RESEARCH ARTICLE

***In vitro* assessment of antioxidative potential of goat milk, casein and its hydrolysates: Comparison of goat milk with bovine and buffalo milk**

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Abstract: The present study was executed with an aim to explore the antioxidative potential of goat, cow, and buffalo milk. Buffalo milk has showed highest antioxidative potential than goat and cow milk as measured by ABTS, ORAC, and DPPH assays, whereas goat milk has showed better antioxidative potential than cow milk when measured by ORAC and DPPH. Further, the effect of temperature on the antioxidative potential of goat milk was assessed. An increase in temperature has a negatively affect the antioxidative potential of goat milk. The antioxidative potential of goat milk was in the following order: raw milk > pasteurized milk > boiled milk. Casein derived from goat milk by isoelectric precipitation was hydrolyzed by gastrointestinal enzymes pepsin (P), trypsin (T), chymotrypsin (C), and their combinations PT, PC, TC, and PTC. Among all the casein hydrolysates, the maximum antioxidative potential was found in PT hydrolysate, further fractionated by 10, 3 and 1 kDa ultrafiltration membranes. 3-10 kDa fraction exhibited maximum antioxidative potential in comparison to other fractions of PT hydrolysate. Our results suggested that antioxidative potential of goat milk and its hydrolysates could be an important mean to obtain natural antioxidative peptides.

Keywords: Antioxidative potential, Buffalo milk, Casein hydrolysates, Cow milk, Goat Milk

Introduction

Oxidative stress is a physiological disproportion of evolvement of reactive oxygen species (ROS) and their neutralization by the antioxidative system. The uncontrolled production of free radicals in organisms can turn into oxidative stress. This imbalance has been closely linked with alzheimer's, atherosclerosis, cardiovascular disease, diabetes, huntington, rheumatoid arthritis, other age-related diseases and break down of vital biochemical compounds such as lipids, cellular proteins, DNA in the body (Fitzpatrick et al. 2009). Proper intake of antioxidants in the form of food enriched in antioxidants may protect the body from abovementioned diseases. Thus, antioxidant-enriched food products are becoming increasingly popular. Previous studies have demonstrated that food components, including ascorbic acid (vitamin C), β -carotenes, tocopherols, polyphenols, and bioactive peptides, have protective effects against oxidative stress and, therefore, act as antioxidants (Simos et al. 2011). Goat milk production has shown an upsurge worldwide and highly preferable owing to its anti-allergenicity especially in infants while comparing with cow milk (Park, 2009; Simos et al. 2011) also anti-platelet, antibacterial, immunomodulatory and antioxidative capacity (Simos et al. 2011). Additionally, goat milk has manifested diverse biological properties in comparison to different species milk including human, such as high buffering capacity, dissimilar alkalinity (Park, 2009), higher digestibility because of small size fat globules (Meena et al. 2014) and higher amount of short and medium chain fatty acids which are linked with hypocholesterolemic function in humans, by preventing cholesterol deposition and enhancing its movement and excretion (Kalyan et al. 2018). Therefore, due to goat milk's nutritional and bio-functional properties, it has received extensive attention. The antioxidative properties of goat milk are reported higher than bovine and donkey milk (Simos et al. 2011). These beneficial effects occur due to the release of bioactive compounds as goat milk is readily digestible and absorbed in the gut and small intestine, respectively (Park, 2009).

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Bioactive peptides from bovine milk are extensively known, but in recent times, studies have also been conducted on non-bovine species such as camel, sheep, donkey and goat (Mudgil et al. 2018; Li et al. 2013). Goat milk proteins have shown presence of peptides with antimicrobial, antioxidative, antidiabetic, ACE inhibitory and immune-modulating properties (Mudgil et al. 2018; Li et al. 2013; Park, 2009). A few studies have reported antioxidative peptides from goat milk protein. A total five antioxidative peptides were identified in goat milk casein hydrolysate after digestion with two enzymes namely, Alcalase and Pronase however, four reported peptides didn't correspond to sequences in goat casein databases (Li et al. 2013). Kusumaningtyas et al. (2016) performed enzymatic digestion with pepsin, trypsin, and chymotrypsin and by *Bacillus* sp.E.13 protease indicating antimicrobial and antioxidative potential of *Bacillus* sp.E.13 protease against other enzymes. The bioactive peptides of non-bovine species milk and their milk products were comprehensively reviewed recently (Guha et al. 2021). Previously, studies pertaining to antioxidative potential of goat milk has been performed. However, in present study all the commonly accepted antioxidative assays (FRAP, ABTS, DPPH and ORAC) have been performed to compare the antioxidative potential of goat milk with cow and buffalo milks. Alongwith, the effect of different degree of thermal treatment on antioxidative capacity of goat milk has also been performed. Further, antioxidative potential of goat milk casein and its hydrolysates and most potent antioxidative fraction of goats' milk casein obtained by enzymatic digestion with pepsin, trypsin and chymotrypsin individually and in combination.

Materials and Methods

Procurement and processing of milk

Raw milk of goat (crossbred of *Alpine* × *Beetal*), cow (*Sahiwal* breed) and buffalo (*Murrah* breed) were procured from the Livestock Research Centre, ICAR-National Dairy Research Institute, Karnal Haryana (India), and fat content was adjusted to 3% for all samples owing to variability in fat-soluble antioxidants such as tocopherols, retinol and carotenoids with varying fat percentage (Chauveau-Duriot et al. 2010). The SNF content of the fat adjusted milk were measured as 8.5, 8.5 and 9.0 % for cow, buffalo and goat milk respectively. The antioxidant potential of raw, pasteurized (63°C/30 min) and boiled milk (100°C for no-hold) were further assessed. All the chemicals were procured from Sigma-Aldrich Chem. Co., St Louis, MO, USA except the pepsin which was procured from Sisco Research Laboratories, Mumbai, India.

Assays to measure Antioxidative capacity of milk from different species

Antioxidant components from milk samples were isolated as per the method of Alyaoubi et al. (2014). The following four different

assays were employed for measuring the Antioxidative potential of milk from different species.

ABTS assay

ABTS assay was executed as per the methodology indicated by Re et al. (1999). Trolox standard was used in the range of 50 to 500 µM at an interval of 50 µM. Trolox equivalent antioxidant capacity (TEAC) of the samples was evaluated from the standard curve ($y = 0.187x - 3.818$ ($R^2 = 0.994$)) of Trolox (50 to 500 µM) based on the inhibition percentage.

Oxygen radical absorbance capacity-Fluorescein (ORAC-FL) assay

ORAC assay was performed via fluorescein as per the procedure of Davalos et al. (2004). The trolox standard was used in the range of 10 to 100 µM at an interval of 10 µM. The standard curve equation was $y = 0.328x + 1.635$ ($R^2 = 0.991$).

Ferric Reducing Antioxidant Power (FRAP) activity

The FRAP activity was performed as per the methodology of Benzie and Strain (1996). The trolox standard was used in the range of 0.08 to 0.72 µM at an interval of 0.08 µM. The standard curve for Trolox was $y = 1.017x + 0.022$ ($R^2 = 0.989$).

DPPH activity

The antioxidant capacity established on DPPH radical for milk samples was analyzed by methods defined by Zhidong et al. (2013). Trolox standard was used in the range of 100-800 µM. The standard curve equation was $y = 0.107x + 3.529$ ($R^2 = 0.993$).

Screening of casein hydrolysates for Antioxidative activity

Isolation of goat milk casein

Isoelectric precipitation method was utilized for separating the casein of goat milk (Davies and Law, 1977). The concentration of goat milk casein was evaluated by Lowery method using the standard curve i.e., $y = 0.001x + 0.054$ ($R^2 = 0.992$). The purity of the casein derived from goat milk was tested by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) in a vertical slab gel electrophoresis system (Laemmli et al. 1970).

In vitro hydrolysis of casein protein

Isoelectrically precipitated casein was hydrolyzed with pepsin, trypsin, chymotrypsin, and their different combinations (Jinsmaa and Yoshikawa, 1999). Before carrying out the hydrolysis of protein by the enzymes and their combinations, the time duration of complete hydrolysis by each enzyme was standardized. Pepsin, trypsin, and chymotrypsin were mixed with pre-incubated casein solution (1 mgmL⁻¹) in the ratio of 1:100 (E:S), followed by incubation at 37°C for 8 h using a water bath with continuous

mixing. Thereafter, samples were collected at varying time breaks (0, 1, 2, 4, 6 and 8h). The degree of hydrolysis was calculated by evaluating the free amino groups released during hydrolysis by the OPA method (Church et al. 1983). Thereafter, casein was hydrolyzed by the enzymes and their combinations for 3h by following the same procedure as described above. For pepsin combination with other enzyme, firstly the casein was hydrolyzed with pepsin at 2.0 pH for 3h followed by increment of pH to 8.0 using 1.0N NaOH and then allowing the digestion with other enzymes subsequently for 3h each. After incubation, the reaction was terminated by enhancing the pH to 8.0 by 1.0N NaOH for pepsin, while trypsin and chymotrypsin activity was inhibited by raising the temperature to 98°C for 10 min. Peptide concentration in the hydrolysates was estimated by Bicinchoninic acid (BCA) assay (Smith et al. 1985). Briefly, 10 µL of sample was mixed with 190 µL BCA reagent (1:19) in 96-well ELISA plate and incubated at 37°C for 30 min. ELISA plate was cooled to room temperature and the absorbance was recorded at 562 nm. The peptide concentration in the hydrolysates was evaluated using the standard curve of BSA ($y = 0.048x + 0.023$ with an R^2 of 0.987). Further, Tricine Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis (Tricine SDS-PAGE) was performed to detect low molecular weight peptides in hydrolysates (Pardo and Natalucci, 2002). Furthermore, isolated casein was used for further studies.

***In vitro* evaluation of the casein and its hydrolysates**

Antioxidative activity of the casein and its hydrolysates was evaluated by ABTS, ORAC and DPPH assay as described above.

Ultrafiltration of Pepsin-Trypsin hydrolysates

Pepsin-Trypsin hydrolysates of casein with maximum Antioxidative activity were purified using ultrafiltration. Pepsin-Trypsin hydrolysate of casein were ultrafiltered by 10, 3 and 1 kDa molecular weight cut-off (MWCO) membrane which fractionated the casein hydrolysate based on their size and also eliminated the enzyme and non-hydrolyzed proteins from the reaction mixture (Segura-Campos et al. 2011). The ultra-filtrated peptide fractions so obtained were labelled as >10 kDa (10 kDa retentate), 3-10 kDa (3 kDa retentate), 1-3 kDa (1 kDa retentate) and <1 kDa (1 kDa permeate) as described in Supplementary Fig. 1. The antioxidant activity of each fraction was measured as discussed previously.

Table 1 TEAC ($\mu\text{mol mL}^{-1}$) of goat raw, pasteurized and boiled milk

Assay	Type of milk				
	Cow raw	Buffalo raw	Goat raw (GR)	Goat pasteurized (GP)	Goat boiled (GB)
ABTS	5430 $\pm$ 39.48 ^a	5571 $\pm$ 32.32 ^b	5416 $\pm$ 18.11 ^a	4964 $\pm$ 36.44 ^b	4749 $\pm$ 21.22 ^c
ORAC	3968 $\pm$ 3.23 ^b	4242 $\pm$ 33.95 ^c	4135 $\pm$ 2.64 ^a	4076 $\pm$ 35.02 ^a	4052 $\pm$ 40.53 ^a
FRAP	0.28 $\pm$ 0.00 ^{ab}	0.26 $\pm$ 0.01 ^b	0.31 $\pm$ 0.01 ^a	0.30 $\pm$ 0.00 ^{ab}	0.29 $\pm$ 0.00 ^b
DPPH	131.9 $\pm$ 0.91 ^b	207.3 $\pm$ 10.80 ^c	180.3 $\pm$ 2.77 ^a	166.2 $\pm$ 6.39 ^b	151.6 $\pm$ 3.19 ^c

Values are expressed as mean $\pm$ SEM (n=3). ^{abc} Dissimilar superscript within a row within an attribute indicate significant differences ($P < 0.05$).

Statistical analysis

The differences among the treatments were evaluated using one way ANOVA and considered statistically significant at 5% level of significance.

Results and Discussion

Antioxidative potential of milk

The antioxidative properties of milk were measured in terms of TEAC using ABTS, ORAC, FRAP and DPPH assay. Results revealed improved antioxidative potential of buffalo milk as compared to cow and goat milk using all the four assays (Table 1). However, goat milk showed better antioxidative potential than cow milk when evaluated using ORAC and DPPH assay. Besides the contribution of water-soluble compounds namely phenols, ascorbate, and low-molecular weight thiols, it is well established that TEAC also depends on milk fat components, which encompasses various fat-soluble antioxidants such as tocopherols, retinol, and carotenoids (Chauveau-Duriot et al. 2010), casein and whey proteins (Pihlanto, 2006), although in present study fat was adjusted to constant value of 3% for all milk samples. Therefore, the detected variations in TEAC might be attributed to variation in species-specific milk composition particularly, protein and other potential antioxidative compounds (Niero et al. 2018). The differences among diverse assays could also be due to their quantifying parameters. Huang et al. (2005) reported that antioxidative assays are majorly of two types i.e., dependent on hydrogen atom transfer reactions and electron relocation reactions. Thus, it is suggested that ABTS, FRAP and DPPH should be utilized to measure antioxidant reducing capacity and ORAC assay to evaluate peroxyl radical scavenging potential of antioxidant. Our results are in confirmation with Alyaqoubi et al. (2014) who had found significantly higher ($p < 0.05$) antioxidant activity of goat milk than cow milk using DPPH and FRAP assay. Similarly, Simos et al. (2011) claimed better antioxidative capacity of goat (Prisca breed) milk as compared to cow milk reflecting higher bioavailability of antioxidant compounds in goat milk. In present study, FRAP assay showed converse result with goat milk possessing significantly ($P < 0.05$) higher antioxidative activity in comparison with buffalo milk but non-significantly ($P > 0.05$) different from cow milk (Table 1).

Fig. 1 (A) SDS-PAGE of Isoelectric Casein isolates (Lane 1 – Low molecular weight markers: Myosin (209 kDa), β -galactosidase (124 kDa), Bovine serum albumin (80 kDa), Ovalbumin (49.1 kDa), Carbonic anhydrase (34.8 kDa), Soybean trypsin inhibitor (28.9 kDa) and Lysozyme (20.6 kDa) from top to bottom; Lane 2-5 – Casein) (B) Tricine SDS-PAGE of casein and its hydrolysates. (Lane 1- Low molecular weight markers; Lane 2- Casein; Lane 3- Casein hydrolyzed by pepsin; Lane 4- Casein hydrolyzed by trypsin; Lane 5- Casein hydrolyzed by chymotrypsin)

Further, antioxidative potential of pasteurized and boiled goat milk declined in comparison to raw goat milk when estimated by ABTS, ORAC, FRAP and DPPH assay. The antioxidative potential by ABTS of pasteurized goat milk and boiled goat milk was 4964 ± 36.44 and $4749 \pm 21.22 \mu\text{mol mL}^{-1}$ respectively thus signifying inverse relationship between antioxidative potential and degree of heat treatment. Raw goat milk had the highest antioxidative activity followed by boiled and pasteurized goat milk as measured by ABTS and DPPH assay (Table 1). Heat treatment has detrimental effects on the antioxidative properties of goat milk and it is reported that higher temperature short time treatments can deplete the overall antioxidative properties of milk. Contrarily, high heat treatment facilitates the Maillard reaction between nucleophilic group of amino acids and carbonyl group of reducing sugars and these Maillard reaction products i.e., brown melanoidins, enhance recovery and possible elevation of antioxidative properties (Zulueta et al. 2009). Alyaqoubi et al. (2014) assessed the impact of heat treatment on antioxidative potential of milk and reported lower antioxidative potential of pasteurized milk than unpasteurized milk and reduced phenol and flavonoids content in pasteurized milk.

Preparation and screening of casein hydrolysates

Goat milk casein was isolated by isoelectric precipitation and concentration in goat milk sodium caseinate was 270 mg mL^{-1} as determined by Lowry method. Then, the purity of the casein samples was evaluated by 15% Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (Fig. 1A). Different digestive enzymes such as pepsin (P), trypsin (T), chymotrypsin (C) and their combinations were employed to hydrolyze isoelectrically precipitated casein for peptides formation. The hydrolysis rate of casein protein by trypsin and chymotrypsin increased with time and almost got saturated after 3 h of incubation (Fig. 2A),

but hydrolysis by pepsin persisted even after 3 h, although the hydrolysis rate was slightly lower. Consequently, duration of hydrolysis was standardized to 3h for the experiments owing to maximal degree of hydrolysis during this duration (Fig. 2A). Shanmugam et al. (2015) also reported similar findings in their study. Similarly, the antioxidant peptides were liberated maximally at 3h while digesting casein with pepsin and trypsin (Ming and Zhi-he, 2012). The degree of hydrolysis of goat milk casein hydrolyzed by P, T, C, PT, PC, TC and PTC were $2.84 \pm 0.210\%$, $5.29 \pm 0.194\%$, $5.85 \pm 0.048\%$, $6.27 \pm 0.127\%$, $5.07 \pm 0.073\%$, $7.19 \pm 0.250\%$ and $8.53 \pm 0.127\%$, respectively (Fig. 2B). The extent of hydrolysis of intact casein using trypsin, chymotrypsin and all the combinations was significantly higher as compared to pepsin. The degree of hydrolysis was in the range of 7.10 to 17.60 % for buffalo milk casein hydrolyzed by P, T, C singly and their combinations (Shanmugam et al. 2015). Variations were attributed to enzyme specificity for cleavage site, enzyme rate of reaction, substrate affinity and the quantum of sites available in respective proteins (Mudgil et al. 2018). Zu et al. (2021) reported that efficiency of enzyme also depends upon denaturing conditions mainly due to heat treatment or pH thereby better accessibility to internal cleavage sites of protein. Preferential cleavage of a peptide bond between hydrophobic amino acids residues of leucine, phenylalanine, and tyrosine is catalyzed by pepsin (Dunn, 2002). Similarly, chymotrypsin hydrolyses the protein sites having hydrophobic amino acids (Cassidy et al. 1997). The specificity of trypsin is solely limited to hydrophilic amino acid residues of arginine and lysine (Olsen et al. 2004). The yield (%) of the peptides after hydrolysis by P, T, C, PT, PC, TC and PTC were 19.51 ± 0.565 , 22.24 ± 1.012 , 20.15 ± 0.548 , 18.90 ± 0.308 , 17.58 ± 0.356 , 18.19 ± 0.638 and $17.83 \pm 0.116\%$ respectively (Fig. 2C) estimated by BCA method. Casein hydrolysis was visually evaluated by tricine SDS-PAGE to determine the low molecular weight peptides in hydrolysate and to ascertain hydrolysis of casein by different

Fig. 2 (A) Rate of hydrolysis of casein by pepsin (P), trypsin (T) and chymotrypsin (C). (B) Degree of hydrolysis of casein hydrolyzed by pepsin (P), trypsin (T), chymotrypsin (C) and their combinations (PT, PC, TC and PTC) as estimated by OPA method. (C) Yield (%) of casein hydrolyzed by P, T, C, PT, PC, TC, and PTC. (D) Antioxidant activity of different fractions (>10 kDa, 3-10 kDa, 1-3 kDa & < 1 kDa) of pepsin–trypsin (PT) hydrolysates. The bars bearing different alphabets differ significantly ($P < 0.05$).

Table 2 TEAC values (μmolmg^{-1}) of casein and its hydrolysates by different assays

Groups	Casein	P	T	C	PT	PC	TC	PTC
Assay								
ABTS	0.52 $\pm$ 0.01 ^a	0.73 $\pm$ 0.01 ^b	0.74 $\pm$ 0.01 ^b	0.77 $\pm$ 0.01 ^{bc}	0.78 $\pm$ 0.02 ^c	0.80 $\pm$ 0.01 ^c	0.74 $\pm$ 0.02 ^b	0.79 $\pm$ 0.01 ^{bc}
ORAC	0.34 $\pm$ 0.0 ^a	0.40 $\pm$ 0.01 ^b	0.39 $\pm$ 0.00 ^{bd}	0.38 $\pm$ 0.0 ^{cd}	0.37 $\pm$ 0.00 ^c	0.36 $\pm$ 0.00 ^c	0.35 $\pm$ 0.00 ^{ac}	0.36 $\pm$ 0.0 ^{ac}
DPPH ($\times 10^{-3}$)	1.59 $\pm$ 0.18 ^a	2.74 $\pm$ 0.07 ^b	4.15 $\pm$ 0.05 ^c	2.76 $\pm$ 0.12 ^b	4.37 $\pm$ 0.14 ^c	2.81 $\pm$ 0.15 ^b	4.38 $\pm$ 0.07 ^c	3.96 $\pm$ 0.01 ^c

Values are expressed as mean $\pm$ SEM ($n=3$).^{abc} Different superscript within a row within an attribute indicate significant differences ($P < 0.05$).

enzymes as previously determined by OPA method. It was observed that casein was completely hydrolyzed during this digestion process by pepsin, trypsin, and chymotrypsin as depicted in Fig. 1B.

Antioxidative property of casein hydrolysates

The antioxidative potential of casein hydrolysates was analyzed on the basis of free radical scavenging activity using ABTS, DPPH, and ORAC (Table 2). In ABTS, ORAC, and DPPH assay, TEAC of hydrolysates was between 0.736 to 0.804 μmolmg^{-1} , 0.352 to 0.402 μmolmg^{-1} and 2.745 to 4.381 $\times 10^{-3}$ $\mu\text{mol mg}^{-1}$ of the casein hydrolysates, respectively, whereas the values of unhydrolyzed casein were 0.528, 0.347, and 1.598 $\times 10^{-3}$ $\mu\text{mol mg}^{-1}$ as measured by ABTS, ORAC, and DPPH assay, respectively. Similarly, the antioxidative activity of casein hydrolysates obtained from P, T, C, and their mixture got enhanced significantly in comparison to unhydrolyzed casein when evaluated using ABTS and DPPH assay (Table 2). The TEAC values of buffalo

milk based hydrolyzed casein obtained by pepsin, trypsin and chymotrypsin enzymes were higher than unhydrolyzed buffalo milk casein (Shanmugam et al. 2015). The ABTS assay yielded higher antioxidative activity of the hydrolysates obtained from PT and PC than P, T, C, TC, and PTC hydrolysates. However, the antioxidative assay of PC hydrolysate was significantly ($P < 0.05$) lower than PT hydrolysate when analyzed using DPPH assay, therefore PT hydrolysate was opted for further purification.

Purification of pepsin–trypsin (PT) hydrolysate of casein by ultrafiltration and assessment of antioxidative potential of different fractions

PT hydrolysate of casein possessed maximum antioxidative activity which was further purified using ultrafiltration. The obtained fractions were further analyzed for their antioxidative capacity. The PT hydrolysate was also ultrafiltered using 10 kDa, 3 kDa, and 1 kDa MWCO to separate enzyme and non-hydrolyzed protein from the reaction mixture and fractionation of casein

hydrolysate based on their size. Antioxidative activity of ultrafiltration fractions of PT hydrolysate was assessed by ABTS assay. Antioxidative activity of 3-10 kDa fraction was significantly ($P < 0.05$) higher than all the other fractions and PT hydrolysate (Fig. 2D). Our findings are in agreement with Shanmugam et al. (2015), who also chose PT hydrolysate of buffalo milk casein for ultrafiltration and reported highest antioxidative activity of 1 kDa permeate as evaluated using ABTS assay. Sun et al. (2011) reported that out of four fractions the PHH-IV fraction that is 3 kDa permeate has higher inhibitory effect of lipid peroxidation and scavenging effect on superoxide radical. Similarly, Kusumaningtyas et al. (2016), observed the variations in the antioxidative capacity of goat milk protein hydrolysates based on the technique used and reported the maximum activity of 10-30 kDa than 3 kDa as analyzed using ABTS and DPPH, respectively. The higher activity of < 1 kDa peptides in antioxidant assays may be due to higher amount of total hydrophobic and aromatic amino acids as these amino acids' types exhibited more antioxidative activity (Ajibola et al. 2011).

Conclusion

The present study revealed better antioxidative potential of goat and buffalo milk in comparison to cow milk. However, it could not be ascertained that which milk species has higher antioxidative potential in totality as TEAC depends on number of compounds besides proteins and their composition. Temperature adversely influences the antioxidative capacity of goat milk and antioxidative capacity decreases with increased temperature. Hence, it can be inferred that antioxidative potential of goat raw milk is better than any heat treatment given. Although, due to their intrinsic pathogenic microbiota, goat raw milk consumption is restricted, which has been related to foodborne disease. Antioxidative potential increased upon hydrolysis of goat milk casein. Further, pepsin-trypsin (PT) hydrolysate of goat milk casein exhibited the maximum antioxidative potential than other hydrolysates. PT hydrolysate fraction 3-10 kDa fraction could be utilized to develop physiologically functional foods or therapy drugs. The identification of more potent antioxidative peptides of 3-10 kDa fraction of PT hydrolysate and their bioavailability and antioxidative effect has to be studied both *ex vivo* and *in vivo*.

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