

Activity of indigenous enzymes in Surti buffalo milk

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Abstract

Pooled milk samples of Surti buffalo were collected from Reproductive Biology Research Unit, Anand Agricultural University, Anand (Gujarat) during the period from August 2019 to March 2020 and analyzed for gross composition and activity of selected indigenous enzymes. Per cent fat, protein, lactose, ash and total solids content of milk ranged from 5.97 to 9.77 (mean 7.38), 3.46 to 4.93 (mean 3.87), 4.54 to 5.98 (mean 5.04), 0.664 to 0.862 (mean 0.753) and 15.32 to 20.67 (mean 17.02), respectively. The titratable acidity and pH of milk ranged from 0.143 to 0.170 per cent lactic acid (mean 0.156) and 6.50 to 6.80 (mean value 6.65), respectively. The alkaline phosphatase and acid phosphatase activity ranged from 227.00 to 442.70 (mean 341.34) and 18.51 to 33.47 (mean 27.31) $\mu\text{g p-nitrophenol/mL}$. The lipase activity ranged from 0.25 to 3.00 (mean 0.97) U/mL. The catalase activity ranged from 0.23 to 0.75 (mean 0.40) catalase unit. The xanthine oxidase and lactoperoxidase activity ranged from 40.16 to 117.72 (mean 70.77) and 61.73 to 160.48 (mean 118.65) mU/mL. The proteolytic activity ranged from 134.00 to 183.08 (mean 158.59) $\mu\text{g tyrosine/mL}$. The activity of the above indigenous enzymes of Surti buffalo milk was in corroboration with studies reported in the literature. However, the variation observed in the activity of some of the indigenous enzymes might be due to the factors like methods used for measurement, lactation stage, individuality and health of animal, etc.

Keywords: Enzyme activity, Indigenous enzymes, Milk composition, Surti buffalo

Highlights

- The gross chemical composition and activity of selected indigenous enzymes in Surti buffalo milk were investigated.
- The mean values of fat, protein, lactose, ash and total solids content of milk were 7.38, 3.87, 5.04, 0.753 and 17.02%, respectively.
- The mean values of activity for different indigenous enzyme found were as follows: alkaline phosphatase 341.34 $\mu\text{g p-nitrophenol/mL}$; acid phosphatase 27.31 $\mu\text{g p-nitrophenol/mL}$; lipase 0.97 U/mL; catalase 0.40 catalase unit; xanthine oxidase 70.77 mU/mL; lactoperoxidase 118.65 mU/mL and proteolytic activity 158.59 $\mu\text{g tyrosine/mL}$.

INTRODUCTION

Buffaloes contribute to a major portion of world's milk production. Two main types of buffaloes, i.e., riverine buffaloes (*Bubalus bubalis*) and swamp buffaloes (*Bubalus carabanesis*), are found in the world. The riverine buffalo is found in South Asia and Southwest Asia, whereas the swamp buffalo is present in East Asia and Southeast Asia. Mediterranean buffaloes evolved from these two major types, and they are found in Italy, the Balkan states, Turkey and some parts of Russia (Khedkar *et al.*, 2016). In India, milk

production during 2018-19 and 2019-20 was 187.75 and 198.40 million tonnes, respectively, showing an annual growth rate of 5.68%. Nearly 49% of milk production was contributed by Indigenous/Non-descript buffaloes, followed by 28% by crossbred cows. Indigenous/Non-descript cows contributed 20% of the total milk production in the country (Annual Report 2020-21).

The composition, properties and processing of buffalo milk and milk products have been the subjects of several reviews (Abd El-Salam and El-Shibiny, 2011; Murtaza *et al.*, 2017;

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Vargas-Ramella *et al.*, 2021; Khetra *et al.*, 2022). Recently, the detailed milk composition of three buffalo breeds (Murrah, Nili-Ravi and Mediterranean) has been compared (Abdel-Hamid *et al.*, 2023).

India has a rich repository of buffalo breeds including 13 recognized breeds. Surti buffalo is a popular breed found in the Charotar track of Gujarat between the Mahi and Sabarmati rivers. Breeding tract of Surti is in and around Kheda, Vadodara, Anand and Bharuch districts of the middle and southern part of Gujarat.

Milk obtained from healthy milch animals contains a number of indigenous enzymes. These enzymes are believed to be derived from the secretory epithelial cells of the mammary gland, and their presence may be regarded as due to a spilling over from these cells and serum during the milk secretion process. The activity of many enzymes can vary significantly due to the stage of lactation, diet, breed, stress, mastitis or other factors (O'Mahoney *et al.*, 2013). Some enzymes are of interest for their beneficial activity (e.g., lactoperoxidase), some for use as an index of processing (e.g., alkaline phosphatase) and some for effects on the quality of dairy products (e.g., plasmin, lipoprotein lipase), which may be either beneficial or undesirable (Fox *et al.*, 2015; Mejares *et al.*, 2022; O'Brien *et al.*, 2022).

The indigenous enzymes present in cow milk have been studied in details (O'Mahoney *et al.*, 2013; Fox *et al.*, 2015). There are differences in the activity of indigenous enzymes in milk of different species (Sharma *et al.*, 2009; Sandilya *et al.*, 2014; Yoganandi *et al.*, 2014a; Prajapati *et al.*, 2017). However, information on enzymes in buffalo milk is very limited (Arora *et al.*, 2022). The data on gross composition and activity of selected enzymes in buffalo milk of Surti breed have been reported in the present study.

MATERIALS AND METHODS

Pooled milk samples of Surti buffalo were collected weekly from the Reproductive Biology Research Unit (RBRU), Anand

Agricultural University, Anand. Fresh milk samples were collected in a clean, dry container during milking time. Total thirty two milk samples were collected during the period from August 2019 to March 2020. Analysis of milk was carried out at the Dairy Chemistry Department, SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat. The ambient temperature during this period ranged between 14.5 to 30.5°C. The samples of milk of Surti buffalo were collected every week throughout the whole lactation period. The animals were fed on dry fodder, green pasture and concentrates during the milking days.

All the chemicals used during the study for the analysis were of 'Analytical grade' (AR). Glassware meant for analysis purposes was washed with diluted nitric acid followed by distilled water and dried in an oven.

Pooled milk samples of Surti buffalo were analyzed for contents of fat, protein, lactose, ash, total solids and titratable acidity as per the procedures described by BIS (1981).

Total nitrogen content in buffalo milk was determined by Micro-Kjeldahl method (AOAC, 2010), which was multiplied by 6.38 to get the protein percentage, pH of buffalo milk was determined using pH meter (LabMan).

Determination of activity of selected indigenous enzymes in milk: Pooled milk samples of Surti buffalo were analyzed for the activity of selected indigenous enzymes. The activities of alkaline phosphatase and catalase enzymes were estimated as per the methods of Sharma and Ganguli (1971), while those of acid phosphatase, lipase, xanthine oxidase and lactoperoxidase enzymes were determined following the methods of Chavarri *et al.* (1998), Kumar *et al.* (2012), Cerbulis and Farrell (1977) and Kumar and Bhatia (1999), respectively. The spectrophotometric method of Hull (1947) was followed to measure the proteolytic activity of Surti buffalo milk samples.

Statistical analysis: Descriptive statistical

measures like mean, standard deviation, coefficient of variation (CV), etc., were obtained for analysis of data (Steel and Torrie, 1980). Coefficient of variation (CV) was calculated by using the following formula:

Coefficient of variation = (Standard deviation / Mean) * 100

RESULTS

Proximate composition and chemical characteristics of milk

The range of fat, protein, lactose, ash and total solids content in Surti buffalo milk were 5.97-9.77 (mean 7.38), 3.46-4.93 (mean 3.87), 4.54-5.98 (mean 5.04), 0.664-0.862 (mean 0.753) and 15.32-20.67 (mean 17.02) %, respectively over the period of study (Table 1).

The titratable acidity and pH of Surti buffalo milk ranged from 0.143-0.170 (mean 0.156 ± 0.01) % lactic acid and 6.50-6.80 (mean 6.65), respectively during the period (Table 1).

Activity of selected indigenous enzymes in milk

The results for activity of selected indigenous enzymes are reported below:

Alkaline phosphatase activity: Alkaline phosphatase activity of Surti buffalo milk was expressed as μg p-nitrophenol formed per mL milk at 37°C in 30 minutes.

The range of alkaline phosphatase activity in Surti buffalo milk was 227.00-442.70 μg p-nitrophenol/mL with a mean value of 341.34

μg p-nitrophenol/mL. Alkaline phosphatase activity was maximum (413.66 μg p-nitrophenol/mL) in the month of January and minimum (245.94 μg p-nitrophenol/mL) in the month of October (Table 2).

Acid phosphatase activity: Acid phosphatase activity of Surti buffalo milk was expressed as μg p-nitrophenol released when 0.5 mL enzyme (of milk) was incubated with 0.5 mL substrate for 1 h at 37°C .

The range of acid phosphatase activity in Surti buffalo milk was 18.51-33.47 μg p-nitrophenol/L with a mean value of 27.31 μg p-nitrophenol/mL. Acid phosphatase activity was maximum (30.74 μg p-nitrophenol/mL) in the month of March and minimum (20.18 μg p-nitrophenol/mL) in the month of November (Table 2).

Lipase activity: One lipase unit was equivalent to a preparation, which could liberate fatty acids requiring one mL of N/100 alcoholic KOH with 1h incubation period at 37°C .

The range of lipase activity in Surti buffalo milk was 0.25-3.00 U/mL with a mean value of 0.97 U/mL. Lipase activity was maximum (2.75 U/mL) in the month of August and minimum (0.34 U/mL) in the months of November and March (Table 2).

Catalase activity: One catalase unit is expressed as mL of N/10 sodium thiosulphate

Table 1. Proximate composition and chemical characteristics of Surti buffalo milk (Pooled milk samples, n=32)

Parameters	Content		CV %
	Range	Mean $\pm$ SD	
Fat (%)	5.97-9.77	7.38 ± 0.75	10.12
Protein (%)	3.46-4.93	3.87 ± 0.35	9.04
Lactose (%)	4.54-5.98	5.04 ± 0.29	5.75
Ash (%)	0.664-0.862	0.753 ± 0.04	5.31
Total solids (%)	15.32-20.67	17.02 ± 1.12	6.58
Titratable acidity (% lactic acid)	0.143-0.170	0.156 ± 0.01	6.41
pH	6.50-6.80	6.65 ± 0.06	0.90

Table 2. Enzyme activity of Surti buffalo milk (Pooled milk samples, n=32)

Months	Alkaline phosphatase activity ($\mu\text{g p-nitrophenol/mL}$)	Acid phosphatase activity ($\mu\text{g p-nitrophenol/mL}$)	Lipase activity (U/mL)	Catalase activity (catalase unit)	Xanthine oxidase activity (mU/mL)	Lactoperoxidase activity (mU/mL)	Proteolytic activity ($\mu\text{g tyrosine/mL}$)
August 2019	255.64 $\pm$ 6.83	27.61 $\pm$ 1.26	2.75 $\pm$ 0.17	0.29 $\pm$ 0.04	101.59 $\pm$ 14.42	94.12 $\pm$ 11.68	134.8 $\pm$ 0.92
September 2019	246.75 $\pm$ 10.31	28.75 $\pm$ 1.59	2.01 $\pm$ 0.31	0.39 $\pm$ 0.04	89.07 $\pm$ 3.27	103.37 $\pm$ 17.01	144.57 $\pm$ 5.53
October 2019	245.94 $\pm$ 14.63	27.57 $\pm$ 4.26	1.14 $\pm$ 0.66	0.44 $\pm$ 0.12	74.94 $\pm$ 6.29	129.61 $\pm$ 15.93	155.85 $\pm$ 5.23
November 2019	385.23 $\pm$ 27.69	20.18 $\pm$ 2.13	0.34 $\pm$ 0.08	0.35 $\pm$ 0.08	62.98 $\pm$ 3.66	148.11 $\pm$ 14.27	163.95 $\pm$ 14.28
December 2019	399.73 $\pm$ 36.03	28.58 $\pm$ 1.02	0.39 $\pm$ 0.02	0.57 $\pm$ 0.12	73.19 $\pm$ 5.19	129.62 $\pm$ 29.39	161.87 $\pm$ 5.70
January 2020	413.66 $\pm$ 9.70	29.21 $\pm$ 1.62	0.42 $\pm$ 0.11	0.35 $\pm$ 0.01	54.86 $\pm$ 14.97	98.74 $\pm$ 39.04	166.95 $\pm$ 1.84
February 2020	388.92 $\pm$ 30.31	25.82 $\pm$ 4.23	0.44 $\pm$ 0.08	0.46 $\pm$ 0.08	53.71 $\pm$ 10.15	125.33 $\pm$ 20.25	167.75 $\pm$ 5.56
March 2020	394.85 $\pm$ 18.85	30.74 $\pm$ 0.94	0.34 $\pm$ 0.06	0.35 $\pm$ 0.06	53.48 $\pm$ 11.45	120.35 $\pm$ 11.80	173.00 $\pm$ 2.82
Mean $\pm$ SD	341.34 $\pm$ 75.29	27.31 $\pm$ 3.75	0.97 $\pm$ 0.91	0.40 $\pm$ 0.11	70.77 $\pm$ 18.52	118.65 $\pm$ 26.03	158.59 $\pm$ 13.56
Range	227.00-442.70	18.51-33.47	0.25-3.00	0.23-0.75	40.16-117.72	61.73-160.48	134.00-183.08
CV%	22.06	13.73	93.81	27.5	26.17	21.94	8.55

required for control determination less the titration value for the experimental solution per 5 mL of the enzyme solution (milk).

The range of catalase activity in Surti buffalo milk determined during the period of study was 0.23-0.75 catalase unit, and the mean value was 0.40 catalase unit. Catalase activity of Surti buffalo milk was maximum (0.57 catalase unit) in the month of December and minimum (0.29 catalase unit) in the month of August (Table 2).

Xanthine oxidase activity: One unit of xanthine oxidase activity is defined as that forming one micromole of urate per min at pH 7.4 and 25°C, the molar absorbance of urate being $1.22 \times 10^4 \text{ LM}^{-1} \text{ cm}^{-1}$.

The range of xanthine oxidase activity in Surti buffalo milk was 40.16-117.72 mU/mL, and the mean value was 70.77 mU/mL. Xanthine oxidase activity was maximum (101.59 mU/mL) in the month of August and minimum (53.48 mU/mL) in the month of March (Table 2).

Lactoperoxidase activity: One unit of lactoperoxidase activity (U) is defined as the amount of enzyme that catalyses the oxidation of 1 μmol of ABTS per min at 20°C in 0.1 mol phosphate buffer (pH 6.0), using a concentration of 1 mmol ABTS and 0.1 mmol hydrogen peroxide in the reaction mixture.

The range of lactoperoxidase activity in Surti buffalo milk was 61.73-160.48 mU/mL with a mean value of 118.65 mU/mL. Lactoperoxidase activity was maximum (148.11 mU/mL) in the month of November and minimum (94.12 mU/mL) in the month of August (Table 2).

Proteolytic activity: The proteolytic activity of milk was expressed as $\mu\text{g tyrosine/mL}$ milk. The range of proteolytic activity in Surti buffalo milk was 134.00-183.08 $\mu\text{g tyrosine/mL}$, and the mean value was 158.59 $\mu\text{g tyrosine/mL}$. Proteolytic activity was maximum (173.00 $\mu\text{g tyrosine/mL}$) in the month of March and minimum (134.8 $\mu\text{g tyrosine/mL}$) in the month of August (Table 2).

DISCUSSION

Proximate composition and chemical characteristics of Surti buffalo milk

The information related to the proximate composition of Surti buffalo milk is limited. Several researchers have analyzed compositional parameters of milk from other breeds of buffalo, which are cited below.

The fat content in buffalo milk was reported to be 7.97% (Mahmood and Usman, 2010), 7.19 to 8.63% (Yadav *et al.*, 2013), 6.38% (Yoganandi *et al.*, 2014c) and 7.9 to 8.8% (Kapadiya *et al.*, 2016). Varrichio *et al.* (2007) reported that the average fat content in buffalo milk was 8.3%, which can reach up to 15% under favourable conditions. Mishra *et al.* (2022) reported the mean fat content in Surti buffalo milk as 7.50%. Protein content in buffalo milk was 3.46 to 3.56% (Yadav *et al.*, 2013), 3.87% (Yoganandi *et al.*, 2014c) and 4.11 to 4.74% (Kapadiya *et al.*, 2016). According to Islam *et al.* (2014), the average protein and casein content in buffalo milk were 3.50 and 2.70%, respectively. Mishra *et al.* (2022) reported the mean protein content in Surti buffalo milk as 4.46%. The values of mean fat and protein content in Surti buffalo milk in our study are in corroboration with the values reported for buffalo milk.

The lactose content in buffalo milk has been reported by different research workers to be 4.49 to 4.73% (Han *et al.*, 2012), 4.36 to 4.60% (Yadav *et al.*, 2013), 4.70% (Yoganandi *et al.*, 2014c) and 4.67 to 5.27% (Kapadiya *et al.*, 2016). Mishra *et al.* (2022) reported the mean lactose content in Surti buffalo milk as 5.02%. Ash content in buffalo milk was 0.81% (Mahmood and Usman, 2010), 0.69% (Yoganandi *et al.*, 2014c) and 0.69 to 0.89% (Kapadiya *et al.*, 2016). Mishra *et al.* (2022) reported the mean ash content in Surti buffalo milk as 0.83%. The total solids content in buffalo milk was 16.39 to 18.48% (Han *et al.*, 2012), 18.45% (Mahmood and Usman, 2010), 15.57% (Yoganandi *et al.*, 2014c), 16.80% (Balasumi, 2015) and 17.88 to 19.18% (Kapadiya *et al.*, 2016). Legesse *et al.* (2017)

found 14.6, 13.65 and 14.25 per cent total solids in cow milk, camel milk and goat milk, respectively. Mishra *et al.* (2022) reported the mean total solids content in Surti buffalo milk as 17.52%. The lactose, ash and total solids content in Surti buffalo milk were in general agreement with the values reported in the literature for buffalo milk.

The titratable acidity (% lactic acid) of the buffalo milk has been reported by different research workers to be 0.21 (Mahmood and Usman, 2010), 0.133 (Yoganandi *et al.*, 2014b) and 0.108 to 0.162 (Prajapati *et al.*, 2017). Mishra *et al.* (2022) reported the mean titratable acidity (% lactic acid) in Surti buffalo milk as 0.175%. The pH of buffalo milk has been reported as 6.75 (Mahmood and Usman, 2010) and 6.65 (Han *et al.*, 2007). Padghan *et al.* (2008) compared physico chemical parameters of Marathwadi buffalo milk with the milk of other buffalo breeds. pH values in milk of various breeds of buffalo like Marathwadi, Jaffrabadi, Surti, Murrah and Nagpuri were 6.544, 6.537, 6.515, 6.526 and 6.556, respectively. Mishra *et al.* (2022) reported a mean pH of 6.73 in Surti buffalo milk. The titratable acidity and pH of Surti buffalo milk were in general agreement with the values reported in the literature for buffalo milk.

Activity of selected indigenous enzymes in milk

The study aimed to determine the activity of selected enzymes in Surti buffalo milk. Various researchers have used different assays for determination of activity of particular enzymes in milk. Further, the results of the same has been expressed variously. Henceforth, it is difficult to compare results from different studies (Kelly and Fox, 2006). However, works related to indigenous enzymes, particularly in buffalo milk, carried out by different researchers are cited below.

Alkaline phosphatase activity: Sharma and Ganguli (1971) reported the range of alkaline phosphatase activity in buffalo milk as 50 to

360 µg p-nitrophenol/mL (average 209 µg p-nitrophenol/mL), whereas that of cow milk as 500 to 1000 µg p-nitrophenol/mL (average 710 µg p-nitrophenol/mL). Sharma *et al.* (2009) reported the alkaline phosphatase activity in buffalo, cow and goat milk as 79.20, 61.39 and 26.62 U/mL, respectively. Yoganandi *et al.* (2014a) reported average alkaline phosphatase activity in the milk of buffalo, cow and goat as 253.75, 575 and 21.31 µg p-nitrophenol/mL, respectively. Prajapati *et al.* (2017) reported the alkaline phosphatase activity in the milk of goat, cow and buffalo as 15.79, 30.59 and 14.46 µg p-nitrophenol/mL, respectively. The results obtained in this study were in corroboration with the above cited values.

Acid phosphatase activity: Rifaat *et al.* (1969b) reported that buffalo milk contained much less acid phosphatase activity (57.5 µg phenol/mL) than cow milk (175.8 µg phenol/mL). Acid whey of buffalo milk contained higher acid phosphatase activity than rennet whey (Ismail *et al.*, 1976), suggesting that the acid phosphatase associated with casein was set free by acidity. Acid phosphatase activity in the milk of Sahiwal dairy animals revealed a declined pattern in conjunction with lactation stages (Hameed *et al.*, 2014). The data for acid phosphatase activity in buffalo milk are limited, hence could not be compared with the results of this study.

Lipase activity: Hofi *et al.* (1976) reported the average lipase activity of bulk and individual buffalo's milk as 1.39 and 1.43 unit/mL, respectively. The lipase activity of buffalo milk increased gradually during the lactation period from 0.86 U/mL in the first month to a maximum of 2.48 U/mL at the sixth month. Lipase activity of buffalo milk was affected by feed, number of lactations, but no changes were observed between morning and afternoon milking (Abd El-Hamid *et al.*, 1977). The lipase activity of bulk milk samples from Murrah buffaloes was 3.73 unit/mL. Feeding protected fat had no significant effect on lipase activity

of buffalo milk (Srivastava *et al.*, 1989). Prajapati *et al.* (2017) reported the lipase activity in the milk of goat, cow and buffalo as 1.2, 1.3 and 2.2 units/mL, respectively. Lipoprotein lipase in cow milk is altered due to the breed, lactation phase, feed and fodder, season, and milk yield (Toušová *et al.*, 2013). Lipase activity (1.55 U/mL) was recorded higher in milk, examined at the last of lactation, followed by other lactations (1.29 and 1.16 U/mL, respectively) (Hameed *et al.*, 2014). The lipase activity of Surti buffalo milk in this study was in general agreement with those reported in the literature for buffalo milk.

Catalase activity: The average catalase activity of buffalo milk was 10.9 U/mL, while that of cow milk was 12.1 U/mL (Rifaat *et al.*, 1969a). Sharma and Ganguli (1971) reported catalase activity in the milk of Murrah buffalo and Tharparker cow in the range of 0.30 to 1.50 (average of 0.56) and 0.42 to 2.0 (average 1.08) catalase unit, respectively. Yoganandi *et al.* (2014a) determined catalase activity in the milk of camel, cow and buffalo and found average catalase activity as 6.20, 0.86 and 0.62 catalase unit, respectively. They concluded that camel milk has higher catalase activity compared to cow and buffalo milk. Prajapati *et al.* (2017) reported the catalase activity in milk of goat, cow and buffalo as 1.03, 1.18 and 0.85 catalase unit, respectively. Cow milk had the highest catalase activity, which was followed by goat milk, and lowest catalase activity was found in buffalo milk. The catalase activity of Surti buffalo milk in this study was within the range of those reported in the literature for buffalo milk.

Xanthine oxidase activity: Xanthine oxidase activity in bulk milks received at the dairy plant ranged from 75.7 to 151 mU/mL with an average of 110 mU/mL. Upon storage of these milk samples at 2°C for 6 days, the average xanthine oxidase activity increased slightly from 110 mU/mL to 124 mU/mL (Cerbulis and Farrell, 1977). The xanthine oxidase in raw

market milk determined by Zittle *et al.* (1956), was found to be 140 units/mL for cream (separated by gravity, about 25% butterfat) and 25 units/mL for skim milk. Sanhotra and Dutta (1986) found lower xanthine oxidase activity in buffalo milk (0.075 U/mL) of indigenous breeds as compared to cow milk (0.093 U/mL). Sharma and Ganguli (1971) reported xanthine oxidase activity in Murrah buffalo milk in the range of 5.33×10^7 to 8.00×10^7 units (average of 7.17×10^7 units), whereas that in Tharparker cow milk, it ranged between 5.66×10^7 to 9.66×10^7 units (average of 7.58×10^7 units). El-Gazzar *et al.* (1999) observed that heat treatment of buffalo milk at 55°C/1 min increased the xanthine oxidase activity by 9.29 per cent. However, it decreased by 75.35 per cent and 89.43 per cent at 70°C/1 min and 80°C/1 min, respectively. Sharma *et al.* (2009) reported xanthine oxidase activity in buffalo, cow and goat milk as 96.08, 82.45 and 45.71 U/mL, respectively. The data for xanthine oxidase activity in the buffalo milk are expressed in different units, hence could not be compared with the results of this study.

Lactoperoxidase activity: The peroxidase activity in Murrah buffalo milk ranged from 45 to 408 (average of 230.7) peroxidase units per mL, whereas that in cow milk, it ranged from 165 to 975 (average of 489.9) peroxidase units per mL (Sharma and Ganguli, 1971). It was observed that lactoperoxidase activity was higher in Murrah buffalo milk than in crossbred cow milk. The average values for lactoperoxidase activity for Murrah buffalo milk and crossbred cow milk were 7.315 and 5.724 U/mL at pH 6.0. On skimming the milk, peroxidase activity was found to increase slightly both in buffalo and cow milk, while about 10-15% loss of activity was observed in the acid whey and rennet whey obtained from the milk (Kumar and Bhatia, 1999). The lactoperoxidase activity of buffalo milk varied widely (mainly due to the assay method used) from 0.9 U/mL (Hamulv and Kandasamy, 1982) to 16.84 U/mL (Tayefi-Nasrabadi and Asdpour, 2008). Sharma *et al.* (2009) studied the activity and thermal stability

of five indigenous enzymes in cow, buffalo and goat milk and found significant differences in lactoperoxidase activity amongst cow, buffalo and goat milk. They reported lactoperoxidase activity in buffalo milk, cow milk and goat milk as 12.02, 6.83 and 0.77 U/mL, respectively. The data for lactoperoxidase activity in the study varied from those reported owing to the use of different assay methods.

Proteolytic activity: Prajapati *et al.* (2017) reported proteolytic activity in goat, cow and buffalo milk. They observed cow milk had the highest proteolytic activity, followed by buffalo and goat milk. They reported proteolytic activity in goat, cow and buffalo milk as 502, 656 and 645 µg tyrosine/mL. The lower proteolytic activity in Surti buffalo milk was observed in the study.

The findings of the study opined that proximate chemical composition and chemical properties (titratable acidity and pH) of Surti buffalo milk were in general agreement with those reported in the literature. Variation observed in different constituents of Surti buffalo milk might be due to factors like season, feed, lactation and individuality of the animal. The activity of some indigenous enzymes in this study was in corroboration with the results reported by other researchers. However, variation in activity of some of the indigenous enzymes was observed, which might be due to factors like methods used for measurement, lactation stage, individuality and health of animal, etc.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contributions: AAH: Involved in the investigation, data generation, and preparing original draft; AKJ, SCP, AIS: Engaged in conceptualization, data curation, supervision and final editing.

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