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Enzymatic Modification of Cow, Buffalo and Goat Milk Proteins and their Effect on Anti-Oxidant Activity and Digestibility

Rajanna M [a++*], H M Jayaprakasha [b], Venkatesh M [b#] and Akshaykumar [c†]

[a] Department of Dairy Chemistry, KVAFSU-Bidar, Dairy Science College, Hebbal, Bangalore, India.
[b] Department of Dairy Technology, KVAFSU-Bidar, Dairy Science College, Hebbal, Bangalore, India.
[c] Department of Dairy Microbiology, KVAFSU-Bidar, Dairy Science College, Mahagaon Cross, Kalaburagi, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Buffalo milk and goat milk were enzymatically hydrolyzed to have 5, 10 and 15 per cent DH to improve the techno-functional properties of milk proteins in order to utilize in production of channa based dairy products.
Study Design: A significant contribution to total milk production of India comes from buffalo milk and followed by cow and goat milk. Inspite of that, Buffalo milk and goat milk are not being utilized

[++] Associate Professor;
[#] Professor;
[†] Assistant Professor;
**Corresponding author: Email: marapparajanna@gmail.com*

for many products in view of their inherent problems associated in production of Chhana based dairy *products*. Buffalo milk and goat milk were enzymatically hydrolyzed to have 5, 10 and 15 per cent DH to improve the techno-functional properties of milk proteins in order to utilize 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: Highest protein content was noted in buffalo casein hydrolysate (83.0 %) than cow (81.0 %) and goat (81.16 %) and lowest nitrogen and ash content was observed in goat casein hydrolysate (13.65 %) and (15.20 %) respectively. (Akshya pl include few lines of enzymatic modification on anti-oxidant property and digestibility copy from discussions)

Conclusion: Though buffalo milk contributes nearly half of the total milk production of India and goat milk carries both nutritional and therapeutic benefits, the potential utilization of both these milks are very much limited due to the inherent problems associated with them. This investigation has shown that by enzymatically hydrolyzing buffalo milk and goat milk to 5 per cent DH results significant improvement in physico-chemical and functional attributes Rasagulla which is on par with cow's milk protein hydrolysates.

Recommendation: Scavenging activity or anti-oxidant activity and Digestibility of hydrolysates has been studied as influenced by the extent of enzymatic modification in this study.

Keywords: Buffalo milk; goat milk; casein hydrolysates; anti-oxidant activity, casein; digestibility.

1. INTRODUCTION

According to the Food and Agriculture Organization Corporate Statistical Database [1], "world milk production increased to about 887 million tonnes in 2021 growing at the rate of 1.1 per cent. (Roughly 81 per cent Cow milk, 15 per cent Buffalo milk, and 4 per cent for Goat, sheep and camel milk combined) India is the largest milk producer in the world contributing 24 Percent of global milk production in the year2021- 22 and is the single largest agricultural commodity contributing 5 per cent of the Indian national economy". 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 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 [1]. They are of great economic importance in India in 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 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 various dairy products" [2].

In order to maximize buffalo milk utilization for the production of dairy products the inherent problems associated with buffalo milk could be minimized by enzymatic modification of proteins through improving the functional properties. The enzymatic hydrolysis of milk proteins has been innovative approach to modify proteins and bring desirable changes in the physico-chemical and

functional properties of milk proteins which may ultimately impart desirable body and texture in the required dairy products.

Enzyme modified casein hydrolysates play crucial role in food industry. The extent of protein hydrolysis, which represents the extent of protein breakdown to peptides and amino acids, is expressed either as per cent amino nitrogen or as degree of hydrolysis (DH). Degree of hydrolysis is the ratio of the number of peptide bonds cleaved and the total number of peptide bonds in the intact protein. DH is one of the important controlling factors, which reflects on the product quality. Proteolytic enzymes have the ability to hydrolyze proteins to peptides and amino acids. The chain length of peptides formed is dependent upon the extent of hydrolysis, condition of hydrolysis, type, concentration and activity of enzyme, and type of protein to be hydrolyzed.

This investigation is taken up with the following objectives to suitably modify buffalo and goat milk proteins to maximize the utilization share of buffalo milk and goat milk after required protein hydrolysis.

1.1 Enzymatic Hydrolysis of Milk Casein of Cow, Buffalo and Goat

"Enzymatic hydrolysis of proteins does not only affect digestibility and allergenicity of proteins but also induces modification of functional properties such as solubility, viscosity, gelation, emulsifying and foaming properties. Hydrolysis of proteins causes changes such as an increase in the number of charged groups, a decrease in the average molecular weight, and exposure of reactive groups, factors that influence emulsion forming and emulsion-stabilizing abilities of protein hydrolysates. Furthermore, the small peptides present in protein hydrolysates are absorbed more rapidly from the intestine than free amino acids or intact proteins" [3].

"Enzymatic hydrolysis of buffalo milk and goat milk is carried out for modification of protein is referred to changes in conformational or structural features which subsequently alter the physico-chemical properties. The enzymatic modification generally involves controlled hydrolysis of protein to yield a mixture of peptides, which can improve desirable functionalities of proteins remarkably" [3]. "Casein and whey proteins were hydrolysed by chymotrypsin and trypsin at 52ºC the substrate concentration ratio was 10 per cent v/v and the enzyme to substrate ratio was 0.02. The pancreatin hydrolysis of casein can be used to enhance the whipping properties of casein with this enzyme range of tailor-made hydrolysates, with defined viscosity and foaming properties can be prepared" [4].

1.2 Bioactive Peptides from Cow, Buffalo and Goat Milk Proteins

"Cow milk proteins are good sources of bioactive peptides which have been reported to have various physiological effects. However, studies on Buffalo milk proteins as sources of bioactive peptides have received much less attention. Based on the similarity of the amino sequences of β-LG and α-LA of Buffalo and Cow milks, it is expected that they would yield similar bioactive peptides. However, no literature on bioactive peptides from Buffalo whey proteins has been cited" [5].

"On the other hand, reports on bioactive peptides from buffalo milk caseins have been cited but are very limited. Treatment of a beta-casomorphine-containing fragment from Buffalo β-casein (residues 49–68) with pancreatic proteases was not able to release β-casomorphine" [6]. "An angiotensin I-converting enzyme inhibitory peptide corresponding to β-CN 58–66 was produced by the action of Lactobacillus helveticus PR4 proteinase on buffalo milk casein. Cationic peptides were separated from the peptic digest of αs1- and αs2-caseins of Buffalo milk which had antibacterial activity. However, the antibacterial activity of αs1- derived cationic peptide was greater than that of αs2- against both gram-positive and gram-negative organisms" [7].

"Peptides, originating from the enzymatic hydrolysis of milk proteins, are able to perform specific biological activities (i.e., antihypertensive, antimicrobial, antioxidant, and immune-modulatory). Such protein fragments, called bioactive peptides, are formed from the inactive precursor protein during gastrointestinal digestion or can be produced following specific events during food processing" [8].

2. 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, 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. 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.

2.1 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 Hippet al., (1952). Whey proteins were separated by precipitation and filtration of whey. The protein was estimated by Kjeldahl Method. Casein fractions were separated on the basis of their differential solubility in urea solution as per the method outlined [9].

SDS-PAGE was carried out to assess the molecular weight ranges of casein fractions by following the method prescribed by Laemmli (1976).

2.2 Enzymatic Hydrolysis of Caseins from Cow, Buffalo and Goat

Whole casein of Cow, Buffalo and Goat milk were dispersed separately in distilled water at 40ºC to give a 5 per cent (w/v) protein concentration and the pH of the solutions was adjusted to optimum as that of the enzyme using 0.1N NaOH. The enzyme neutrase was added (1µl of enzyme/5g of protein) at pH 7.5 and temperature 45ºC was maintained. Enzymatic hydrolysis of caseins of different species were carried out at an enzyme substrate (E: S) ratio of 1:25 [10]. The pH of the protein solutions was maintained constant by addition of 0.1N NaOH. The samples treated with enzymes were maintained at an optimum pH and temperature for a period of 90 min, to obtain required percentage of Degree of Hydrolysis (5, 10 and 15 per cent DH). Then the protein solution was treated with enzymes brought to pH 4.6 to restrict enzymes activity and to precipitate the partially hydrolyzed casein. The hydrolyzed casein was further used for the study of antioxidant property, in-vitro digestibility and characterization of Bio-active peptides (BAPs).

Antioxidant property was determined by (DPPHH) 2, 2, Dipheny-1-picrylhydrazyl (DPPH) radical scavenging method. The scavenging activity of hydrolyzed casein dispersions was assessed slight modifications [11]. 1.5 ml of hydrolysates suspension (20 mg/ml) was mixed with 1.5 ml of 0.15 mmol/L DPPH solution. The mixture was vortexed thoroughly and incubated at 25ºC in the dark for 30 min followed by filtration using

Whatman filter No 4 to remove the suspended particles. The absorbance was measured at 517 nm. The control was prepared by using distilled water in place on sample. Where A *control* and A *sample* are the absorbance of control and test sample respectively.

In-vitro **digestibility (IVDP)** Hydrolysates was sequentially digested with pepsin and pancreatin according to the modified method of Liu et al., (2016). The digestion of hydrolsate suspension (2.0 %, W/V) was carried out using pepsin (E/S; 0.02(W/W), pH 1.2-2.0) at 37ºC for 120 min. After 120 min of digestion the reaction mixture was subsequently adjusted to pH 8.1 with 2.0 mo1/L NaHCO3 solution and then pancreatin was added at E/S ratio of 0.02 (W/W). The suspension was incubated for further digestion at 37ºC for 2 h. Digestion was terminated by adding 30 g/100 mL, trichloroacertic acid. The mixture was mixed properly and incubated for 15 min at 4ºC, followed by the centrifugation at 10,000 g for 30 min. The protein content of the resultant precipitate was determined and IVDP was evaluated as follows:

IVDP, % = (A-B) /A 100

Where; A is the protein content (mg) of the sample and B is the protein content (mg) of Precipitate.

2.3 Quantification of Bio-active Peptides (BAPs)

The quantification of BAPs from casein and whey fractions was carried out by adopting the method suggested by Bradford (1976). The BAPs were isolated from cow, buffalo and goat casein hydrolysate fractions by adapting the method [12], which is based on the principle that BAPs are soluble at pH 4.6 and aggregated with divalent cations such as calcium at neutral pH of 7.0. BAPs obtained by ethanol extraction were dried overnight in an oven maintained at a temperature of 70±1ºC and stored at 4ºC before use. Hydrolysates of Casein fractions were subjected for centrifugation (6000 rpm/10 min). The obtained supernatant was adjusted to pH 7.0, then1 per cent calcium chloride was added and kept for 1h. Ethanol (50 per cent v/v) was added to the supernatant to yield BAPs of respective fractions separately.

2.4 Determination of Chemical Composition of BAPs

The total ash and moisture content of BAPs obtained from hydrolyzed casein protein fractions were evaluated separately as per the procedure of AOAC (1984). Whereas, nitrogen and protein content by micro-kjeldhal method as per the procedure of AOAC (1980). Bioactive peptides were analyzed for nitrogen and protein content by micro-kjeldhal method. The total ash, and moisture content were determined gravimetrically as per the reference procedure

“The digestibility of hydrolysates increased (P< 0.05) from 71.7 per cent to 95.6 per cent with increasing DH. The soluble nitrogen of hydrolysate was more easily released during digestion indicating excellent In-Vitro digestibility of Protein (IVDP) of hydrolysate. IVDP increased in hydrolysate due to the increasing protein flexibility (α-helix and random coil) and solubility, whereas decreased in β-sheets (structural stability) and hydrophobicity” [13].

“Apart from these factors, peptide sizes and composition, protein unfolding, break down of sulfide and disulfide bonds might also be major factors to make more susceptible to digestibility. The strong negative relationship between the β-sheets content and IVDP also stated that enzymatic hydrolysates improved the IVDP” [14]. “High hydrophobic character of protein is the function of β-sheets, the main component of protein which increases the protein structure stability by promoting interaction and aggregation among protein molecules and decreases protein digestibility by reducing the access of proteases to susceptible sites. It is suggested that lower β-sheet structures and hydrophobicity were the major responsible factors for increasing the digestibility of hydrolystes” [15]

3. RESULTS AND DISCUSSION

Extent of hydrolysis of Cow, buffalo and goat milk protein were studied after being hydrolyzed by Neutrase enzyme by employing optimum enzyme: substrate (E: S) ratio of 1:25 at a pH of 7.5 and a temperature of 45ºC. The extent of hydrolysis was measured at an interval of every 5 minutes and the hydrolysis is attained is expressed as degree of hydrolysis (DH). The results are presented in Table 1.

Cow milk casein showed 2.7 and 3.82 Per cent of hydrolysis at 5- and 10-minute duration of incubation, respectively, which was more than buffalo milk casein which was 2.6 per cent at 5 min and 3.14 per cent at 10 minute followed by goat milk casein 2.2 percentage 5 min and 2.98 percent at 10 minute. More than 35 min hydrolysis of proteins resulted in higher degree of hydrolysis as against buffalo milk and goat milk casein. From 40 min onwards, the Degree of hydrolysis was more in buffalo milk casein (7.65 %) and goat milk casein (7.71%) as compared to cow milk casein (7.46%) which was significantly lesser. This trend was observed till the end of hydrolysis of 90 min where, cow milk protein had 14.72 percent hydrolysis against buffalo milk of 15.01 percent and15.32 percent for goat milk. To obtain approximately 5, 10 and15 percent DH, the duration required 25, 60 and 90 minutes, respectively, irrespective of source of casein used.

It can be clearly seen from the table 1 that the extent of hydrolysis is directly proportional to the time of hydrolysis. The casein from milk of cow was found to be more susceptible for hydrolysis in the first 10 minutes as compared to buffalo milk or goat milk.

Cow milk caseins needed a time of 25 minutes to get 5.32 per cent DH while in the same time duration buffalo milk caseins were hydrolysed to

5.01 per cent. Goat milk caseins were hydrolyzed to 5.23 per cent at 25 minutes. To obtain a 10 per cent DH 60 minutes of duration was required for all the three types with goat milk showing higher hydrolysis of 10.45 per cent and cow milk showing 10.2 per cent and buffalo milk casein showing 10.32 per cent. The 5 per cent DH attained at the end of 25 min, for all the three caseins of different species milk by using neutrase enzyme was 5.32, 5.01 and 5.23 per cent for cow, buffalo and goat respectively. This showed that for the given incubation time the per cent DH with neutrase was found to be more in cow milk casein as compared to buffalo and goat milk casein thus indicating that cow milk casein was more susceptible to enzymatic hydrolysis by neutrase. These results are in conformity with the findings of earlier workers [16]. who also reported similar findings on enzymatic hydrolysis by different enzymes.

Table 1. Effect of duration of enzymatic hydrolysis on extent of hydrolysis (DH)

Time of Hydrolysis (Min)	Cow milk casein	Buffalo milk casein	Goat milk Casein
	Neutrase % Degree hydrolysis (DH)		
5	2.70	2.60	2.20
10	3.82	3.14	2.98
15	4.12	4.65	3.82
20	4.92	4.49	4.16
25	**5.32**	**5.01**	**5.23**
30	6.15	5.89	5.92
35	6.89	6.75	6.58
40	7.46	7.65	7.71
45	7.89	8.02	8.31
50	8.41	8.54	9.01
55	9.03	9.31	9.84
60	**10.2**	**10.32**	**10.45**
65	10.50	11.07	11.40
70	11.10	11.90	12.03
75	11.95	12.75	12.95
80	12.76	13.50	13.95
85	13.96	14.20	14.65
90	**14.72**	**15.01**	**15.32**

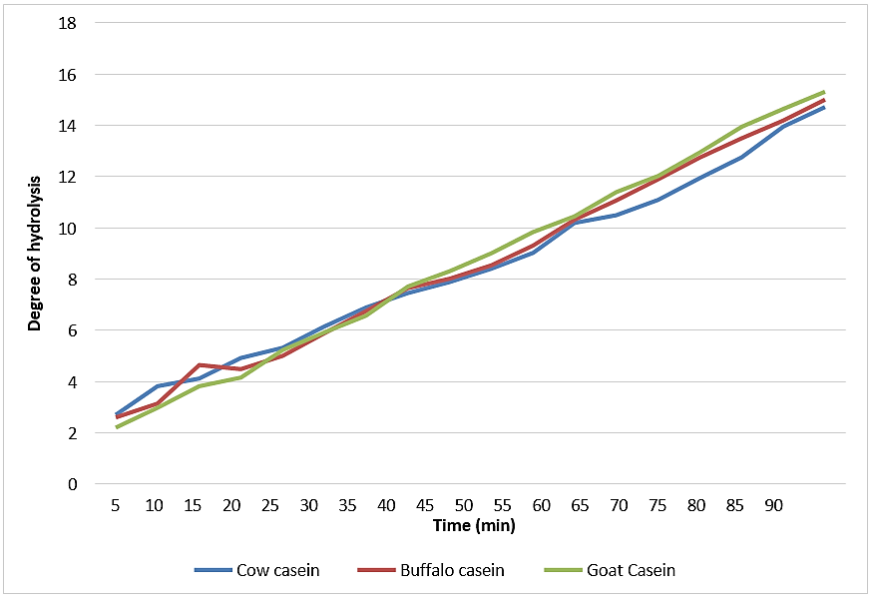

Fig. 1. Effect of duration of enzymatic hydrolysis on extent of hydrolysis (DH)

Table 2. Effect of extent of hydrolysis on antioxidant activity of casein hydrolysate of cow, buffalo and goat

Degree of hydrolysis (DH %)	Cow milk casein hydrolysate	Buffalo milk casein hydrolysate	Goat milk casein hydrolysate
Scavenging activity (%)			
Control	5.53[a]	5.65[a]	5.85[a]
5	13.25[b]	13.53[b]	14.85[b]
10	15.81[c]	14.5[c]	16.92[c]
15	36.85[d]	37.6[d]	37.5[d]
CD (p<0.05)	**1.02**	**0.85**	**0.75**

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

3.1 Anti-Oxidant Property

Effect of extent of hydrolysis on antioxidant activity of casein hydrolysate of cow, buffalo and goat is shown in Table 2. The un-hydrolyzed (control) samples of cow, buffalo and goat milk showed antioxidant activity were 5.53, 5.65 and 5.85 per cent, respectively. At 5 per cent DH the antioxidant activity values of cow milk casein hydrolysate was observed to be 13.25 per cent, while at 10 per cent DH it was 15.81 per cent and at 15.per cent DH it was 36.85 per cent, Similar trend was observed for buffalo milk casein hydrolysates, The antioxidant activity was found to be 13.53, 14.5 and 37.6 per cent at 5, 10 and 15 per cent DH.

Results for goat milk casein hydrolysate also showed a similar trend with control showing antioxidant activity of 5.85 per cent, whereas it was 14.85, 16.92 and 37.50 per cent at 5, 10 and 15 per cent DH, for 5 per cent degree hydrolyzed showed 14.85. At 10 per cent degree hydrolyzed showed 16.92 and 15 per cent showed 37.5 as observed before, the degree of hydrolysis affected absorbance values among all the three treatments. Degree of hydrolysis was found to have significant effect of antioxidant activity irrespective of source of casein hydrolysates.

The improved antioxidant activity of hydrolyzed milk has been explained by the greater exposure of the anti-oxidative amino acid residues due to peptide bonds cleavage. Additionally, it has been reported that during hydrolysis, the protein chains unfolded and resulted in the development of hydrophobicity. The balance between hydrophilic and hydrophobic forces of peptides has a crucial influence on the solubility of protein hydrolysate as well as antioxidant activity. The antioxidant activity of protein hydrolysate is also influenced by characteristics and sequences of amino acid in the derived peptides, which arise from the specificity of protein enzyme used for hydrolysis.

The enzymatic hydrolysis of whole casein from Buffalo and Cow milk with proteolytic enzymes alcalase, and trypsin to assess the antioxidant activity of casein hydrolysate was studied by Shukla et al (2019). The degree of hydrolysis of alcalase was higher than trypsin hydrolysis for both Buffalo and bovine casein (92.26 and 86.43 per cent) respectively. Fractions 2 and 3 from bovine casein hydrolysate by trypsin were confirmed to have the highest antioxidant activity (92.54 and 92.59 per cent), respectively.

The anti-oxidative properties of peptides. They observed that peptides can be released from caseins by hydrolysis with digestive enzymes and by proteolytic LAB in fermented milks have been shown to possess free radical scavenging activities and to inhibit enzymatic and non-enzymatic lipid peroxidation. Anti-oxidative peptides may also find their applications as ingredients, e.g. in the prevention of oxidation in fat-containing foodstuffs, cosmetics and pharmaceuticals [17].

3.2 Effect of Enzymatic Hydrolysis on Digestibility of Casein Hydrolysates of Cow, Buffalo and Goat

Studies on in-vitro digestibility of casein hydrolysates of Cow, Buffalo and Goat milk as affected by extent of hydrolysis is presented Table 3. The digestibility of hydrolyzed protein is measured in per cent. The digestibility for unhydrolyzed (control) Cow milk casein was 75.35 per cent, whereas at 5, 10 and 15 per cent DH it was 79.51, 83.14 and 86.35 per cent respectively. There was significant difference in digestibility of casein hydrolysates amongst 5, 10 and 15 per cent DH Similarly digestibility of

buffalo milk casein hydrolysate was 74.85, 78.32, 82.54 and 85.86 per cent, at 5, 10 and 15 per cent DH, respectively, while goat milk unhydrolyzed (control) sample showed 76.50 percent, while at 5 per cent DH, it was 80.45, at 10 per cent DH, it was 85.12 per cent and at 15 % DH the digestibility was observed to be 89.51 per cent. The DH was found to have significant effect on digestibility irrespective of source of casein hydrolysates and extent of hydrolysis.

The digestibility of all the three types of casein hydrolysates increased as the DH increased. This may be due to release of soluble nitrogen as the result of hydrolysis resulting higher digestibility of hydrolysates. These findings are in agreement with the results of earlier workers (Adil *et al.,*2015 and Shu *et al.,*.2018). Apart from these factors digestibility increased in all the casein hydrolysates, this could be ascribed to increase in the protein flexibility (α-helix and random coil) and solubility, whereas decreased in β-sheets (structural stability) and hydrophobicity along with decrease in peptide size, protein folding, and breakdown of sulfide and disulfide bonds which are the major factors responsible for increase in digestibility. Similar findings are reported and also stated that enzymatic hydrolysis improves the digestibility [18].

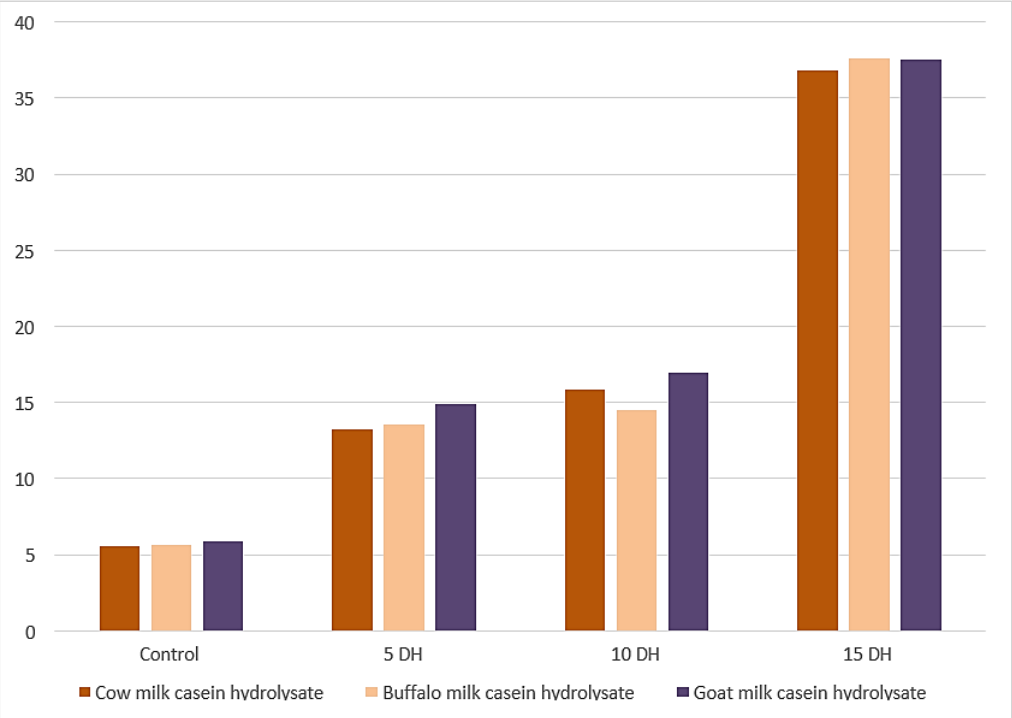

Fig. 2. Effect of extent of hydrolysis on antioxidant activity of casein hydrolysate of cow, buffalo and goat

Table 3. Effect of enzymatic hydrolysis on digestibility of casein hydrolysates of cow, buffalo and goat milk

Degree of hydrolysis (DH %)	Cow milk casein hydrolysate (%)	Buffalo milk casein hydrolysate (%)	Goat milk casein hydrolysate (%)
Control (Unhydrolysed)	75.35[a]	74.85[a]	76.50[a]
5	79.51[b]	78.32[b]	80.45[b]
10	83.14[c]	82.54[c]	85.12[c]
15	86.35[d]	85.86[d]	89.51[d]
CD(p<0.05)	**0.58**	**0.46**	**0.51**

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

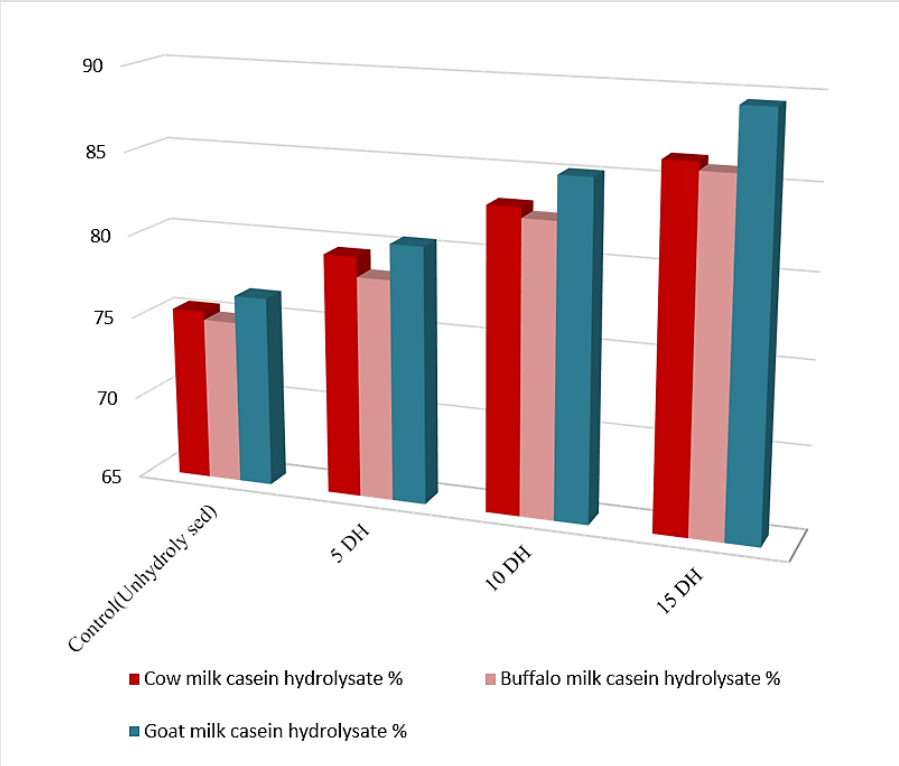

Fig. 3. Effect of enzymatic hydrolysis on digestibility of casein hydrolysates of cow, buffalo and goat

4. CONCLUSION

Scavenging activity or antioxidant activity of casein hydrolysates increased as the degree of hydrolysis increased in all the three samples. Highest scavenging activity was observed in Buffalo milk casein hydrolysate (37.6 %) at 15 % DH. *In-vitro* digestibility of casein hydrolysates increased as the degree of hydrolysis increased in all the three samples. Highest *In-vitro* digestibility was observed in Goat milk casein hydrolysate (89.51 %) at 15 % DH followed by Cow milk casein hydrolysate (86.35 %) and buffalo milk casein hydrolysate (85.86 %). Higher levels of BAPs were noted in casein hydrolysates as the degree of hydrolysis increased in all the three samples. Highest number of BAPs was observed in Goat milk casein hydrolysate (92.53 µ/ml) followed by buffalo milk casein hydrolysate (89.24 µ/ml) and cow milk casein hydrolysate (83.45 µ/ml) at 15 % DH.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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