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.Buffalomilkandgoatmilkweresubjectedto physico-chemical attribute studies and a detailed protein profile namely total protein,casein and whey protein along with its various fractions of casein viz; α, β and κ-casein.Casein fractions separated were α-, β- and κ 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 lowlowest contents (19.29 %) Among casein fractions α fraction (16.64%) was found maximum in Buffalo milk followed by cow (14.92 %) and goat milks (5.42 %). Goat milk sample showed highest β 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. Inspite 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, Bengalore. Commercially available pureNeutrase 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 proteinscontent of different species. Highest quantity of total caseins (34.30g/l) and wheyprotein (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 bythesourceofmilk obtained.

Comment [PB1]: Modify title. Whey protein and casein fractions of cow, buffalo and goat milk

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Comment [PB3]: Fractionation

Comment [PB4]: Cow milk is not included. ; Space between subjected to; physico chemical attribute is not studied. ; modify sentence accordingly.

Comment [PB5]: Change sentence format; a,b and k caseins were fractionated by urea fractionation method.

Comment [PB6]: Cow not cow's ; buffalo lower case and goat lower case ; breed should be mentioned.

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Comment [PB11]: how many samples and breed of goat, cow, buffalo should be mentioned.

Comment [PB12]: Remove pure; enzyme activity should be mentioned

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Comment [PB15]: PMWM range should be given; and results are not found in the paper. Hence not required here.

Comment [PB16]: Sources of protein from different species

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Conclusion: Thus this investigation has shown that both buffalo and goat milk milkcould beuninterruptedly used in the preparation of *Rasagulla*, without comprismisingany of thequality characteristics, which means that the utilization share of buffalo and goat milkcouldbeenhanced besides adding onto thebetternutritional profile.

Recommendation: *Rasagulla*will be consumeduniversallybyallagegroupsglobally.Thus,consumptionof*Rasagulla*helpsinprovidingoverall nutritional requirement. However, the study has been conducted on a pilot scale.Moreover, commercial production may necessiates huge cost on enzymatic modificationofproteinsandseparationpeptidesforvalueaddition.

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

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INTRODUCTION

MilkproductioninIndiaincreasedto221.0milliontonnesintheyear2021-22withgrowth rate of 6.38 per cent per annum.The share of milk contribution by Cow, Buffaloand Goat to India's milk production is 51.85 per cent, 44.84 per cent and 2.93 percent,respectively.Among the species, indigenous Buffaloes have highest share of milkproduction in India with 32.13 per cent in the fiscal year 2022, followed by cross breedcows 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 production of milkand milk products. The richness of buffalo milk makes it highly suitable for processing ifproperprocessingtechnologies areexploited.

Buffalomilk,likecow'smilk,canbeutilizedforthemanufactureofawidevarietyof 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 processingstrategies.However,processingtechnologyandequipmentdesignedforcowmilkproductmanufacture areoftennotadequatelysuitableforproductionofcertaindairyproductsusingBuffalo milk due to the differences in compositional, physico-chemical, and functionalproperties.Buffalomilkcompositionalvariationsgreatlyaffecttheprocessingandyieldofcertaindairyproducts.OutoftotalmilkproducedinIndiamorethan50percentofmilkisbeingutilizedforthepreparation ofdairy products.

Buffalomilkisoftennotconsideredasanidealfluidforthemanufactureofseveraltypes of cheeses, milk powders, evaporated, condensed milk, infant formulae and *Chhana*baseddairysweets,duetothehighconcentrationofcalcium,protein,fatandlargersizeofcasein micelles,whichproducesundesirablequality,attributesthuscausingtexturaldefectsindairyproducts.Therefore,theconventionalprocessingtechnologiesareoftenunsuitableandcannotbeapplieddirectlyforproductionof*chhana*and*chhanabased*sweets.Patternofmilkconsumptioninindiaindicatesthatabout6percentmilkisconvertedintoChhanaandChhana based products[6].

Generally, cow milk is preferred for *chhana* making as it produces soft body andsmooth texture product which is highly suitable for *chhana* based sweets particularly*rasagulla*.However,buffalomilkbecauseofmanyinherentdifferencesinphysico-chemical make up as compared to cow milk, poses many technological problems inpreparation of good quality *chhana* and *rasagulla.*Hence processing of buffalo milk intoproducts needs suitable modification with respect to composition similar to that of cowmilk, thus makes it suitable to produce *chhana* and *chhana* based sweets particularly*rasagulla.*

Goatmilkdiffersfromcoworbuffalomilkinhavingbetterdigestibility,alkalinity,buffering capacity and certain therapeutic values in medicine and human nutrition. Goatmilk is considered to be an ideal food for people suffering from cow milk allergies andother gastro-intestinal ailments. Feeding goat milk to infants provides significantly higherdigestibilityascomparedtocowmilk.Thechildrenfedongoatmilksurpassedthoseon cowmilkinweightgain,skeletalmineralizationandbloodserumcontentofvitaminA,calcium,thiamin,riboflavin, niacinand hemoglobin[7].

Goat milk proteins are similar to the cow milk proteins fractions such as α-casein,β-casein,k-casein,β-lactoglobulin,α-lactalbumin,buttheydifferwidelyingeneticpolymorphisms and their frequencies in the goat population. Peptides formed from theenzymatic cleavage of caseins of goat milk have greater advantages than those from cowmilk casein. Goat milk fat differs in contents of fatty acids profile significantly fromaverage 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 bebeneficialforcardiovascular 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, 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

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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 .

Preparationofwholecasein andwheyproteins

Whole casein and whey proteins were prepared by coagulation of buffalo and goatskim milk separately at pH 4.6 using 10 per cent dilute hydrochloric acid. Cool thesuspension to room temperature and leave it for 5 min. Filter through muslin cloth andcasein precipitate was washed 2 to 3 times with cold distilled water to remove traces ofacid.Theresultantproductwasfreezedriedasperthemethod[5].Wheyproteins were separated by precipitation and filtration of whey. The protein was estimatedbyKjeldahl Method.

Fractionationofcaseinsbyureasolubilitymethod

Casein fractions were separated on the basis of their differential solubility in ureasolutionas perthemethod outlined[5].

Analysisofcasein fractionsbySDS-PAGE

SDS-PAGEwascarriedouttoassessthemolecularweightrangesofcaseinfractions by following the method [9].The following reagentswereemployed for analysis.

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RESULTS AND DISCUSSION

Effectofsourceofmilkproteinonyieldofcaseinsandwheyproteins

Comment [PB33]: remove. Yield of casein and whey proteins from different species milk sources

Caseinandwheyproteinswereisolatedfromcow,buffaloandgoatmilk.Theyieldof total protein, casein, and whey protein's are presented in Table (1). As observed fromthe table, buffalo milk resulted in significantly higher protein content (42.50 g/l) followedby 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, 34.30g/l, and 28.45 g/l. The caseinyieldwashighestinbuffalomilk(80.71%)followedbycowmilk(79.76%)andgoatmilk(79.42%).Theproteinc ontentofcow,buffaloandgoatmilkwasobservedtobe3.58,4.25and3.68percentrespectively.Thewheyproteinsyi eldwasobservedtobe7.24,8.20and7.37g/l,respectively,forcow,buffaloandgoatmilk.Wheyproteinyieldvariedb etween19.29to20.58percentas against yield ofcaseinwhichvariedbetween79.42to80.71 percent.

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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 Cow (28.52 g/l) and goat milk (28.45g/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.

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Effectofsourceofprotein onyieldofvariousfractions ofcasein

The effect of source of milk protein on yield of various fractions of protein ispresentedin Table (2)and Fig (2).Thetotalcaseincontentofcow,buffaloandgoatmilkwasrecordedtobe27.47g/l,34.13g/l,and26.62g/l,respectively.Thetotalαcaseincontentinbuffalomilk(16.64g/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 β 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) andcow milk (3.17 g/l). There was a significant difference in k-casein content of buffalo milk(5.19g/l)ascomparedtocow(3.17g/l)andgoatmilk(3.35g/l).Significantdifferencewasobservedin the proteincontent 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 sourceswas subjected to fractionation and the yield of various fractions viz α casein, β casein and k casein were estimated. It is pertinent to note that buffalo milk resulted in higher yield of total α 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 (5.42g/l and 3.35 g/l). This could be due to genetic inheritance of buffalo milk, which carry higher proportion of α casein and k-casein than cow and goat milk. The results are in agreement with earlier workers[8&9].Similarly, β 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 β 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 α-casein as compared to cow and buffalo milk. This may be due to variation from species specific[2& 7].

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Table1:Effect ofsourceofmilk proteinon yield ofcaseinsand wheyproteins.

Sourceo fMilk	TotalPro tein(g/l)	Protein(%)	Caseins(g/l)	Casein Yield(%)	WheyProt eins(g/l)	WheyProteinsYie ld (%)
Cow	35.76[a]	3.58[a]	28.52[a]	79.76[a]	7.24[a]	20.24[a]
Buffalo	42.50[b]	4.25[b]	34.30[b]	80.71[a]	8.20[b]	19.29[a]
Goat	35.82[a]	3.68[a]	28.45[a]	79.42[a]	7.37[ac]	20.58[a]
CDp<0.05)	**0.57**	**0.51**	**0.55**	**0.53**	**0.53**	**0.58**

- Allthevaluesareaverageofthreetrails.
- Similarsuperscriptsindicatenon-significantatcorrespondingcriticaldifference(CD)

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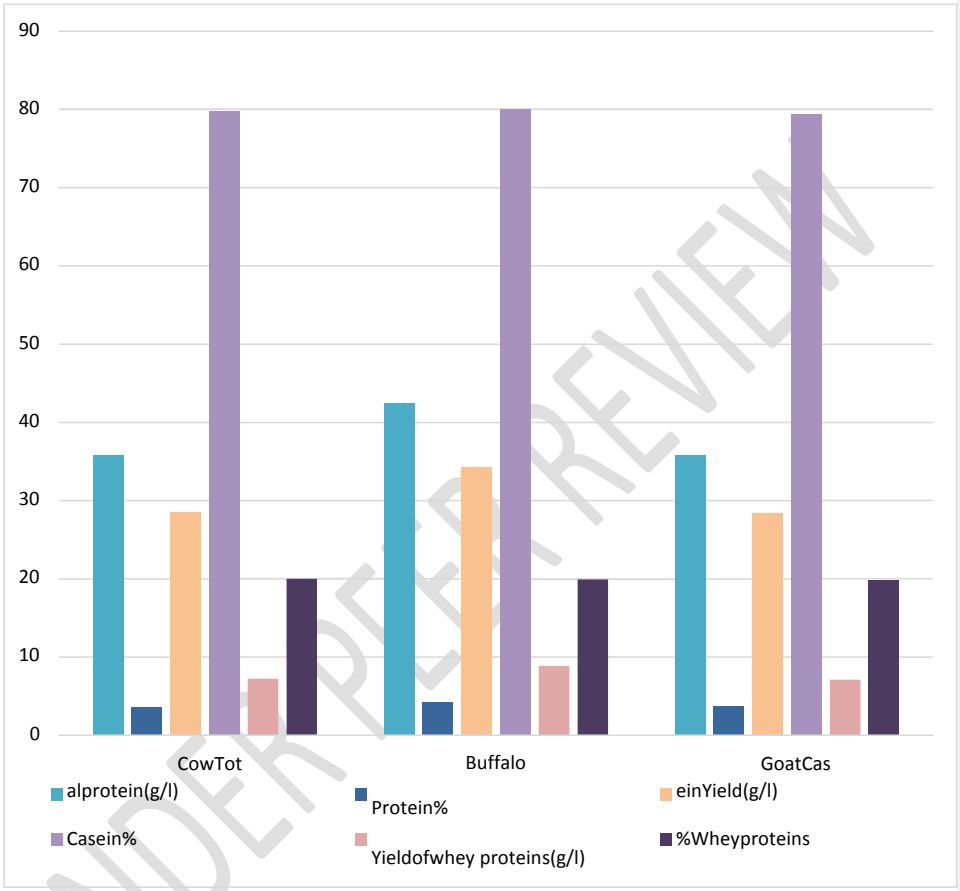

Fig.1.Effectofsourceofmilkproteinon yield ofcaseinsandwheyprotein.

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Table2:Effect ofsourceofproteinon yield ofvariousfractions ofcasein

Sourceof CaseinFractions	TotalCasein(g/l)	Yield(%)	Totalα-Casein (g/l)	Yield(%)	Totalβ-Casein (g/l)	Yield(%)	Totalk-Casein (g/l)	Yield(%)
Cow	27.47[a]	76.83[a]	14.92[a]	54.31[a]	9.38[a]	34.14[a]	3.17[a]	11.53[a]
Buffalo	34.13[b]	76.70[a]	16.64[b]	48.75[b]	12.30[b]	36.03[b]	5.19[b]	15.20[b]
Goat	26.62[c]	72.33[b]	5.42[c]	20.36[c]	17.85[c]	54.05[c]	3.35[ac]	20.49[c]
CD($p<0.05$)	**0.53**	**0.54**	**0.47**	**0.60**	**0.56**	**0.50**	**0.55**	**0.49**

- Allthevaluesareaverageofthreetrails.
- Similarsuperscriptsindicatenon-significantatcorrespondingcriticaldifference(CD)

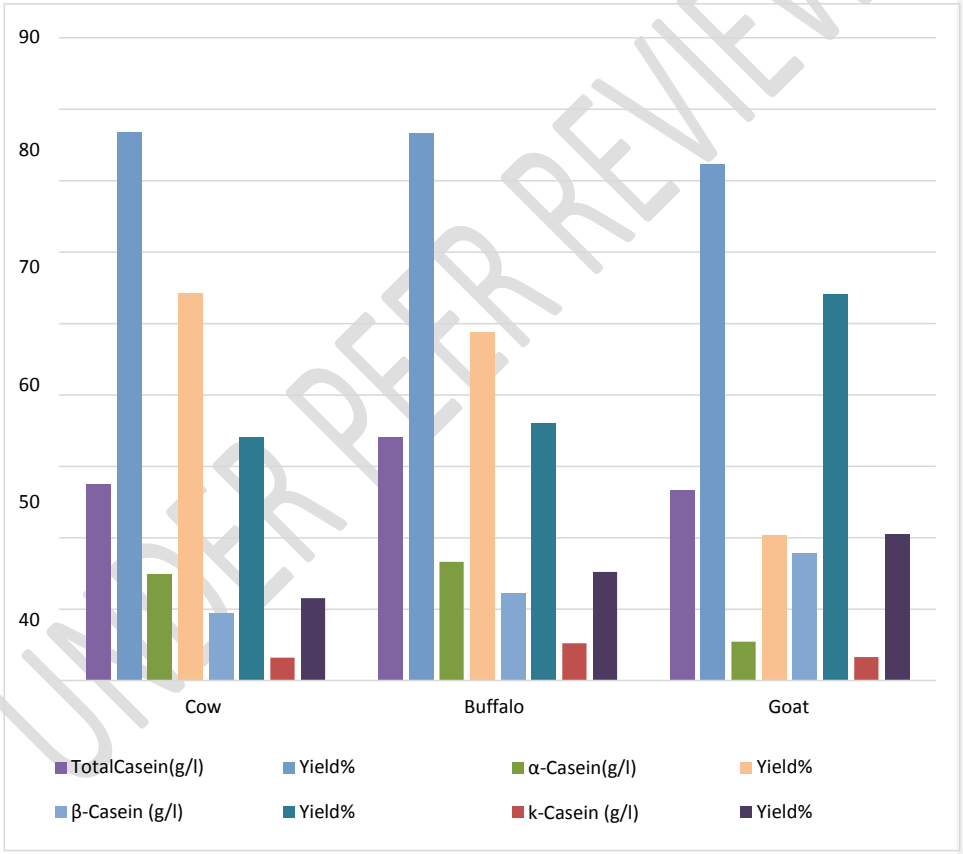

Fig.2.Effectofsourceofproteinon yield ofvarious fractionsofcasein

CONCLUSION:

Caseinobtainedfromcow,buffaloandgoatmilkwerefractionatedbyureasolubilitymethodandthefractions werequantified.Thesignificanteffectofsourceonproteinyieldandfractionsofcaseinswasobserved.Higheryieldo fα-casein(54.31%)wasobserved in cow milk than buffalo milk (48.95 %) and lower yield (20.36 %) wasfoundingoatmilk.Amongstthethreespecies,highestpercentofβcaseinwasnotedingoatmilk (54.05%)followedbybuffalo (36.03%)andcowmilk (34.14%), The highest molecular weight in respect of α-casein (23.82 kDa) was observed incowmilkfollowedbygoatmilk(23.61KDa)andbuffalomilk(22.74kDa).Whereas the molecular weight of β casein in respect of cow milk was 24.31, and it was 23.84for buffalo milk and 23.82 for goat milk.There was no wide variation in molecularweight of k-casein irrespective of source of milk. The molecular weight of k- caseinvariedbetween 19.15 to19.38kDa.

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