified with AA and CA separately until pH was reached to 5.4. Then rennet enzyme was added at the rate of 1.5 g/100 L of milk. Approximately 40 minutes after rennet addition, curd was cut into small cubes, cooked at 38°C for 20 minutes, whey was drained off and then curd cubes were immersed in hot water (80-85°C). All three types of cheeses were moulded separately into spherical shape. The cheese balls were kept in the brine solution (20-22% salt) for 3-4 h. At last, the processed cheeses were covered in polyethylene and stored in a freezer at -18°C.

### Cheese Yield, pH and Composition

Cheese yield was calculated as per the method given by Seth and Bajwa (2015). Briefly it was determined by dividing the weight of cheese by the weight of milk used to make cheese, multiplied by 100. Cheese pH, total solids (TS), fat, protein and ash content were measured after one day of processing. pH of Mozzarella cheese was determined as per the method explained by Abd El Aziz and Abo-srea (2014). Total solids and ash content were determined according to AOAC (1990). The protein content was determined by micro Kjeldahl method (Kirk and Sawyer, 1991). The fat content was determined by Soxhlet method (Strugnell, 1989).

### Meltability

Meltability of Mozzarella cheese was measured after one day of processing. The Schreiber melt test was carried out on Mozzarella cheese samples using modified method from Muthukumarappan et al. (1999). In brief, cheese samples were cut into 39 mm diameter and 17 mm height discs. Discs with equal weights were selected and placed on aluminum plates. The discs were then covered with a glass petri plate and kept at room temperature for 30 minutes. After that samples were heated in an oven at 100°C for 5 minutes. Final diameter was determined at 4 different locations of the cheese discs after reached to the room temperature and average value was considered as meltability in cm.

### Instrumental Colour

Instrumental colour analysis of the Mozzarella cheese samples were performed by using PCE-CSM 2 colourimeter calibrated with the white calibration plate provided and as specified by the manufacturer. CIELab color scale was used to get  $L^*$ ,  $a^*$  and  $b^*$  values. The  $L^*$  value ranges from 0 to 100, indicates the brightness from black to white; the  $a^*$  value indicates the variation from red ( $+a^*$ ) to green ( $-a^*$ ); the  $b^*$  value indicates the variation from yellow ( $+b^*$ ) to blue ( $-b^*$ ) in the CIELab colour scale.

### Sensory Evaluation

Mozzarella cheese samples topped on pizza base were baked and subjected to sensory evaluation for attributes namely flavor, colour and appearance, body and texture

and overall acceptability after one day of storage by 30 untrained panelists. Non smoking healthy adults who were willing to participate in the test were selected from the Faculty of Agriculture, University of Ruhuna, Sri Lanka. Individual sensory booths were prepared with the samples, drinking water to wash off the mouth and a sensory evaluation sheet. The place was well ventilated and ample lighting was provided to eliminate disturbances from nuisance odors and insufficient light conditions, respectively. Each sample was labeled with random three-digits. Panelists were advised to evaluate the samples in a quick manner but not in a hurry using a 5-point hedonic scale (1= dislike extremely, 2=dislike slightly, 3=neither like nor dislike, 4=like slightly and 5= like extremely).

### Statistical Analysis

A two factor (3×3) factorial experiment in a completely randomized design was utilized to find out the combined effect of milk source and the method of acidification of cheese milk on properties of Mozzarella cheese. The parametric data collected from the study was subjected to the analysis of variance (ANOVA) and sensory data were analysed using Friedman nonparametric test using SPSS Software version 25. Each experiment was carried out in duplicate for 2 different batches of milk, except sensory evaluation.

## Results and Discussion

### Cheese Yield and Composition

The yield and the composition of Mozzarella cheese made by changing the milk source and the acidification method of cheese milk are summarized in the Table 1. Yield is a valuable parameter for improving the process and cost benefit balance of a dairy manufacturing company

(Sales et al., 2017). Therefore, expressing cheese yield is of utmost importance. As depicted in the Table 1, there was no statistically significant ( $P>0.05$ ) interaction effect between milk source and the method of acidification of cheese milk for the cheese yield; however, the main effects of milk source as well as acidification method of cheese milk expressed statistically significant ( $P<0.05$ ) differences. Even though fat was standardized to 3% in cheese milk of each treatment, the yield of BM cheese was significantly ( $P<0.05$ ) higher than that of the cheese made from cow and the mixed milk. It is well documented that CM and BM are inherently different and the amount of TS, casein, fat and calcium content in BM are higher than that of cow milk which enables efficient production and quality of dairy products compared to cow milk (Abd El-Salam and El-Shibiny, 2011; Mona et al., 2011; Cecchinato and Bittante, 2016; Ahmad and Saleem, 2020). The higher recovery of milk solids causes a higher cheese yield (El-Gawad and Ahmed, 2011). Pazzola et al (2019) reported that the recovery of fat is improved by increasing the milk protein content than by increasing milk fat content. Further, they mentioned that the increase in milk protein content affect the recovery of fat, TS and Energy in the cheese curd. Casein number, calculated as casein to protein ratio strongly influences the fat recovery in cheese rather than protein (Pazzola et al., 2019) and in BM, casein number is higher than that of cow milk. All the above lead to higher recovery of milk solids and thereby the cheese yield.

Further, acidification method of cheese milk significantly ( $P<0.05$ ) affected the yield of Mozzarella cheese. The cheese milk acidified using CA and AA showed a higher cheese yield compared to that of the cheese milk acidified with SC. This is in contrast to the findings of Metzger et al. (2000).

Table 1. Mean±SD of yield and composition of Mozzarella cheese manufactured by changing milk source and acidification method of cheese milk.

| Source of variance                 | Yield (%)   | Composition (%) |             |             |            |
|------------------------------------|-------------|-----------------|-------------|-------------|------------|
|                                    |             | TS              | Fat         | Protein     | Ash        |
| Milk Source                        |             |                 |             |             |            |
| BM                                 | 13.97±0.78  | 51.92±0.89      | 26.00±0.25  | 21.28±0.94  | 2.95±0.08  |
| CM                                 | 08.88±0.52* | 47.12±0.93*     | 25.60±0.15* | 16.62±1.18* | 2.27±0.06* |
| BM:CM (1:1)                        | 09.96±0.54* | 49.15±0.88*     | 25.64±0.12* | 19.27±0.77* | 2.72±0.07* |
| P value                            | 0.000       | 0.000           | 0.000       | 0.000       | 0.000      |
| Acidification method               |             |                 |             |             |            |
| SC                                 | 10.15±2.18  | 50.34±2.05      | 25.78±0.17  | 20.34±1.85  | 2.68±0.30  |
| AA                                 | 11.41±2.43* | 49.55±2.10*     | 25.83±0.35  | 18.58±2.06* | 2.69±0.30  |
| CA                                 | 11.26±2.27* | 48.29±2.04*     | 25.63±0.18* | 18.25±2.07* | 2.57±0.29* |
| P value                            | 0.000       | 0.000           | 0.003       | 0.000       | 0.000      |
| Milk Source × Acidification method |             |                 |             |             |            |
| BM × SC                            | 13.01±0.50  | 52.86±0.06      | 25.90±0.18  | 22.54±0.04  | 2.98±0.04  |
| BM × AA                            | 14.64±0.16  | 52.11±0.09      | 26.28±0.15* | 20.80±0.09* | 3.00±0.04  |
| BM × CA                            | 14.26±0.10  | 50.79±0.07      | 25.83±0.13  | 20.49±0.03* | 2.86±0.07  |
| CM × SC                            | 08.19±0.09  | 48.12±0.50      | 25.70±0.22  | 18.20±0.03* | 2.30±0.03  |
| CM × AA                            | 09.25±0.11  | 47.22±0.04      | 25.55±0.06* | 15.10±0.08* | 2.30±0.03  |
| CM × CA                            | 09.21±0.10  | 46.03±0.10      | 25.55±0.13* | 15.67±0.04* | 2.20±0.06  |
| BM:CM × SC                         | 09.25±0.13  | 50.34±0.06      | 25.75±0.06  | 20.29±0.04* | 2.77±0.05  |
| BM:CM × AA                         | 10.33±0.13  | 49.34±0.30      | 25.65±0.06  | 18.93±0.08* | 2.76±0.03  |
| BM: CM × CA                        | 10.31±0.14  | 48.06±0.18      | 25.53±0.09* | 18.58±0.05* | 2.65±0.04  |
| P value                            | 0.057       | 0.921           | 0.002       | 0.000       | 0.899      |

Means with \* within a column differ significantly ( $P<0.05$ ) from the control. (BM= buffalo milk, CM= cow milk, BM: CM (1:1) = buffalo and cow milk mixture, SC= starter culture, AA= acetic acid, CA= citric acid and TS= Total solids).

However, they reported the composition adjusted cheese yield and not the actual cheese yield. The actual yield of cheese has a direct relation to moisture content and SNF level. A reduction in calcium content in Mozzarella cheese made from milk that had been acidified before setting was observed depending on the content of pH reduction at setting and the type of acid used (Najafi et al., 2006; Metzger et al., 2000). Relatively low calcium to casein ratio in directly acidified cheese is conducive to a greater degree of casein hydration (Najafi et al., 2006). Hence, the observed results might be due to the higher degree of casein hydration. Further, El-Abd et al. (2003) reported that addition of some SCs reduced the coagulation time, yield as well as moisture content of some fresh cheeses. Keller et al. (1974) found that the yield of Mozzarella cheese made with various acidulants differ significantly ( $P<0.05$ ).

An interaction effect was not observed between milk source and acidification method of cheese milk on the TS content of Mozzarella cheese. However, the milk source had a significant ( $P<0.05$ ) effect on TS content. The Mozzarella cheese made from buffalo milk had higher TS content compared to cow milk and their mixture. As Ganguli (1992) observed, it is attributable to the higher TS content and recovery of more milk solids in buffalo milk as previously mentioned under the cheese yield in this article. Similar observations were reported by El Batawy et al. (2004) who mentioned that Mozzarella cheese manufactured from cow milk and mixture of cow and buffalo milk (1:1) showed higher moisture content and less solids than cheese made from buffalo milk.

Furthermore, acidification method of cheese milk significantly ( $P<0.05$ ) affected the TS content of the resultant cheese. The cheese made from milk acidified with SC showed higher TS content even though the yield is lower compared to that of the cheese made from other two methods whereas cheese made from milk acidified with CA had the lowest TS content and is in agreement with previous findings. Najafi et al. (2006) reported that the total composition of cheese is affected according to the type of acid used to acidify the cheese milk. Further they reported that the acid type and the pH used for the initial pH adjustment of milk for cheese making significantly affected the Ca concentration of cheese. In similar pH, Ca content of cheese produced by CA was less than the one which was produced by LA and finally has an effect on TS. Abbas et al. (2014) produced Mozzarella cheese using SC, CA, LA and lemon juice and they observed that the cheese manufactured using CA had lower TS than cheese milk acidified with SC. In the current study even though, the TS level is lower, the higher yield in the cheese made from CA and AA acidification might be due to the presence of high moisture content compared to the cheese made by milk acidifying with SC. Keller et al. (1974) also reported that Mozzarella cheese manufactured using CA and LA had high moisture content and was softer compared to the cheese produced by phosphoric acid.

A significant ( $P<0.05$ ) interaction effect between milk source and acidification method of cheese milk was observed on fat content of Mozzarella cheese. Cheese made from buffalo milk acidified using AA had the highest fat percentage whereas cow milk acidified with AA, cow milk acidified with CA and mixed milk acidified with CA

had lower fat content compared to that of the control with a significant ( $P<0.05$ ) difference. When main effect is considered, it was observed that the cheese made from buffalo milk had significantly ( $P<0.05$ ) higher fat even though, cheese milk was standardized to the similar fat content during the processing. This could be due to the presence of higher total casein content, especially  $\alpha_{s2}$  and  $\kappa$ -casein of buffalo milk than that of cow milk (Claeys et al., 2014) and as Fangmeier et al. (2019) mentioned, this may have a favourable effect on the retention of fat and ash during the coagulation process in preparation of cheese. Pazzola et al. (2019) also mentioned that increase in milk protein content and casein number affect the recovery of fat. The protein content and the casein number of BM are higher than that of CM and the observed results might be due to those reasons.

Even though cheese milk was standardized, there was a significant ( $P<0.05$ ) difference in fat content of cheese samples made using different acidification methods as well. The cheese made from milk acidified with SC possessed significantly ( $P<0.05$ ) higher fat content than cheeses made using milk acidified with CA. This is in line with the findings of Bhaskaracharya (2004). However, statistically significant differences were not observed in fat content of cheese samples made by milk acidified with SC and AA. As reported by Najafi et al. (2006), this may be due to the effect of acid type used in cheese milk acidification on the total composition of cheese in constant pH.

Significant ( $P<0.05$ ) interaction effect was found between the milk source and the acidification method of cheese milk on protein content of Mozzarella cheese (Table 1). It was observed that the protein content was significantly ( $P<0.05$ ) higher in control cheese made by buffalo milk in which bio acidification by SC was used as the acidification method, compared to the other treatments. There was a statistically significant ( $P<0.05$ ) difference in the protein content of Mozzarella cheese samples made using different milk sources. As expected, protein content of buffalo milk Mozzarella cheese had the highest value compared to the cheese made from cow milk and their mixture (1:1). Previous studies also proved that buffalo milk Mozzarella cheeses were associated with higher fat, protein, ash and TS content as compared to cow milk cheese (Bhattarai and Acharya, 2010; Mijan et al., 2010). Further, it was observed that the cheese made by milk acidified using SC obtained the highest protein content. However, this result is in contrast with the findings of Jooyandeh et al. (2016) who reported that the Mozzarella cheese made by milk acidified with SC gives lower protein content compared to that of the cheese made by direct acidification and by combined method.

Interaction effect between milk source and the method of acidification of cheese milk was not observed in the ash content of Mozzarella cheese. However, statistically significant ( $P<0.05$ ) difference was observed in the ash content of cheese samples made with different milk sources. Ash content of buffalo milk Mozzarella cheese was observed to be higher compared to that of the cheese made from cow as well as mixed milk. Compared to cow milk, buffalo milk is richer in fat, lactose, protein, vitamins and minerals (Ahmad et al., 2008; Fundora et al., 2001). Further, total casein,  $\alpha_{s2}$  and  $\kappa$  casein in buffalo milk are higher than those reported in cow milk (Claeys et al.,

2014). As mentioned elsewhere in this article, Fangmeier et al. (2019) reported that the protein composition in buffalo milk may have favoured the retention of fat and ash in the coagulation process during preparation of cheese. The observed differences might be attributable to the above mentioned facts. Concerned with the acidification method of the cheese milk, it was observed that significantly ( $P<0.05$ ) lower ash content of cheese made by milk acidified with CA, while no difference was observed with AA compared to the control (Table 1). This may be due to the role of acid in removing some of the calcium phosphate

complex from casein (Emam and Nasser, 2019). Further, Metzger et al. (2000) mentioned that the reduction in cheese calcium is greater when used CA for the milk acidification compared to that of the AC

### *pH, Meltability and Instrumental Colour of Mozzarella Cheese*

Table 2 shows the pH, meltability and instrumental colour of Mozzarella cheese made by changing milk source and acidification method of cheese milk.

Table 2. Mean $\pm$ SD of pH, meltability and instrumental colour of Mozzarella cheese manufactured by changing milk source and acidification method of cheese milk.

| Source of variance                        | pH               | Meltability      | Instrumental colour |                   |                   |
|---|------------------|------------------|---------------------|-------------------|-------------------|
|   |                  |                  | <i>L</i> *          | <i>a</i> *        | <i>b</i> *        |
| Milk Source                               |                  |                  |                     |                   |                   |
| BM  | 5.30 $\pm$ 0.09  | 4.48 $\pm$ 0.61  | 85.32 $\pm$ 4.21    | -2.15 $\pm$ 1.22  | 12.52 $\pm$ 2.20  |
| CM  | 5.30 $\pm$ 0.09  | 6.98 $\pm$ 1.66* | 82.19 $\pm$ 5.29    | 0.17 $\pm$ 0.76*  | 20.91 $\pm$ 2.33* |
| BM:CM (1:1)                               | 5.30 $\pm$ 0.09  | 4.38 $\pm$ 0.54  | 82.11 $\pm$ 5.63    | -1.42 $\pm$ 1.27  | 15.70 $\pm$ 4.17* |
| P value                                   | 1.000            | 0.000            | 0.092               | 0.000             | 0.000             |
| Acidification method                      |                  |                  |                     |                   |                   |
| SC  | 5.20 $\pm$ 0.00  | 4.48 $\pm$ 0.70  | 86.73 $\pm$ 4.85    | -0.36 $\pm$ 0.99  | 17.66 $\pm$ 3.68  |
| AA  | 5.35 $\pm$ 0.06* | 4.84 $\pm$ 1.18* | 83.20 $\pm$ 2.91    | -1.35 $\pm$ 1.14* | 15.90 $\pm$ 4.13  |
| CA  | 5.35 $\pm$ 0.05* | 6.51 $\pm$ 1.94* | 79.69 $\pm$ 5.07*   | -1.70 $\pm$ 1.84* | 15.57 $\pm$ 5.78  |
| P value                                   | 0.000            | 0.000            | 0.001               | 0.006             | 0.107             |
| Milk Source $\times$ Acidification method |                  |                  |                     |                   |                   |
| BM $\times$ SC                            | 5.20 $\pm$ 0.00  | 4.03 $\pm$ 0.05  | 88.25 $\pm$ 5.97    | -1.10 $\pm$ 0.21  | 14.98 $\pm$ 1.37  |
| BM $\times$ AA                            | 5.35 $\pm$ 0.06  | 4.10 $\pm$ 0.00  | 83.24 $\pm$ 1.34    | -2.33 $\pm$ 0.94  | 11.34 $\pm$ 1.48  |
| BM $\times$ CA                            | 5.35 $\pm$ 0.06  | 5.30 $\pm$ 0.00* | 84.46 $\pm$ 3.08    | -3.01 $\pm$ 1.42  | 11.24 $\pm$ 1.22  |
| CM $\times$ SC                            | 5.20 $\pm$ 0.00  | 5.43 $\pm$ 0.05* | 87.14 $\pm$ 6.29    | 0.38 $\pm$ 1.12   | 19.53 $\pm$ 0.92  |
| CM $\times$ AA                            | 5.35 $\pm$ 0.06  | 6.40 $\pm$ 0.48* | 80.81 $\pm$ 1.33    | -0.10 $\pm$ 0.59  | 20.19 $\pm$ 2.80* |
| CM $\times$ CA                            | 5.35 $\pm$ 0.06  | 9.13 $\pm$ 0.28* | 78.61 $\pm$ 3.01    | 0.22 $\pm$ 0.58   | 22.99 $\pm$ 1.46* |
| BM:CM $\times$ SC                         | 5.20 $\pm$ 0.00  | 4.00 $\pm$ 0.00  | 84.79 $\pm$ 1.69    | -0.35 $\pm$ 0.91  | 18.47 $\pm$ 5.64  |
| BM:CM $\times$ AA                         | 5.35 $\pm$ 0.06  | 4.03 $\pm$ 0.00  | 85.55 $\pm$ 3.54    | -1.62 $\pm$ 0.23  | 16.16 $\pm$ 0.37  |
| BM:CM $\times$ CA                         | 5.35 $\pm$ 0.06  | 5.10 $\pm$ 0.12* | 75.10 $\pm$ 5.06    | -2.29 $\pm$ 1.58  | 12.48 $\pm$ 2.79  |
| P value                                   | 1.000            | 0.000            | 0.087               | 0.364             | 0.008             |

Means with \* within a column differ significantly ( $P<0.05$ ) from the control. (BM= buffalo milk, CM= cow milk, BM: CM (1:1) = buffalo and cow milk mixture, SC= starter culture, AA= acetic acid and CA= citric acid). *L*\* = brightness from black (0) to white (100); *a*\* = red (+*a*\*) to green (-*a*\*); *b*\* = yellow (+*b*\*) to blue (-*b*\*).

#### *Cheese pH*

Chemical interactions between key structural components of cheese are pH-dependent. Therefore, pH directly influences the structural and rheological properties of cheese (Yazici et al., 2010). It is clear from the Table 2 that there was no statistically significant ( $P>0.05$ ) interaction effect of milk source and acidification method of cheese milk on Mozzarella cheese pH. Further, milk source did not have any influence on pH of cheese. Similar results were observed by Sameen et al. (2008) and reported that pH of Mozzarella cheese manufactured using buffalo and cow milk were not affected significantly. However, as depicted in Table 2, cheese pH was significantly ( $P<0.05$ ) influenced by acidification method of cheese milk. Cheese made using milk acidified by CA and AA had higher and similar pH compared to the cheese made using milk acidified by SC. These results are in accordance with the results obtained by Emam and Nasser (2019). Abd El Aziz and Abo-srea (2014) also reported that Mozzarella cheese made by milk acidified with AA had higher pH compared to the cheese made by milk acidified using SC.

#### *Cheese Meltability*

Meltability is an important functional property of cheese especially when it is used in foods consumed after heating (Altan, 2005). The term meltability has been used to indicate the extent to which melted cheese flows and spreads upon heating (Kuo et al., 2001; Abbas et al., 2014). Since Mozzarella cheese is used in many food applications that are consumed after heating, meltability is a vital functional property. In the present study, it was observed that there is a statistically significant ( $P<0.05$ ) interaction effect between milk source and method of acidification of cheese milk on Mozzarella cheese meltability. Highest meltability of 9.13 $\pm$ 0.28 cm was observed in Mozzarella cheese manufactured using cow milk acidified with CA. Further, a significant ( $P<0.05$ ) main effect of milk source was observed on meltability. Cheese manufactured using cow milk showed a higher meltability than the cheese manufactured from buffalo milk. Jana and Tagalpallewar (2017) also reported that cow milk Mozzarella cheese shows higher meltability. This is possibly due to the higher amount of moisture content (Fife et al., 1996; McMahon

and Oberg, 1998) and less ash content (Bhattarai and Acharya, 2010) in cow milk compared to that of buffalo milk. Fat or unbound water act as a lubricant and increases the ability of cheese to melt (Fife et al., 1996). Even though the cheese made using buffalo milk contains higher amount of fat (Table 1), the protein content was also observed to be higher and in turn making greater water binding capacity (Fife et al., 1996) of cheese. This might be the reason to have higher meltability in cheese made using cow milk. These results are in parallel with the findings of El-Batawy et al. (2004) who manufactured Mozzarella cheese using buffalo, cow and their mixture (1:1) which were standardized to 3% fat and observed that the cow milk Mozzarella cheese showed higher meltability values.

Emam and Nasser (2019) reported that acids used in the production of Mozzarella cheese affects its functionality. In the current study, it could be observed that Mozzarella cheese manufactured using milk acidified with CA and AA had higher meltability compared to the cheese made using milk acidified with SC with a significant ( $P < 0.05$ ) difference. A greater variation was observed when CA was used for the milk acidification in cheese making. Jana and Mandal (2011) reported that pizza cheese manufactured using CA as pH regulator had the highest calcium concentration and pronounced meltability and stretchability. However, contrasting results were reported by Jooyandeh et al. (2016) who studied the effect of direct acidification, SC method and their combination on quality of cow milk Mozzarella cheese and observed that the SC method yielded the cheese with highest meltability. Furthermore, they reported that the observed results were due to the significantly higher fat content in the cheese made with SC.

#### *Instrumental Colour*

Colour is one of the most important properties of foods including cheese which are directly related to product quality and consumer acceptance (Jooyandeh et al., 2016). The  $L^*$  parameter indicates lightness and the capacity of an object to reflect or transmit light based on a scale ranging from 0 to 100. Therefore, higher lightness values resulted in clearer objects (Queiroga et al., 2013). The results with respect to  $L^*$  value or the degree of lightness of Mozzarella cheese revealed that there is no significant ( $P > 0.05$ ) combined effect of milk source and acidification method. Furthermore, main effect of milk source was also observed to be non significant ( $P > 0.05$ ) even though cheese manufactured from buffalo milk has slightly higher lightness (Table 2) which is due to the presence of pre formed vitamin A which is more light in colour. However, it was observed that acidification method of cheese milk showed a significant impact on lightness of Mozzarella cheese. Cheese made by milk acidified with SC showed higher lightness value followed by cheese made by milk acidified with AA and CA. Higher concentration of soluble casein in the serum phase lower the  $L^*$  value of the unmelted cheese (Metzger et al., 2001). Furthermore, the lightness of cheese can be affected by fat and protein matrix.

$a^*$  value is the indicator of greenness (-) and redness (+) which explains green colour intensity of samples. Results reported in Table 2 revealed that, there was no statistically significant ( $P > 0.05$ ) interaction effect between milk source and method of acidification on  $a^*$  value of Mozzarella cheese. However, there was a significant ( $P < 0.05$ ) difference

of  $a^*$  value of cheese made from different milk sources. Cheese made from buffalo milk showed significantly ( $P < 0.05$ ) lower  $a^*$  value compared to that of the cheese made from cow milk. Buffalo milk contains the pigment biliverdin which is blue green in colour (Abd El-Salam and El-Shibiny, 2011) and the observed results might be due to that. Furthermore, higher amount of carotene in cow milk which is lack in buffalo milk may also contribute.  $a^*$  value of the cheese made from mixed milk was not statistically different ( $P > 0.05$ ) compared to the control. However, the acidification method of cheese milk had a significant ( $P < 0.05$ ) effect on  $a^*$  value. Cheese milk acidified using AA and CA showed a significantly ( $P < 0.05$ ) lower  $a^*$  value compared to the control.

$b^*$  value is the indicator of yellowness which explains yellow colour intensity of a given sample. Results with respect to  $b^*$  value (Table 2) stated that, there was a significant ( $P < 0.05$ ) interaction effect between milk source and the method of acidification of cheese milk. Further, all the  $b^*$  values were observed to be positive indicating the yellowness of the cheese samples. CMxAA and CMxCA samples are the only samples which were having significantly ( $P < 0.05$ ) higher  $b^*$  values compared to the control. Main effect of milk source showed a significant ( $P < 0.05$ ) effect on  $b^*$  value. Cow and mixed milk had higher ( $P < 0.05$ )  $b^*$  value compared with that of buffalo milk. This may be due to the presence of  $\beta$ -carotene in cow milk (Abd El-Salam and El-Shibiny, 2011). However, there was no statistically significant difference ( $P > 0.05$ ) for milk acidification method on  $b^*$  value of the resultant cheese.

#### *Sensory Properties*

Sensory attributes are vitally important and directly affect consumer acceptance and market success of cheese (Young et al., 2004; Yates and Drake, 2007; Wadhvani and McMahon, 2012). The results of the sensory evaluation of the experimental Mozzarella cheese topped on pizza base are shown in Table 3.

It was observed that some treatment combinations used in the current study resulted in significant ( $P < 0.05$ ) changes in the organoleptic characteristics of the final cheese compared to that of the control which was made by buffalo milk acidified with SC. Accordingly, flavor score was observed to be highest and significantly ( $P < 0.05$ ) different in the cheese made by mixed milk acidified using CA followed by cheese made by mixed milk acidified with SC compared to the control. Similarly, highest score for colour and appearance was obtained by Mozzarella cheese manufactured using mixed milk acidified with CA followed by cheese made using cow milk acidified with SC. Highest ( $P < 0.05$ ) score for body and texture as well as for overall acceptability was also obtained by Mozzarella cheese made using mixed milk acidified with CA. Based on the sensory scores, it can be concluded that the cheese made using mixed milk acidified with CA were preferred over control cheese made using buffalo milk acidified with SC. However, Jana and Tagalpallear (2017) reported that the cheese made from cow milk had superior overall sensory score compared to buffalo milk cheese. Further, some authors also reported that cow milk cheese was preferred over buffalo milk cheese for pizza toppings (Bhattarai and Acharya, 2010; Zedan et al., 2014).

Table 3. Mean±SD of sensory scores of Mozzarella cheese manufactured by changing milk source and acidification method of cheese milk, topped on a pizza base.

| Milk Source | Acidification method | Sensory Score <sup>#</sup> |                       |                  |                       |
|-------------|----------------------|----------------------------|-----------------------|------------------|-----------------------|
|             |                      | Flavour                    | Colour and appearance | Body and texture | Overall acceptability |
| BM          | SC                   | 3.23±0.86                  | 3.30±0.88             | 3.33±0.76        | 3.43±0.82             |
|             | AA                   | 3.23±0.90                  | 3.17±0.83             | 3.37±0.72        | 3.37±0.72             |
|             | CA                   | 3.30±1.12                  | 3.23±0.86             | 3.33±0.80        | 3.40±0.93             |
| CM          | SC                   | 3.17±1.15                  | 3.97±1.22*            | 3.97±0.93*       | 3.53±0.94             |
|             | AA                   | 3.43±1.04                  | 3.80±0.81             | 3.57±0.97        | 3.53±0.82             |
|             | CA                   | 3.67±1.09                  | 3.60±0.89             | 3.87±0.97*       | 3.83±0.91             |
| BM:CM (1:1) | SC                   | 3.70±0.95*                 | 3.93±0.87*            | 3.57±1.17        | 3.67±0.89             |
|             | AA                   | 3.40±1.07                  | 3.40±0.89             | 3.37±0.99        | 3.40±0.90             |
|             | CA                   | 3.90±1.09*                 | 4.03±0.89*            | 3.97±0.99*       | 3.90±0.06*            |

Means with \* within a column differ significantly ( $P<0.05$ ) from the control. (BM= buffalo milk, CM= cow milk, SC= starter culture, AA= acetic acid and CA= citric acid; #5-point hedonic scale was used where 1= dislike extremely and 5= like extremely)

## Conclusions

There was a significant ( $P<0.05$ ) interaction effect between milk source and method of acidification of cheese milk on fat and protein percentages, meltability and  $b^*$  value of Mozzarella cheese. In compositional point of view, fat% was significantly ( $P<0.05$ ) higher in Mozzarella cheese made from buffalo milk acidified with AA whereas the protein% in control. Meltability was superior in Mozzarella cheese manufactured from cow milk acidified using CA compared to the control. Cheese made from cow milk acidified using AA and CA showed significantly ( $P<0.05$ ) higher  $b^*$  value compared to the control. Highest mean score for all the sensory attributes was obtained by Mozzarella cheese manufactured from mixed milk acidified using CA and was significantly ( $P<0.05$ ) superior compared to the control. Further, there was no significant ( $P>0.05$ ) combined effect between milk source and method of acidification of cheese milk on yield, %TS and ash, pH,  $L^*$  and  $a^*$  values of Mozzarella cheese, even though main effects were significant ( $P>0.05$ ) in between some treatments. Finally, it can be concluded that the milk source and the method of acidification are closely linked to the yield and the quality characteristics of Mozzarella cheese and hence, careful selection of raw materials and manipulation of processing conditions are required to get an optimum quality end product.

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