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Physico-chemical characterization of various fractions of cow, Buffalo and goat milk casein

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Abstract
Study of Physico-chemical characterization of various fractions of cow, Buffalo and goat milk casein such as α, β and k-casein. Buffalo milk is nutritionally rich and has many health benefits. Inspite of that, half of buffalo milk produced in India is utilised for production of various dairy products. In spite of several positive effects of goat milk on human health, Goat milk is not being used in c*hhana* and channa based sweets. Therefore, in this investigation the attempt has been made to make use of the milk by suitable modification through enzymatic hydrolysis so as to induce desirable changes in the protein conformation leading to improvement in techno-functional properties of milk protein. 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 α -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 per cent of β 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 α-casein (23.82 kDa) was observed in cow milk followed by goat milk (23.61KDa) and buffalo milk (22.74 kDa). Whereas the molecular weight of β 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 k-casein irrespective of source of milk. The molecular weight of k- casein varied between 19.15 to 19.38kDa.

Keywords: Cow milk, buffalo milk, goat milk, milk casein, α-casein; β-casein and k-casein

Introduction
FSSAI, 2011, defines milk as the normal mammary secretion derived from complete milking of healthy milch animal. It should be free from colostrum. Milk which is adjusted for milk fat or milk SNF content or both, may also be named "milk" provided that the minimum and maximum limits for fat and SNF are as per the standards of milk.

Milk is biologically complex fluid, constituted mainly of water, proteins, lactose, fat and inorganic compounds. Caseins and whey proteins are the main proteins present in various milk of different species in different proportion. The ratio of casein and whey proteins are 40:60 in human milk, 50:50 in equine milk, while in milk of cow, sheep, goat and buffalo it is 80:20. Proteins and peptides present in milk have important nutritional, functional, biological and technological properties (Hadohum *et al.*, 2017) [1]. Caseins are highly digestible than whey proteins and are important for growth and development of infants. (Holt *et al.*, 2016) [2].

Caseins in milk are present in the form of micelles which are composed of αSl- casein (αSl-CN), αS2-casein (αS2-CN), β-casein and *k*-casein. Whey proteins contain four major proteins *i.e.* α-lactalbumin (α-lac), β- lactoglobulin (β-lg), blood serum albumin (BSA) and immunoglobulins (Ig). Besides these, the whey fraction contains proteoses and peptones (PP), lactotransferin, serotransferin, osteoportins, vitamin binding proteins, lactoferrin and about 60 indigenous enzymes (Zeynep *et al*., 2017) [5].

Buffalo milk is nutritionally rich and has many health benefits. Inspite of that, half of buffalo milk produced in India is utilised for production of various dairy products. Buffalo milk utilization in production of *chhana* and *chhana* based sweets is very much limited due to inherent properties that produce poor quality of final products. This is largely attributed to the basic compositional difference of buffalo milk as compared to cow milk. High protein and calcium content in buffalo milk renders dairy products hard, rubbery and coarse texture in c*hhana* and *chhana* based sweets. In spite of several positive effects of goat milk on human health, Goat milk is not being used in c*hhana* and channa based sweets. Therefore, in this investigation the attempt has been made to make use of the milk by suitable modification

through enzymatic hydrolysis so as to induce desirable changes in the protein conformation leading to improvement in techno-functional properties of milk proteins.

Law *et al.,* (2007) [4] and Hayem *et al.,* (2014) [3] demonstrated that αs1-CN of BM consists of a single 199 amino acid residues. However, the buffalo αs1-CN exhibited lower mobility in alkaline PAGE than its bovine counterpart but with isoelectric focusing, they had shown similar pI. The primary structure of water buffalo αs1-CN was different to that as evident from nucleotide sequencing of Indian River buffalo αs1-CN. Three isoforms were found in buffalo αs1-CN containing 8, 7, and 6 phosphate groups, respectively. Phosphorylation occurs in similar sites as in cow αs1-CN, i.e., at Ser 41, 46, 48, 64, 66, 67, 68, and 75 respectively but not at Ser 115 as in cow αs1-CN being three, two, and one phosphate group respectively (at Ser 15, 17, 18, 19, and 35). The lack of putative phosphorylation sites within the Buffalo β-CN amino acid sequence resulted in a reduced degree of phosphorylation of this protein with respect to that observed in β-CN of other ruminants (Jitendra *et al.,* 2013) [6].

The complete sequence of buffalo β-CN has been done by a combination of mass spectrometry and Edman degradation (Jitendra *et al.,*.2013) [6] and by DNA sequencing in Indian and German water buffalo (Post *et al.,* 2009) [8]. Both cow and buffalo milk β-CN has a very similar amino acid composition and sequence. Only six single substitutions were found in the two β-CNs indicating high homology (95%) between the two proteins. Two genetic variants (A and B) were identified in Buffalo β-CN (Post *et al.,* 2011) [7].

κ-CN is the casein fraction characterized by having a carbohydrate moiety and all the N-acetylneuramenic acid present in casein micelles. An early study showed differences in the C-terminal amino acid sequence of buffalo and cow κ-CN. K-CN consists of a single surrounded with hydrophobic amino acids. The absence of phosphorylation at Ser 115 strengthens the hydrophobic nature of buffalo αs1-CN. This may explain the higher sensitivity of buffalo αs-CN to Ca++in comparison to that of Cow milk (Feligini *et al*., 2009) [9].

β-CN of buffalo milk is a single polypeptide chain of 209 residues. Several isoforms have been identified (Anamaria *et al.,* 2011) in β-CN of BM which contained five, four, polypeptide chain of 169 amino acid residues in buffalo and cow κ-CNs. Buffalo/Cow κ- CNs showed substitutions in 13 sites and 92.6 per cent homology. Six to seven components were separated from buffalo κ-CN Buffalo κ-CN had less sialic acid content than Cow κ- CN (Adamson *et al.,* 1995) [11]. The carbohydrate free fraction of κ-casein represents 40 per cent of κ-CN in buffaloes where it accounted for only 25 per cent of total κ-CN in cow's milk (Fox,, 2003) [13], which explains the low sialic acid content of Buffalo κ-CN. Based on sequence analysis and coding region, Pticek, *et al.,* 2016 [14] concluded that κ-CN of Indian river Buffalo seems to be an intermediate between A and B variants of Cow κ-CN. On the other hand, Samir *et al*., 2009 [19] demonstrated that Egyptian Buffalo bulls were monomorphic for the κ-CN gene and that they possessed the only B allele variant. Ahmed *et al.,* (2013) [12] observed that Brazilian Buffaloes had only κ-CN B allele.

The physico-chemical characteristics of many dairy products depend on the properties of milk proteins. During the classical cheese making process, it is the casein fraction which constitutes cheese curd after enzyme-triggered milk coagulation step (Berghe *et al.,* 2018) [15].

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, Bengalore. 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 elcterophorectic 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 of Hipp *et al.,* (1952) [16]. 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 by Hipp *et al.,* (1952) [16].

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 prescribed by Laemmli (1976) [18]. The following reagents were employed for analysis.

Reagents

The reagents were prepare as detailed between

a) **30%acrylamide mix:** 29 g. of acryl amide and 1 gm of Bis-acrylamide were dissolved in 100 ml of distilled water
b) **1.5M Tris (pH 8.8):** 18.17 g. of Tris was dissolved in distilled water and pH adjusted with HCL before adjusting the final volume to 100 ml.
c) **10%SDS (Sodium dodecyl sulphate):** 10 g. of sodium lauryl sulphate was dissolved in 100 ml distilled water
d) **10%Ammonium persulphate:** 1 g. of ammonium persulphate was dissolved in 10ml of water
e) **1 M Tris (pH 6.8):** 12.114 g of Tris was dissolved in distilled water. PH was adjusted to 6.8 with dilute HCL

before adjusting the final volume of buffer to 100 ml.

f) TEMED: N,N,N[1],N[1] tetra methyl ethylene diamine

Results and Discussion

Physico-chemical characteristics of various fractions of cow milk casein

The physico-chemical characteristics of various fractions of cow milk casein are presented in Table (4.5), (4.5a) and plate (1). The respective molecular weights of α, β and k case infractions of casein from cow milk, were found to be 23.82, 24.31 and 19.15 KDa. Amongst β and k, β casein fractions had significantly higher molecular mass (24.31kD) followed by α casein (23.82kDa) and k-casein (19.15kDa). There was a significant difference in the molecular mass between α and k caseins. The phosphorous and nitrogen content of α, β and k casein obtained from cow milk were found to be 0.69, 0.35, 0.10 per cent and 14.85, 12.55, 12.75, respectively, Nitrogen content was significantly higher in α casein (14.85%) followed by k- casein (12.75%). From the table (4.5) it is evident that there is no significant difference in the pH of β and k-casein. However, there is a significant difference in α and β casein. There was no significant difference in the iso-electric point of α (4.55) and β caseins (4.73) but isoelectric point of k casein was however significantly higher (5.50) as compared to other fractions as shown in plate 1.

Table 1: Physico-chemical characteristics of various fractions of cow milk casein

Type of casein fractions	Mol wt (kDa)	pI	pH	Phosphorous Content (%)	Nitrogen content (%)
α-Casein	23.82[a]	4.55[a]	4.95[a]	0.69[a]	14.85[a]
β-Casein	24.31[a]	4.73[a]	5.44[b]	0.35[b]	12.55[b]
k-Casein	19.15[b]	5.50[b]	5.73[b]	0.10[c]	12.75[c]
CD (p<0.05)	0.63	0.50	0.45	0.22	0.55

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

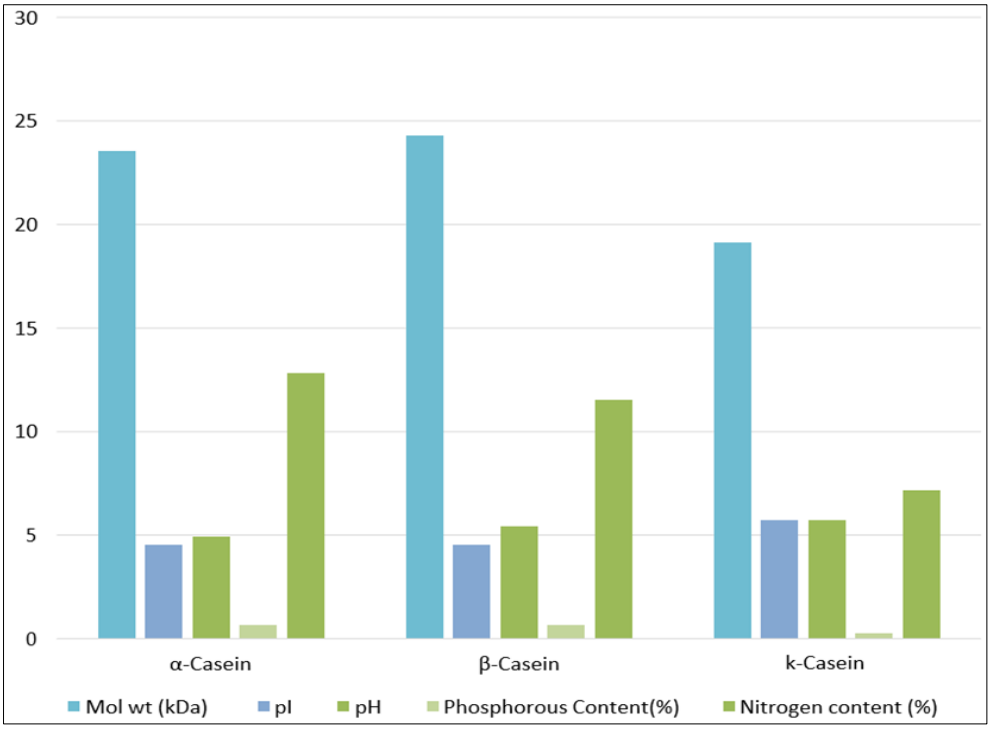

Fig 1: Physico-chemical characteristics of various fractions of cow milk casein

Table 2: Molecular weight profile of casein fractions of cow, buffalo and goat milk

Molecular weight range Da	Cow milk			Buffalo milk			Goat milk		
	α Casein	β Casein	k- Casein	α Casein	β Casein	k- Casein	α Casein	β Casein	k- Casein
< 1800-19500	-	-	+	-	-	-	-	-	+
19500-21500	-	-	-	-	-	-	-	-	-
21500- 22500	-	-	-	-	-	+	-	-	-
22500-23500	-	-	-	+	-	-		-	-
23500-24500	+	-	-	+	+	-	+	+	-
24,500-25,000	-	+	-	+	-	-	-	-	-

+ Presence of bands
- Absence of bands

Physico-chemical characteristics of various fractions of buffalo milk casein

The physico-chemical characteristics of various fractions of buffalo milk casein are depicted in Table (1), fig (1) and plate (1). The molecular weight, of α, β and k-casein were noted to be 22.74, 23.58, 19.20kDa, whereas the isoelectric point of α, β and k-casein fractions were found to be 4.44, 4.63, 5.74 and pH of these fractions were observed to be 4.86, 5.66 and 5.95 respectively. A significant difference in molecular mass was observed amongst α, β and k-caseins, but no significant difference was observed between α and β casein in respect of isoelectric point. The compositional difference between α casein, β casein and k- caseins of different species with respect to phosphorous and nitrogen content were noted to be 0.78, 0.38, 0.12 and 12.66, 12.15, 13.35 Per cent respectively. There was significant difference in the composition of β casein as compared toα and k-casein with respect to molecular weight, pH, phosphorus, nitrogen contents of all casein fractions was observed.

Physico-chemical characteristics of various fractions of goat milk casein

The physico- chemical characteristics of various fractions of goat milk casein were studied and the results are depicted in Table (2), Fig (2) and plate (1). The molecular weight, iso-electric point and pH of α casein, β and k-caseins obtained from goat milk were 23.61, 4.45 and 4.92 whereas it was 23.82, 4.54 and 5.43 for β casein and 19.38, 5.55, and 5.73 for k-casein respectively.

There was a significant difference in molecular mass of β -caseins of Goat milk as compared to k casein. But no significant difference was observed between α casein and βcasein. The phosphorous content of α-casein was found to be 0.45, 0.76, 0.21 per cent, respectively, Whereas the respective nitrogen content was observed to be 12.54, 14.55 and 13.50 per cent. A significant difference was observed in phosphorus and nitrogen content amongst α, β and k-casein as compared to α and k-casein.

Table 3: Physico-chemical characteristics of various fractions of buffalo milk casein

Type of casein fractions	Mol wt (kDa)	PI	pH	Phos Content (%)	Nitrogen Content (%)
αs-Casein	22.74^{a}	4.44^{a}	4.86^{a}	0.78^{a}	12.66^{a}
β-Casein	23.58^{b}	4.63^{a}	5.66^{b}	0.38^{b}	12.15^{b}
k-Casein	19.20^{ac}	5.74^{b}	5.95^{b}	0.12^{c}	13.35^{c}
CD (p<0.05)	0.69	0.60	0.50	0.24	0.51

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

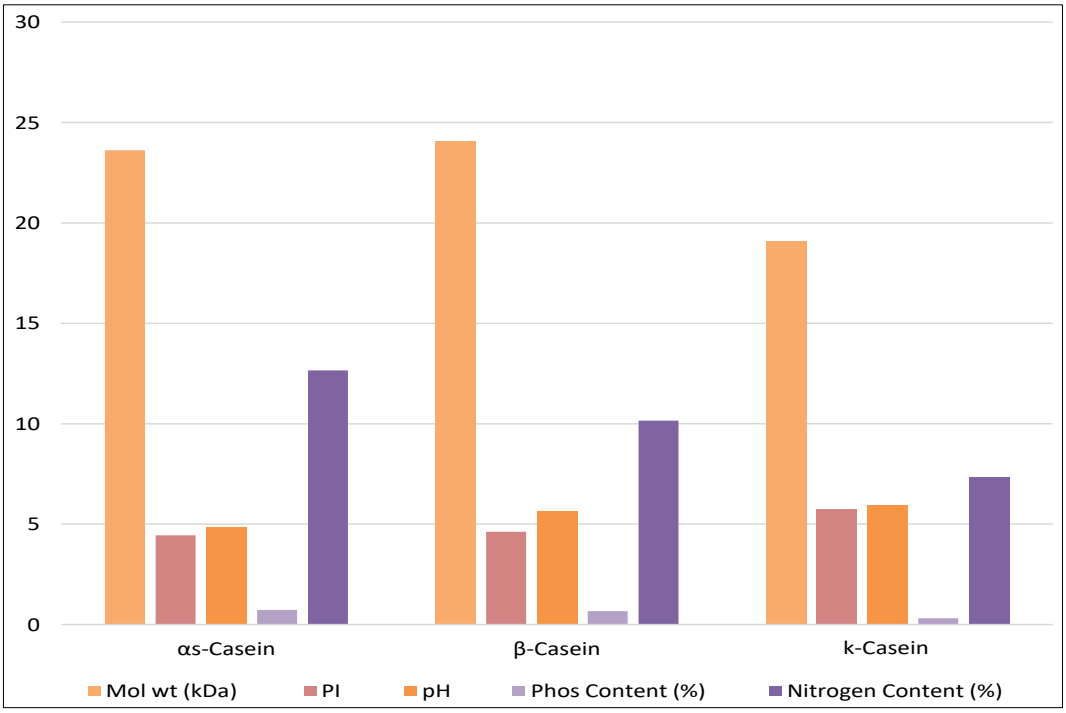

Fig 2: Physico-chemical characteristics of various fractions of buffalo milk casein

Table 4: Physico-chemical characteristics of various fractions of goat milk casein

Casein fractions	Mol wt (kD)	pI	pH	Phosphorus Content (%)	Nitrogen content (%)
α-Casein	23.61^{a}	4.45^{a}	4.92^{a}	0.45^{a}	12.54a
β-Casein	23.82^{a}	4.54^{a}	5.43^{b}	0.76^{b}	14.55^{b}
k-Casein	19.38^{b}	5.55^{b}	5.73^{b}	0.21^{c}	13.50^{c}
CD (p<0.05)	1.20	0.54	0.48	0.20	0.53

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

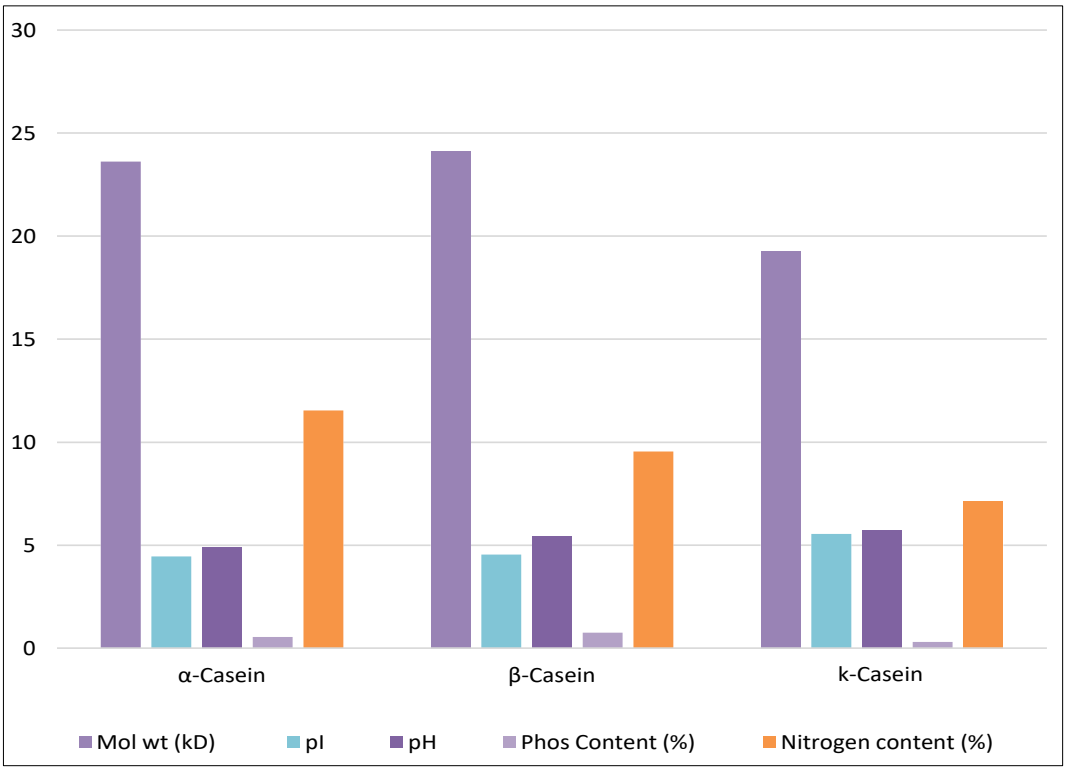

Fig 3: Physico-chemical characteristics of various fractions of goat milk casein

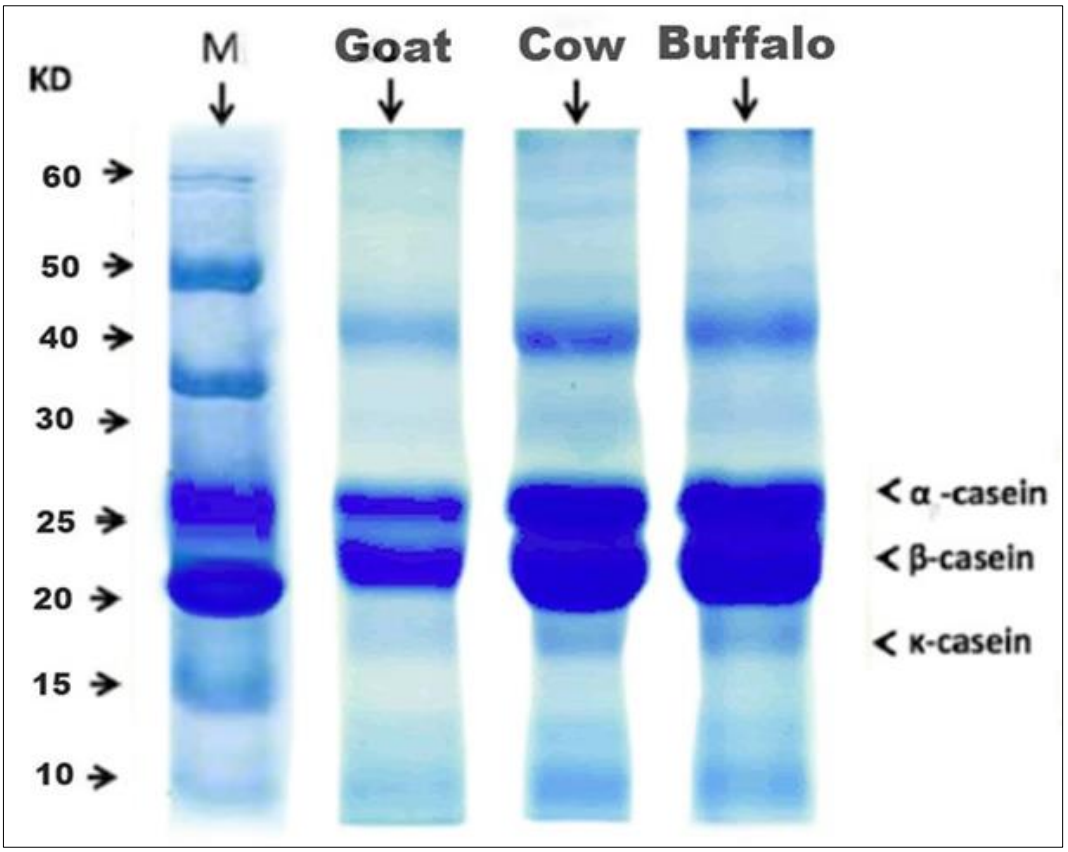

Plate 1: Characterization of casein fractions of cow, buffalo and goat milk proteins by SDS-PAGE

Conclusion

The functional properties of Buffalo and Goat milk casein fractions were explored to improve the quality of *Rasagulla*. Besides, Bioactive peptides derived from enzymatic hydrolysis of casein being nutritionally superior in enhancing the bioavailability of micronutrients. Such as calcium, iron and zinc for value addition. Such developed -*Rasagulla* may help in combating several gasto-interstinal disorders as also promote proper development of hard tissues (Bones and teeth) especially in growing children. *Rasagulla* will be consumed universally by all age groups globally. Thus, consumption of *Rasagulla* helps in providing overall nutritional requirement.

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Competing interests
Authors have declared that no competing interests exist.

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