

# Effect of milk sources on whey protein and fractions of casein content

## ABSTRACT:

**Aim:** Milk from Cow, Buffalo and goat were used for the study of isolation and fraction of casein and whey proteins. Buffalo milk and goat milk were subjected to physico-chemical attribute studies and a detailed protein profile namely total protein, casein and whey protein along with its various fractions of casein viz;  $\alpha$ ,  $\beta$  and  $\kappa$ -casein. Casein fractions separated were  $\alpha$ -,  $\beta$ - and  $\kappa$  casein by urea fractionation. The aim of this study was to compare various casein fractions isolated from cow's, Buffalo and Goat milk. The method of separating casein into its fractions is based on the solubility of the individual components in urea solution, the separation of casein fractions was conducted by changing the urea concentration in acid media and the yield of fractions and total protein percentages were calculated. Buffalo and goat milks have comparatively higher total protein and whey protein content as compared to cow milk. Maximum whey proteins were found in the goat milk (20.58 %) whereas cow milk had low lowest contents (19.29 %). Among casein fractions  $\alpha$  fraction (16.64%) was found maximum in Buffalo milk followed by cow (14.92 %) and goat milks (5.42 %). Goat milk sample showed highest  $\beta$  casein (17.81 %) and lowest found in cow milk (9.38%).

**Study Design:** A significant contribution to total milk production of India comes from buffalo milk and goat milk. In spite of that, Buffalo milk and goat milk are not being utilized for many products in view of their inherent problems associated in production of *products*.

**Place and Duration of Study:** Karnataka Veterinary Animal and Fisheries Sciences University (KVAFSU), Dairy Science College, Hebbal, Bangalore Karnataka, India

**Methodology:** The Goat milk samples were collected from Sinchana Goat and sheep farm, Marenahalli village (Bengaluru Rural Dist) and Buffalo milk was obtained from Country Delight Pvt. Ltd., J. P. Nagar, Bengaluru, Karnataka. Cow milk used in this investigation was collected from SEDP, Dairy Science College, Hebbal, Bangalore. Commercially available pure Neutrase enzyme was purchased from DSM Nutritional Products India Pvt. Ltd, Bangalore. All the glassware used were soaked in chromic acid solution, repeatedly washed with water, rinsed with distilled water and dried before use. For microbiological analysis dried test tubes, conical flask, pipettes were cotton plugged and sterilized in hot air oven. The chemicals and reagents used in this study were mainly of analytical grade procured from Prince Laboratory Company Pvt limited, Bangalore. The protein molecular weight markers were used for the electrophoretic study were procured from Bangalore Genei Pvt Ltd.

**Results:** The sources of protein had significant influence on total casein and whey protein content of different species. Highest quantity of total caseins (34.30g/l) and whey protein (8.87 g/l) were noted in buffalo milk than cow (28.52 g/l) and Goat milk (28.45 g/l). Total protein, casein and whey protein contents were greatly affected by the source of milk obtained.

**Conclusion:** Thus this investigation has shown that both buffalo and goat milk could be uninterruptedly used in the preparation of *Rasagulla*, without compromising any of the quality characteristics, which means that the utilization share of buffalo and goat milk could be enhanced besides adding onto the better nutritional profile.

**Recommendation:** *Rasagulla* will be consumed universally by all age groups globally. Thus, consumption of *Rasagulla* helps in providing overall nutritional requirement. However, the study has been conducted on a pilot scale. Moreover, commercial production may necessitate huge cost on enzymatic modification of proteins and separation peptides for value addition.

**Keywords:** *Rasagulla; Chhana; Buffalo milk; Goat milk; Milk protein; Whey Protein and Casein*

## INTRODUCTION

Milk production in India increased to 221.0 million tonnes in the year 2021-22 with a growth rate of 6.38 per cent per annum. The share of milk contribution by Cow, Buffalo and Goat to India's milk production is 51.85 per cent, 44.84 per cent and 2.93 per cent, respectively. Among the species, indigenous Buffaloes have the highest share of milk production in India with 32.13 per cent in the fiscal year 2022, followed by cross breed cows accounting for over 29.31 per cent of the total milk production in the country [3]. They are of great economic importance in India in the production of milk and milk products. The richness of buffalo milk makes it highly suitable for processing if proper processing technologies are exploited.

Buffalo milk, like cow's milk, can be utilized for the manufacture of a wide variety of dairy products such as cream, butter, butter oil (clarified butter or ghee), UHT cream, ice cream, yoghurt and some cheeses without changing the equipment or processing strategies. However, processing technology and equipment designed for cow milk product manufacture are often not adequately suitable for the production of certain dairy products using Buffalo milk due to the differences in compositional, physico-chemical, and functional properties. Buffalo milk compositional variations greatly affect the processing and yield of certain dairy products. Out of total milk produced in India more than 50 per cent of milk is being utilized for the preparation of dairy products.

Buffalo milk is often not considered as an ideal fluid for the manufacture of several types of cheeses, milk powders, evaporated, condensed milk, infant formulae and *Chhana* based dairy sweets, due to the high concentration of calcium, protein, fat and larger size of casein micelles, which produces undesirable quality, attributes thus causing textural defects in dairy products. Therefore, the conventional processing technologies are often unsuitable and cannot be applied directly for production of *chhana* and *chhana* based sweets. Pattern of milk consumption in India indicates that about 6 percent milk is converted into *Chhana* and *Chhana* based products [6].

Generally, cow milk is preferred for *chhana* making as it produces soft body and smooth texture product which is highly suitable for *chhana* based sweets particularly *rasagulla*. However, buffalo milk because of many inherent differences in physico-chemical make up as compared to cow milk, poses many technological problems in preparation of good quality *chhana* and *rasagulla*. Hence processing of buffalo milk into products needs suitable modification with respect to composition similar to that of cow milk, thus makes it suitable to produce *chhana* and *chhana* based sweets particularly *rasagulla*.

Goat milk differs from cow or buffalo milk in having better digestibility, alkalinity, buffering capacity and certain therapeutic values in medicine and human nutrition. Goat milk is considered to be an ideal food for people suffering from cow milk allergies and other gastro-intestinal ailments. Feeding goat milk to infants provides significantly higher digestibility as compared to cow milk. The children fed on goat milk surpassed those on cow milk in weight gain, skeletal mineralization and blood serum content of vitamin A, calcium, thiamin, riboflavin, niacin and hemoglobin [7].

Goat milk proteins are similar to the cow milk proteins fractions such as  $\alpha$ -casein,  $\beta$ -casein,  $\kappa$ -casein,  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin, but they differ widely in genetic polymorphisms and their frequencies in the goat population. Peptides formed from the enzymatic cleavage of caseins of goat milk have greater advantages than those from cow milk casein. Goat milk fat differs in contents of fatty acids profile significantly from average cow milk fat. Goat milk has higher content of monounsaturated fatty acids, polyunsaturated fatty acids, medium chain fatty acids, than cow milk which are proven to be beneficial for cardiovascular disorders.

## **MATERIALS AND METHODS**

The materials used and methods followed in this investigation for production of protein hydrolysates and bioactive peptides from Buffalo milk and Goat milk. The Goat milk samples were collected from Sinchana Goat and sheep farm, Marenahalli village (Bengaluru Rural Dist) and Buffalo milk was obtained from country Delight Pvt. Ltd., J. P. Nagar, Bengaluru, Karnataka. Cow milk used in this investigation was collected from SEDP, Dairy Science College, Hebbal, Bengaluru. Commercially available pure Neutrase enzyme was purchased from DSM Nutritional Products India Pvt. Ltd, Bangalore. All the glassware used were soaked in chromic acid solution, repeatedly washed with water, rinsed with distilled water and dried before use. For microbiological analysis dried test tubes, conical flask, pipettes were cotton plugged and sterilized in hot air oven. The chemicals and reagents used in this study were mainly of analytical grade procured from Prince Laboratory Company Pvt limited, Bangalore. The protein molecular weight markers used for the electrophoretic study were procured from Bangalore Genei

Pvt Ltd. All the necessary reagents were prepared in distilled or double glass distilled water for all analytical purposes and freshly prepared reagents were used in the study. Standard procedures (IS 1479) 2001 were followed for analysis milk .

#### **Preparation of whole casein and whey proteins**

Whole casein and whey proteins were prepared by coagulation of buffalo and goat skim milk separately at pH 4.6 using 10 per cent dilute hydrochloric acid. Cool the suspension to room temperature and leave it for 5 min. Filter through muslin cloth and casein precipitate was washed 2 to 3 times with cold distilled water to remove traces of acid. The resultant product was freeze dried as per the method [5]. Whey proteins were separated by precipitation and filtration of whey. The protein was estimated by Kjeldahl Method.

#### **Fractionation of caseins by urea solubility method**

Casein fractions were separated on the basis of their differential solubility in urea solution as per the method outlined [5].

#### **Analysis of casein fractions by SDS-PAGE**

SDS-PAGE was carried out to assess the molecular weight ranges of casein fractions by following the method [9]. The following reagents were employed for analysis.

### **RESULTS AND DISCUSSION**

#### **Effect of source of milk protein on yield of caseins and whey proteins**

Casein and whey proteins were isolated from cow, buffalo and goat milk. The yield of total protein, casein, and whey protein's are presented in Table (1). As observed from the table, buffalo milk resulted in significantly higher protein content (42.50 g/l) followed by goat milk (35.82 g/l) and cow milk (35.76 g/l). The respective casein content of cow, buffalo and goat milk were observed to be 28.52 g/l, 34.30 g/l, and 28.45 g/l. The casein yield was highest in buffalo milk (80.71%) followed by cow milk (79.76%) and goat milk (79.42%). The protein content of cow, buffalo and goat milk was observed to be 3.58, 4.25 and 3.68 percent respectively. The whey protein yield was observed to be 7.24, 8.20 and 7.37 g/l, respectively, for cow, buffalo and goat milk. Whey protein yield varied between 19.29 to 20.58 percent as against yield of casein which varied between 79.42 to 80.71 percent.

The cow, buffalo and goat milk used in this investigation were analysed for total protein, casein and whey proteins content, and the results are presented in Table (1.). It is pertinent to note that buffalo milk resulted in highest yield of total protein (42.50 g/l) as compared to cow's (35.76 g/l) and goat milk (35.82 g/l). Higher yield of total protein in buffalo milk is mainly attributed to higher initial protein content in buffalo milk. The buffalo milk resulted in higher yield of casein (34.30 g/l) as compared to Cow (28.52 g/l) and goat milk (28.45 g/l). Buffalo milk yielded not only higher casein but also higher whey protein content. In case of buffalo milk the yield of whey protein was higher (8.20 g/l) as against cow's milk (7.24 g/l) and goat milk (7.37 g/l), these results are in agreement with the earlier workers [1, 4 & 9]. The Buffalo milk possessed higher casein and whey protein content as compared to cow's milk and goat milk.

### Effect of source of protein on yield of various fractions of casein

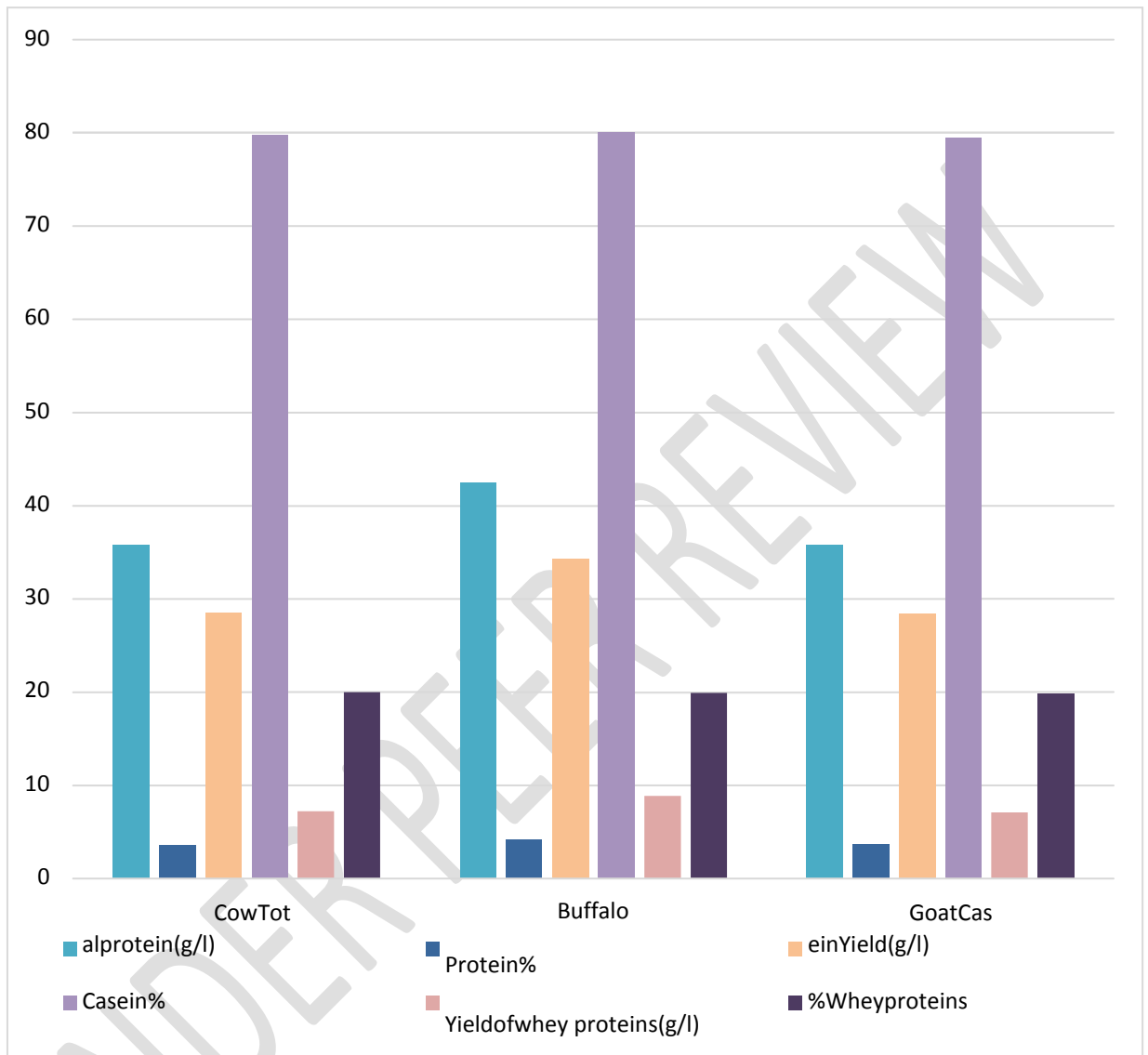
The effect of source of milk protein on yield of various fractions of protein is presented in Table (2) and Fig (2). The total casein content of cow, buffalo and goat milk was recorded to be 27.47 g/l, 34.13 g/l, and 26.62 g/l, respectively. The total casein content in buffalo milk (16.64 g/l) and cow milk (14.92 g/l) were significantly higher as compared to goat milk (5.42 g/l). Goat milk had significantly higher  $\beta$  casein (17.85 g/l) as compared to buffalo (12.30 g/l) and cow milk (9.38 g/l). It is observed from the table (3) that the concentration of k-casein was much higher in buffalo milk (5.19 g/l) as compared to goat milk (3.35 g/l) and cow milk (3.17 g/l). There was a significant difference in k-casein content of buffalo milk (5.19 g/l) as compared to cow (3.17 g/l) and goat milk (3.35 g/l). Significant difference was observed in the protein content amongst Cow, Buffalo and Goat.

The effect of source of milk proteins on yield of various fractions of casein was studied and results are presented in Table (2). The casein obtained from various sources was subjected to fractionation and the yield of various fractions viz  $\alpha$  casein,  $\beta$  casein and k casein were estimated. It is pertinent to note that buffalo milk resulted in higher yield of total  $\alpha$  casein and k-casein (16.64 and 5.19 g/l) as compared to cow (14.92 and 3.17 g/l) and goat milk (5.42 g/l and 3.35 g/l). This could be due to genetic inheritance of buffalo milk, which carry higher proportion of  $\alpha$  casein and k-casein than cow and goat milk. The results are in agreement with earlier workers [8&9]. Similarly,  $\beta$  casein content was also higher in buffalo milk (12.30 g/l) as compared to cow milk (9.38 g/l). But in goat milk  $\beta$  casein (17.85 g/l) content was significantly higher as compared to cow (9.38 g/l) and buffalo milk (12.30 g/l), though goat milk yielded lowest per cent of  $\alpha$ -casein as compared to cow and buffalo milk. This may be due to variation from species specific [2& 7].

**Table1:Effect ofsourceofmilk proteinon yield ofcaseinsand wheyproteins.**

Source of Milk	Total Protein(g/l)	Protein(%)	Caseins(g/l)	Casein Yield(%)	Whey Proteins(g/l)	Whey Proteins Yield (%)
Cow	35.76 <sup>a</sup>	3.58 <sup>a</sup>	28.52 <sup>a</sup>	79.76 <sup>a</sup>	7.24 <sup>a</sup>	20.24 <sup>a</sup>
Buffalo	42.50 <sup>b</sup>	4.25 <sup>b</sup>	34.30 <sup>b</sup>	80.71 <sup>a</sup>	8.20 <sup>b</sup>	19.29 <sup>a</sup>
Goat	35.82 <sup>a</sup>	3.68 <sup>a</sup>	28.45 <sup>a</sup>	79.42 <sup>a</sup>	7.37 <sup>ac</sup>	20.58 <sup>a</sup>
<b>CD<sub>p&lt;0.05</sub></b>	<b>0.57</b>	<b>0.51</b>	<b>0.55</b>	<b>0.53</b>	<b>0.53</b>	<b>0.58</b>

- All the values are average of three trails.
- Similar superscripts indicate non-significant at corresponding critical difference (CD)

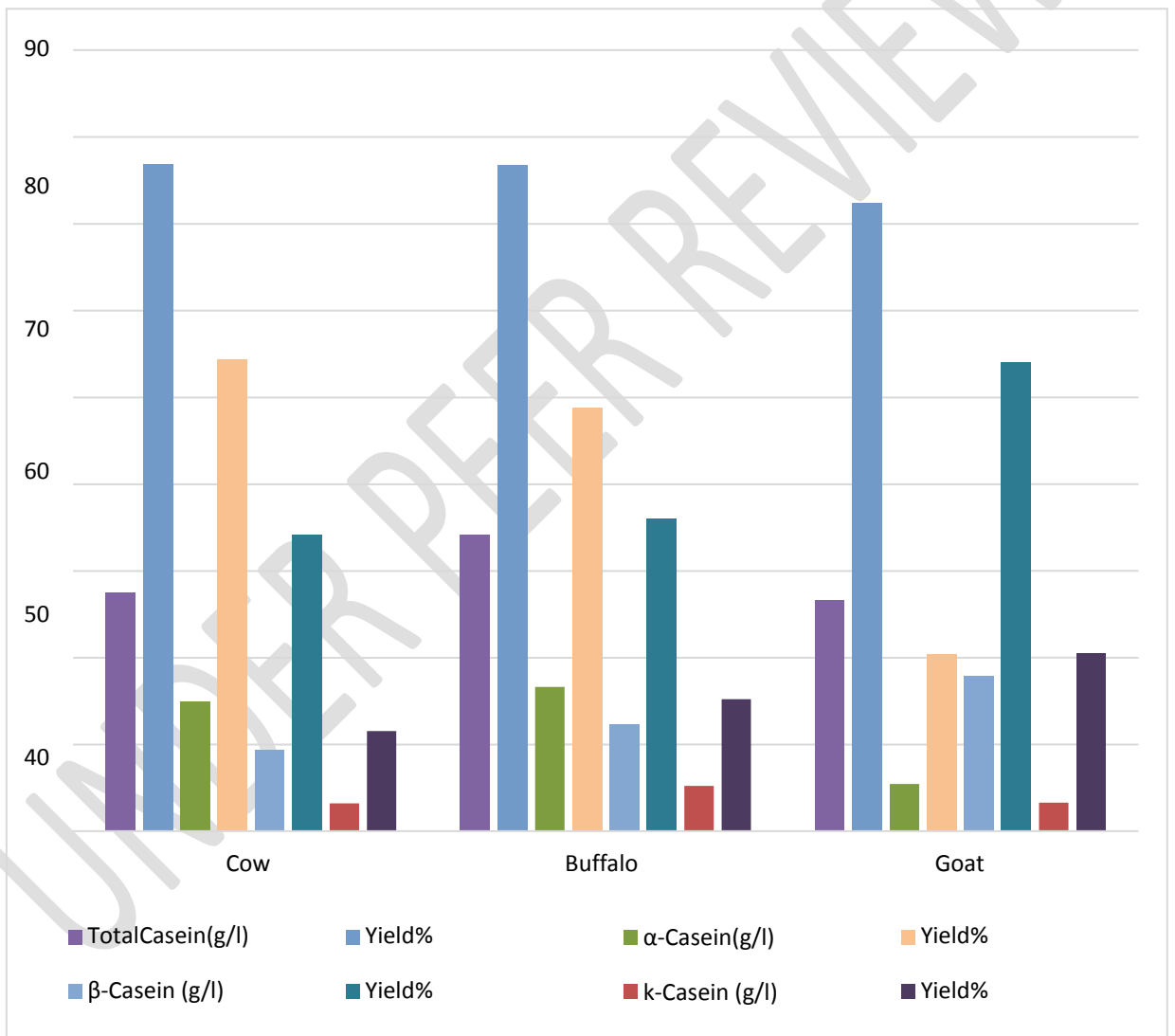


**Fig.1.Effectofsourceofmilkproteinon yield ofcaseinsandwheyprotein.**

**Table 2: Effect of source of protein on yield of various fractions of casein**

Source of Casein Fractions	Total Casein (g/l)	Yield (%)	Total $\alpha$ -Casein (g/l)	Yield (%)	Total $\beta$ -Casein (g/l)	Yield (%)	Total $\kappa$ -Casein (g/l)	Yield (%)
Cow	27.47 <sup>a</sup>	76.83 <sup>a</sup>	14.92 <sup>a</sup>	54.31 <sup>a</sup>	9.38 <sup>a</sup>	34.14 <sup>a</sup>	3.17 <sup>a</sup>	11.53 <sup>a</sup>
Buffalo	34.13 <sup>b</sup>	76.70 <sup>a</sup>	16.64 <sup>b</sup>	48.75 <sup>b</sup>	12.30 <sup>b</sup>	36.03 <sup>b</sup>	5.19 <sup>b</sup>	15.20 <sup>b</sup>
Goat	26.62 <sup>c</sup>	72.33 <sup>b</sup>	5.42 <sup>c</sup>	20.36 <sup>c</sup>	17.85 <sup>c</sup>	54.05 <sup>c</sup>	3.35 <sup>ac</sup>	20.49 <sup>c</sup>
<b>CD (p &lt; 0.05)</b>	<b>0.53</b>	<b>0.54</b>	<b>0.47</b>	<b>0.60</b>	<b>0.56</b>	<b>0.50</b>	<b>0.55</b>	<b>0.49</b>

- All the values are average of three trails.
- Similar superscripts indicate non-significant at corresponding critical difference (CD)



**Fig.2. Effect of source of protein on yield of various fractions of casein**

## CONCLUSION:

Casein obtained from cow, buffalo and goat milk were fractionated by urea solubility method and the fractions were quantified. The significant effect of source on protein yield and fractions of caseins was observed. Higher yield of  $\alpha$ -casein (54.31%) was observed in cow milk than buffalo milk (48.95 %) and lower yield (20.36 %) was found in goat milk. Amongst the three species, highest percent of  $\beta$  casein was noted in goat milk (54.05%) followed by buffalo (36.03%) and cow milk (34.14%), The highest molecular weight in respect of  $\alpha$ -casein (23.82 kDa) was observed in cow milk followed by goat milk (23.61 kDa) and buffalo milk (22.74 kDa). Whereas the molecular weight of  $\beta$  casein in respect of cow milk was 24.31, and it was 23.84 for buffalo milk and 23.82 for goat milk. There was no wide variation in molecular weight of  $\kappa$ -casein irrespective of source of milk. The molecular weight of  $\kappa$ -casein varied between 19.15 to 19.38 kDa.

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