



Quality and storage stability of goat meat emulsion during refrigerated storage upon incorporation of α -chymotrypsin hydrolysed camel milk casein

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Received: 13 December 2017; Accepted: 9 July 2018

ABSTRACT

Three different levels, viz. T1 (0.03%), T2 (0.06%) and T3 (0.09%) (w/w) of α -chymotrypsin hydrolysed camel milk casein was incorporated into goat meat emulsion, and compared with control (C: 0% hydrolysate) and positive control (PC: 0.02% butylated hydroxytoluene (BHT), w/w) for changes in quality at $4\pm 1^\circ\text{C}$. During storage, the water activity, extract release volume and emulsion stability decreased significantly, while pH increased. Except in T3, improvement in antioxidant potential of treated emulsions was recorded. Lower fatty acid oxidation was recorded in treated emulsions during storage. The treated emulsions had better instrumental colour profile, however, lightness (L^*), redness (a^* value) and yellowness (b^*) values decreased with the advancement of storage period. The microbiological counts in treated emulsions were initially reduced, and at the end of storage, significantly lower counts were recorded. In microbial challenging test (MCT), the colony forming units in treated emulsions decreased upto 4th day for all the tested pathogens, thereafter increased significantly on 6th day except in T3, whereas, in C and PC groups, the counts increased significantly throughout the storage period. The findings suggested that camel milk casein hydrolysate with α -chymotrypsin could be used as a potential food ingredient to improve its quality.

Key words: Antimicrobial activity, Antioxidant activity, Camel milk casein, Meat emulsion, Physico-chemical properties

Goat meat (Chevon) is the most preferred and widely consumed meat in India and worldwide without any religious taboo (Kumar *et al.* 2015). Unlike other meat, chevon is generally lean and somewhat tougher when derived from aged/spent animals. To improve quality of such kind of meat, the most efficient technique evolved was emulsion based products. However, storage and marketing of uncooked emulsions is another difficulty because of the problems of microbial, oxidative and other deteriorative changes during refrigerated storage. The freezing of raw emulsion may cause several changes in meat emulsion which may not be desirable for further processing. Therefore, it becomes necessary to extend the shelf life of raw emulsions during refrigerated storage by applying suitable techniques. To extend the shelf-life of meat emulsion, several synthetic/natural antioxidants and

antimicrobials have been tried (Kumar and Tanwar 2011, Oliveira *et al.* 2014, Kumar *et al.* 2016d), however, limited literatures are available on application of food derived bioactive peptides in meat emulsion. Therefore, this study was carried out to look at the possibility of use of camel milk casein hydrolysate obtained from enzymatic hydrolysis by α -chymotrypsin for extension of shelf life of goat meat emulsion. Camel milk is reported to have unique properties, higher antioxidant and antimicrobial properties as compared to other milk (Salami *et al.* 2009, Kumar *et al.* 2016). *In vitro* antioxidant and antimicrobial activity of camel milk casein was also reported by several authors (Salami *et al.* 2011, Kumar *et al.* 2016a, 2016b, 2016c, 2017a & 2017b). Therefore, in this experiment, camel milk casein hydrolysed by α -chymotrypsin (CCHC) was used in goat meat emulsion to study the effect of incorporation on different quality attributes at refrigeration temperature.

MATERIALS AND METHODS

Chemical and reagents: Fine laboratory chemicals such as 2,4,6-tripyridyl-s-triazine (TPTZ), α -chymotrypsin (EC 3.4.21.1, activity 35 units/mg protein) were purchased from MP Biomedicals, France, whereas, 2,2-azinobis (3-

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ethylbenzthiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma–Aldrich Chemical Co. Other chemicals and dehydrated culture media used were procured from reputed firms. The cultures of *Escherichia coli* (MTCC 2991), *Bacillus cereus* (MTCC 6728), *Staphylococcus aureus* (MTCC 7443) and *Listeria monocytogenes* (MTCC 657) were procured from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology (IMTECH), Chandigarh, in freeze dried form.

Camel milk casein hydrolysates (CCHC) powder: CCHC was prepared according to the methods described by Kumar *et al.* (2016a). Briefly, freeze dried camel milk casein powder was dissolved (5% total solid) in phosphate buffer (pH 8.0) and enzyme (α -chymotrypsin) was added at an enzyme:substrate ratio (E:S ratio) of 1:100. The hydrolysis was carried out for 4 h by incubating the samples at constant temperature (37°C) in stirred water bath. Hydrolyzed sample was immediately heated to 85°C for 15 min in water bath to denature the residual enzyme. Then, the samples were cooled and centrifuged in a refrigerated centrifuge (Eltek, Model:MP 400R, Elektrocraft (India) Pvt. Ltd., Mumbai, India) at 10,000 rpm for 25 min, supernatant was collected, dried in vacuum oven (Narang Scientific Works, New Delhi, India) at 50°C and 600 mm Hg vacuum for 12–14 h and ground to make fine powder and stored at –20°C till further use.

Goat meat (Chevon) emulsion: Castrated Beetal bucks of 12–15 months of age, weighing 25–30 kg were procured from Goat Farm of Department of Livestock Products Technology (LPM), College of Veterinary Science (CoVS), Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana. The animals were slaughtered in experimental slaughter house of Department of LPM, CoVS, GADVASU, Ludhiana, Punjab, following the standard procedure while keeping animal welfare aspects in consideration. The carcasses were hot deboned manually and the fascia, external fat and other connective tissues were removed. The recovered deboned meat was chilled over-night in refrigerator, packed in low-density polyethylene (LDPE) bags (200 gauge) and stored under frozen condition (–18°C) until use.

The frozen boneless lean meat was thawed overnight at 4±2°C, cut into small chunks and minced twice through 6 mm sieve plate in a meat mincer (MADO ESKIMO, MEW714, Germany). Meat emulsions were made in 5 groups containing minced meat (83.5%), oil (10%), ice (5%) and salt (1.5%). Based on preliminary trials conducted, 3 different levels of hydrolysate powder were incorporated in goat meat emulsion (T1, 0.03%; T2, 0.06% and T3, 0.09% w/w) and compared with control (without hydrolysates) and positive control (BHT 0.02%). After proper mixing, the emulsions were packed aerobically in LDPE bags and stored under refrigerated conditions (4±2 °C). The samples drawn on 0, 2nd, 4th and 6th day were evaluated for various physico-chemical (pH, water activity: a_w , emulsion stability: ES, extract release volume: ERV and instrumental colour

profile), lipid peroxidation (thiobarbituric acid reactive substances: TBARS, free fatty acid: FFA and peroxide value: PV), antioxidant assays (2,2-Diphenyl-1-picrylhydrazyl: DPPH, 2,2-Azinobis-3-ethylbenzthiazoline-6-sulphonic acid: ABTS, Ferric Reducing Antioxidant Power: FRAP and Superoxide Anion Scavenging Activity: SASA), microbial quality parameters (Standard plate count, Coliform count and Yeast and Mould count).

Microbial challenge testing (MCT): The MCT was conducted as per the method described by Abed *et al.* (2014) with slight modifications. The flash heat treatment of fresh meat cuts was carried out at 100°C for 5 min. The water required for emulsion (10%) was divided into two equal portion and one half was used to dissolve required amount of salt (1.5%) and another half for reconstitution of hydrolysate powder for each groups. Salt-water solution and refined oil were sterilized in autoclave (121 psi, 15 min), chilled and stored. The reconstituted protein hydrolysates were passed through 0.45 µm membrane filter before use. The decontaminated meat chunks were minced, blended properly with required proportion of water-salt solution, refined oil, hydrolysate solution and a known concentration of active live cultures of *E. coli* (MTCC 2991), *S. aureus* (MTCC 7443), *L. monocytogenes* (MTCC 657) and *B. cereus* (MTCC 6728), separately to reach a final concentration of 10⁴–10⁵ cfu/g. The batches were prepared separately for each microorganism tested, packed aerobically and stored at refrigeration temperature. The samples drawn on 0, 2nd, 4th and 6th day were evaluated for survival/inhibition of specific organism challenged in the study.

Physico-chemical analysis: The pH of goat meat emulsion was determined as per Trout *et al.* (1992) using digital pH meter. Emulsion stability (ES) was determined as per the method described by Baliga and Madaiah (1970). Potable digital water activity meter (Rotonix HYGRO Palm AW1 Set) was used to determine the water activity (a_w) of meat emulsion. The standard method of Jay (1964) was followed for ERV estimation.

Instrumental colour profile analysis: Colour profile was measured using Lovibond Tintometer (Lovibond RT-300, Reflectance Tintometer, United Kingdom) set at 2°C of cool white light (D65) and known as L^* , a^* , b^* values. L^* value denotes (brightness 100), or lightness (0), a^* (0+ redness/-greenness), b^* (+ yellowness/-blueness) values were recorded for goat meat emulsion kept in petri plates. The hue and chroma (saturation) values were calculated using the formula $[(\tan^{-1} (b/a))]$ (Little 1975) and $(a^2 + b^2)^{1/2}$ (Froehlich *et al.* 1983) respectively.

Antioxidant and lipid peroxidation assay: The ABTS, DPPH and FRAP assay was carried out as per the method described in Kumar *et al.* (2016b). SASA was determined by the method described by Kumar and Chattopadhyay (2007). The extraction method described by Witte *et al.* (1970) was used for TBARS estimation. PV and FFA in meat emulsion was estimated by methods described by Koniecko (1979).

Microbiological quality parameters: Standard plate counts, *Staphylococci* count, coliforms count, *Listeria monocytogenes* count, *Bacillus cereus* count and yeasts and mold counts of the samples were enumerated as per the methods described by American Public Health Association (APHA 1984).

Statistical analysis: All the experiments were repeated 3 times and all parameters were analysed in duplicate (n=6). Data were expressed as means with standard error. Analysis of variance (Two-way ANOVA) was done for comparing the means by using Duncan's multiple range test (DMRT), at 95% confidence level using SPSS package (SPSS 17.0 for Windows, SPSS Inc., USA).

RESULTS AND DISCUSSION

Physico-chemical quality parameters: The different physico-chemical parameters of goat meat emulsion incorporated with CCHC powder as well as control and positive control are presented in Table 1. The pH of C, PC and treated emulsion did not differ significantly on 0, 2nd and 4th day of storage, however, it varied significantly (P<0.05) on 6th day of storage. The pH of treated emulsion as well as C and PC increased significantly during storage, but the rate of increase was lower in treated emulsion and significantly (P<0.05) lower pH was maintained in treated emulsion upto 6th day of storage. This result are in

accordance with the reports of Das *et al.* (2011) in refrigerated minced chevon meat which might be attributed to bacterial activity. Jin *et al.* (2015) reported a dose dependent decrease in rate of pH increment in pork sausages incorporated with mechanically deboned chicken meat (MDCM) hydrolysates.

Water activity (a_w) of control and treated goat meat emulsion did not vary significantly (P<0.05) during entire storage period, however, a significant decrease in a_w was recorded from day 0 to 6 for all the groups except T2. Lower rate of decrease in a_w of treated emulsion might be due to the antimicrobial properties of incorporated protein hydrolysates.

Extract release volume (ERV) of goat meat emulsion decreased significantly during storage period, however, the treated emulsion maintained significantly (P<0.05) higher ERV as compared to C and PC. T2 and T3 maintained comparatively higher ERV throughout the storage period. The decrease in ERV of meat emulsion might be because of microbial growth/spoilage, which causes alteration in structure of proteins and pH of meat.

A non-significant increase in emulsion stability (ES) was observed upon incorporation of casein hydrolysates in goat meat emulsion. A significant (P<0.05) decreasing trend was also observed during storage period irrespective of treatments and comparatively higher ES was recorded for

Table 1. Physico-chemical quality changes in goat meat emulsion incorporated with CCHC powder during refrigerated storage (Mean±SE).

Group	Storage period (days)			
	0	2	4	6
	<i>pH</i>			
C	5.68±0.08 ^a	5.92±0.08 ^b	6.05±0.05 ^{bc}	6.22±0.05 ^{Bc}
PC	5.72±0.06 ^a	5.91±0.09 ^{ab}	6.00±0.06 ^{bc}	6.14±0.04 ^{ABc}
T1	5.63±0.03 ^a	5.76±0.04 ^b	5.93±0.03 ^c	6.12±0.02 ^{ABd}
T2	5.71±0.07 ^a	5.84±0.07 ^{ab}	5.98±0.06 ^{bc}	6.11±0.03 ^{Ac}
T3	5.77±0.04 ^a	5.90±0.08 ^{ab}	5.99±0.04 ^{bc}	6.09±0.02 ^{Ac}
	<i>Water activity (a_w)</i>			
C	0.973±0.003 ^c	0.964±0.003 ^{bc}	0.956±0.005 ^{ab}	0.948±0.005 ^a
PC	0.966±0.006 ^b	0.951±0.005 ^{ab}	0.946±0.004 ^a	0.943±0.005 ^a
T1	0.968±0.006 ^c	0.958±0.006 ^{bc}	0.947±0.007 ^{ab}	0.940±0.006 ^a
T2	0.960±0.006	0.952±0.006	0.948±0.007	0.944±0.006
T3	0.970±0.004 ^b	0.956±0.006 ^a	0.952±0.005 ^a	0.944±0.004 ^a
	<i>Extract release volume (ml)</i>			
C	42.17±0.75 ^{Bc}	39.50±0.85 ^{Ab}	38.00±0.97 ^{ab}	36.00±0.52 ^{Aa}
PC	40.33±0.88 ^{Ab}	39.83±0.87 ^{Ab}	38.50±0.56 ^{ab}	36.67±0.49 ^{Aa}
T1	44.67±0.61 ^{Cc}	40.67±0.92 ^{ABb}	38.83±0.54 ^b	36.50±0.89 ^{Aa}
T2	44.50±0.62 ^{Cb}	42.83±1.01 ^{Bb}	39.00±0.52 ^a	37.17±1.08 ^{Aa}
T3	45.83±0.79 ^{Cb}	41.83±0.65 ^{ABa}	40.00±0.93 ^a	39.50±0.76 ^{Ba}
	<i>Emulsion stability (%)</i>			
C	72.09±2.00 ^c	69.32±1.11 ^{bc}	67.65±1.01 ^{ab}	64.54±1.16 ^a
PC	72.28±1.21 ^b	69.86±1.35 ^{ab}	67.60±1.72 ^{ab}	65.72±1.87 ^a
T1	72.46±1.80 ^b	70.31±1.62 ^{ab}	69.22±1.82 ^{ab}	66.41±0.88 ^a
T2	72.86±1.55 ^b	70.79±1.41 ^{ab}	69.62±1.57 ^{ab}	66.89±1.05 ^a
T3	73.15±1.42 ^b	70.89±1.48 ^{ab}	69.85±1.49 ^{ab}	67.27±1.73 ^a

Mean±SE values bearing same superscripts row-wise (small alphabets) and column-wise (capital alphabets) do not differ significantly (P<0.05) (n=6); C, control emulsion without hydrolysate; PC, emulsion containing 0.02% BHT; T1, emulsion containing 0.03% CCHC; T2, emulsion containing 0.06% CCHC; T3, emulsion containing 0.09% CCHC.

treated emulsion as compared to C and PC throughout the storage period. The decrease in ES during storage might be due to the microbial decomposition of the protein, enzymatic and non-enzymatic lipid oxidation in meat emulsion. The higher ES in the treated emulsions might be due to functionalities of protein hydrolysates such as the better water holding capacity and emulsifying properties, which prevents leaching of lipid during heating. An increase in the number of peptide molecules and exposed hydrophobic amino acid residues due to hydrolysis of proteins would also contribute to an improved emulsion. Similar results were also reported by Kumar *et al.* (2016d & 2017b). Pena-Ramos and Xiong (2003) reported 12.9% improvement in cooking yield of pork patties upon incorporation of whey protein hydrolysates.

Antioxidant quality parameters: Goat meat emulsion was used as medium for *in vivo* investigation of antioxidant activity of CCHC powders. ABTS, DPPH, FRAP and SASA were estimated to access the antioxidant activity in this study and the data obtained were statistically analyzed, and presented in Table 2. The ABTS⁺ inhibition activity of treated emulsions as well as C and PC decreased significantly ($P<0.05$) with advancement in storage period and the differences among them were also significant during entire storage period. The ABTS⁺ inhibition activity of treated emulsions was recorded significantly ($P<0.05$)

higher than C and PC on 0 and 2nd day, and on 4th and 6th day, the antioxidant activity got decreased for T3, however, T2 maintained higher activity next to PC through storage period. The decrease in ABTS⁺ percent inhibition value during the storage was positively correlated with the concentration of the added protein hydrolysate except for T3 on day 4 and 6. These findings were in accordance with the findings of Rossini *et al.* (2009) who reported a significant increase ($P<0.05$) in the % inhibition of ABTS⁺ values in dose dependent manner.

Similarly, DPPH inhibition activity varied among treatments however it followed a decreasing trend throughout the storage period, irrespective of type and dose of protein hydrolysates. However, among treated emulsion, T2 had significantly higher activity next to PC. Jin *et al.* (2015) also reported significantly higher DPPH radical scavenging activity of sausages containing synthetic antioxidants (ascorbate and sodium erythorbate), compared to the other sausage samples before and after storage.

The FRAP activity was also recorded significantly ($P<0.05$) lower for C as compared to other groups and also decreased significantly during storage period. PC and T1 had comparable activity throughout the storage. Similar trend was also observed in T2 and T3. Serpen *et al.* (2012) measured the FRAP values of raw and cooked meats and reported that raw beef samples had highest FRAP values

Table 2. Changes in antioxidant properties of goat meat emulsion incorporated with CCHC powder during refrigerated storage (Mean±SE).

Group	Storage period (days)			
	0	2	4	6
<i>ABTS (% inhibition)</i>				
C	50.28±0.47 ^{Ac}	43.30±0.55 ^{Ac}	37.51±0.50 ^{Bb}	30.03±0.44 ^{Ba}
PC	82.40±0.49 ^{Dd}	79.52±0.49 ^{Dc}	75.21±0.67 ^{Db}	73.17±0.37 ^{Da}
T1	78.26±0.42 ^{Bd}	75.88±0.68 ^{Bc}	71.64±0.57 ^{Cb}	68.07±0.29 ^{Ca}
T2	79.02±0.38 ^{BCd}	76.57±0.63 ^{BCc}	72.05±0.47 ^{Cb}	69.71±0.53 ^{Ca}
T3	80.14±0.61 ^{Cd}	77.62±0.40 ^{Cc}	61.74±0.40 ^{Ab}	58.52±0.38 ^{Aa}
<i>DPPH (% inhibition)</i>				
C	14.29±0.55 ^{Ad}	11.34±0.33 ^{Ac}	8.03±0.33 ^{Bb}	6.07±0.30 ^{Aa}
PC	42.86±0.84 ^{Dd}	39.50±0.79 ^{Cc}	32.25±0.86 ^{Eb}	25.34±0.55 ^{Da}
T1	33.25±0.53 ^{Bd}	28.97±1.08 ^{Bc}	24.35±0.57 ^{Cb}	17.85±0.76 ^{Ba}
T2	38.05±0.70 ^{Cd}	31.30±0.87 ^{Bc}	26.12±0.53 ^{Db}	20.97±0.82 ^{Ca}
T3	39.13±0.61 ^{Cc}	20.47±0.19 ^{Ab}	13.10±0.36 ^{Aa}	11.93±0.89 ^{Aa}
<i>FRAP (mM Equivalent to FeSO₄·7H₂O)</i>				
C	10.07±0.28 ^{Ab}	7.92±0.25 ^{Ab}	6.84±0.30 ^{Aa}	5.86±0.08 ^{Aa}
PC	17.55±0.72 ^{Bc}	15.75±0.50 ^{Bb}	14.36±0.28 ^{Bab}	13.10±0.25 ^{Ba}
T1	17.98±0.58 ^{Bc}	15.53±0.38 ^{Bb}	13.95±0.61 ^{Ba}	12.72±0.43 ^{Ba}
T2	21.12±0.31 ^{Cb}	20.16±0.21 ^{Cb}	18.22±0.29 ^{Ca}	17.66±0.67 ^{Ca}
T3	22.05±0.24 ^{Cc}	21.29±0.32 ^{Cc}	18.82±0.18 ^{Cb}	16.71±0.76 ^{Ca}
<i>SASA (% inhibition)</i>				
C	15.53±0.94 ^{Ac}	12.79±0.73 ^{Ab}	10.72±0.77 ^{Ab}	8.01±0.17 ^{Aa}
PC	27.06±0.83 ^{Bc}	23.81±2.10 ^{Bbc}	20.37±1.99 ^{Bb}	16.01±1.36 ^{Ba}
T1	36.09±0.61 ^{Cd}	31.55±1.91 ^{Ccb}	25.89±1.15 ^{Cb}	21.99±0.95 ^{Ca}
T2	42.56±1.00 ^{Dc}	40.17±0.76 ^{Dc}	35.93±1.05 ^{Db}	32.78±0.87 ^{Ea}
T3	46.49±0.74 ^{Ed}	39.17±0.65 ^{Dc}	34.79±1.06 ^{Db}	28.45±0.97 ^{Da}

Mean±SE values bearing same superscripts row-wise (small alphabets) and column-wise (capital alphabets) do not differ significantly ($P<0.05$) (n=6); C, control emulsion without hydrolysate; PC, emulsion containing 0.02% BHT; T1, emulsion containing 0.03% CCHC; T2, emulsion containing 0.06% CCHC; T3, emulsion containing 0.09% CCHC.

(4.9±0.2 mmol Trolox Eq./kg d.w.) whereas lowest value of 3.0±0.1 mmol Trolox Eq./kg (d.w.) for fish samples.

The SASA (% inhibition) on day 0 followed a pattern as C<PC<T1<T2<T3. On day 6, T2 recorded higher among all the treatments. Similarly, Onuh *et al.* (2014) estimated SASA in chicken skin protein hydrolysates and its fractions and reported higher activity in hydrolysates as compared to control.

Lipid peroxidation parameters: Protein hydrolysates have been shown to possess radical scavenging activity (ABTS, DPPH etc.) suggesting that it could be utilized as natural antioxidants for improving shelf-life of lipid-rich food products. The oxidative stability exhibited in the present study is presented in Table 3. The PV (meq/kg sample) followed an increasing trend during storage, however, PC and treated emulsion maintained significantly (P<0.05) lower PV as compared to C except on day 0. In this study, it was also observed that the peroxide values of treated emulsions varied in a dose-dependent manner throughout the storage period. This indicated that the casein protein hydrolysates could reduce the lipid oxidation due to its antioxidant and antimicrobial properties as evident from previous experiments. Li *et al.* (2015) also reported that incorporation of grass carp protein hydrolysate (GPH) in grass carp mince resulted in significantly lower PV than control group throughout the frozen storage (-10°C) for 10 weeks.

TBARS value of C, PC as well as treated emulsion increased significantly (P<0.05) with advancement of

Table 3. Changes in lipid peroxidation properties of goat meat emulsion incorporated with CCHC powder during refrigerated storage (Mean±SE).

Group	Storage period (days)			
	0	2	4	6
<i>PV (meq/kg sample)</i>				
C	3.97±0.11 ^a	5.28±0.24 ^{Bb}	7.28±0.43 ^{Bc}	8.97±0.32 ^{Bd}
PC	3.94±0.08 ^a	4.01±0.26 ^{Aa}	5.23±0.15 ^{Ab}	7.07±0.39 ^{Ac}
T1	3.83±0.15 ^a	4.62±0.26 ^{ABb}	5.29±0.21 ^{Ac}	6.73±0.19 ^{Ad}
T2	3.78±0.11 ^a	4.44±0.18 ^{Ab}	5.35±0.22 ^{Ac}	6.33±0.26 ^{Ad}
T3	3.69±0.22 ^a	4.66±0.18 ^{ABab}	5.42±0.23 ^{Ab}	7.35±0.63 ^{Ac}
<i>TBARS (mg malonaldehyde/kg)</i>				
C	0.405±0.007 ^a	0.787±0.068 ^{Bb}	0.977±0.068 ^{Bb}	1.229±0.107 ^{Bc}
PC	0.380±0.013 ^a	0.461±0.024 ^{Aa}	0.622±0.033 ^{Ab}	0.794±0.034 ^{Ac}
T1	0.390±0.015 ^a	0.570±0.039 ^{Ab}	0.634±0.025 ^{Ab}	0.805±0.026 ^{Ac}
T2	0.378±0.013 ^a	0.568±0.014 ^{Ab}	0.592±0.02 ^{Ab}	0.790±0.04 ^{Ac}
T3	0.392±0.010 ^a	0.589±0.035 ^{Ab}	0.643±0.053 ^{Ab}	0.860±0.056 ^{Ac}
<i>FFA (% Oleic acid)</i>				
C	0.117±0.007 ^a	0.188±0.017 ^{Bb}	0.230±0.010 ^{Cc}	0.407±0.009 ^{Cd}
PC	0.105±0.004 ^a	0.134±0.015 ^{Aab}	0.189±0.015 ^{Bb}	0.314±0.032 ^{Bc}
T1	0.110±0.011 ^a	0.133±0.007 ^{Aa}	0.179±0.015 ^{ABb}	0.283±0.010 ^{ABc}
T2	0.101±0.004 ^a	0.129±0.006 ^{Aa}	0.177±0.012 ^{ABb}	0.239±0.016 ^{Ac}
T3	0.101±0.004 ^a	0.135±0.007 ^{Ab}	0.151±0.005 ^{Ab}	0.238±0.007 ^{Ac}

Mean±SE values bearing same superscripts row-wise (small alphabets) and column-wise (capital alphabets) do not differ significantly (P<0.05) (n=6); C, control emulsion without hydrolysate; PC, emulsion containing 0.02% BHT; T1, emulsion containing 0.03% CCHC; T2, emulsion containing 0.06% CCHC; T3, emulsion containing 0.09% CCHC.

Table 4. Changes in lipid peroxidation properties of goat meat emulsion incorporated with CCHC powder during refrigerated storage (Mean±SE).

Group	Storage period (days)			
	0	2	4	6
<i>PV (meq/kg sample)</i>				
C	3.97±0.11 ^a	5.28±0.24 ^{Bb}	7.28±0.43 ^{Bc}	8.97±0.32 ^{Bd}
PC	3.94±0.08 ^a	4.01±0.26 ^{Aa}	5.23±0.15 ^{Ab}	7.07±0.39 ^{Ac}
T1	3.83±0.15 ^a	4.62±0.26 ^{ABb}	5.29±0.21 ^{Ac}	6.73±0.19 ^{Ad}
T2	3.78±0.11 ^a	4.44±0.18 ^{Ab}	5.35±0.22 ^{Ac}	6.33±0.26 ^{Ad}
T3	3.69±0.22 ^a	4.66±0.18 ^{ABab}	5.42±0.23 ^{Ab}	7.35±0.63 ^{Ac}
<i>TBARS (mg malonaldehyde/kg)</i>				
C	0.405±0.007 ^a	0.787±0.068 ^{Bb}	0.977±0.068 ^{Bb}	1.229±0.107 ^{Bc}
PC	0.380±0.013 ^a	0.461±0.024 ^{Aa}	0.622±0.033 ^{Ab}	0.794±0.034 ^{Ac}
T1	0.390±0.015 ^a	0.570±0.039 ^{Ab}	0.634±0.025 ^{Ab}	0.805±0.026 ^{Ac}
T2	0.378±0.013 ^a	0.568±0.014 ^{Ab}	0.592±0.02 ^{Ab}	0.790±0.04 ^{Ac}
T3	0.392±0.010 ^a	0.589±0.035 ^{Ab}	0.643±0.053 ^{Ab}	0.860±0.056 ^{Ac}
<i>FFA (% Oleic acid)</i>				
C	0.117±0.007 ^a	0.188±0.017 ^{Bb}	0.230±0.010 ^{Cc}	0.407±0.009 ^{Cd}
PC	0.105±0.004 ^a	0.134±0.015 ^{Aab}	0.189±0.015 ^{Bb}	0.314±0.032 ^{Bc}
T1	0.110±0.011 ^a	0.133±0.007 ^{Aa}	0.179±0.015 ^{ABb}	0.283±0.010 ^{ABc}
T2	0.101±0.004 ^a	0.129±0.006 ^{Aa}	0.177±0.012 ^{ABb}	0.239±0.016 ^{Ac}
T3	0.101±0.004 ^a	0.135±0.007 ^{Ab}	0.151±0.005 ^{Ab}	0.238±0.007 ^{Ac}

Mean±SE values bearing same superscripts row-wise (small alphabets) and column-wise (capital alphabets) do not differ significantly (P<0.05) (n=6); C, control emulsion without hydrolysate; PC, emulsion containing 0.02% BHT; T1, emulsion containing 0.03% CCHC; T2, emulsion containing 0.06% CCHC; T3, emulsion containing 0.09% CCHC.

storage period, however, PC and treated emulsion maintained significantly lower value as compared to C except on day 0. A sharp increase in TBARS value was observed in control emulsion (0.405 on day 0 to 1.229 mg malonaldehyde/kg on day 6) but PC and treated emulsions maintained a lower value during refrigerated storage. Maintenance of lower TBARS values in treated emulsions might be due to antioxidant activity of casein protein hydrolysates confirmed the results of previous experiments of this study. Similar observations were also reported by Rossini *et al.* (2009) in casein hydrolysate incorporated ground beef and MDPM and Sakanaka and Tachibana (2006) in egg-yolk protein hydrolysate incorporated beef homogenate.

The FFA (% oleic acid) of goat meat emulsion increased significantly (P<0.05) with the advancement of storage period. On day 2 onward, the FFA value of control remained significantly (P<0.05) higher throughout the storage and that of PC and treated emulsions were comparable. In this study, it was observed that the FFA values of treated emulsions varied in a dose-dependent manner throughout the storage period. These results were in agreement with the findings of Dey and Dora (2014) in Croaker fillet pre-soaked with sodium erythroborate and incorporation of shrimp waste protein hydrolysate.

Instrumental colour profile: The statistically analyzed data recorded for instrumental colour profile of goat meat emulsion are presented in Table 4. The lightness (L^*) values did not differ significantly among different treatments as well as PC and C throughout the storage period. It was also

Table 5. Changes in microbiological quality parameter of goat meat emulsion incorporated with CCHC powder during refrigerated storage (Mean±SE).

Group	Storage period (days)			
	0	2	4	6
<i>SPC (log₁₀cfu/g)</i>				
C	4.30±0.22 ^a	4.81±0.20 ^{Db}	5.34±0.03 ^{Cc}	6.34±0.03 ^{Dd}
PC	4.29±0.23 ^a	4.76±0.16 ^{Db}	5.22±0.18 ^{Cc}	6.27±0.05 ^{Dd}
T1	4.17±0.20 ^b	4.05±0.17 ^{Ca}	4.23±0.22 ^{Bb}	4.96±0.17 ^{Cc}
T2	4.15±0.33 ^b	3.88±0.21 ^{Ba}	4.16±0.22 ^{ABb}	4.65±0.19 ^{Bc}
T3	4.06±0.21 ^b	3.55±0.23 ^{Aa}	3.92±0.18 ^{Aab}	4.37±0.15 ^{Ac}
<i>Coliform count (log₁₀cfu/g)</i>				
C	1.86±0.10 ^a	2.03±0.10 ^{Db}	2.18±0.05 ^{Dbc}	2.29±0.05 ^{Dc}
PC	1.87±0.11 ^a	1.93±0.07 ^{Da}	2.14±0.05 ^{Db}	2.23±0.01 ^{Db}
T1	1.70±0.14 ^b	1.64±0.09 ^{Ca}	1.76±0.05 ^{Cb}	2.07±0.05 ^{Cb}
T2	1.63±0.11 ^b	1.48±0.06 ^{Ba}	1.60±0.05 ^{Bb}	1.87±0.04 ^{Bc}
T3	1.56±0.13 ^b	1.30±0.05 ^{Aa}	1.46±0.06 ^{Aab}	1.63±0.04 ^{Abc}
<i>Yeast and mold count (log₁₀cfu/g)</i>				
C	0.88±0.18 ^{Aa}	1.15±0.07 ^{Aa}	1.18±0.11 ^{Ba}	1.57±0.05 ^{Bb}
PC	0.67±0.21 ^{Aa}	1.10±0.06 ^{Ab}	1.28±0.06 ^{Bbc}	1.52±0.03 ^{Bc}
T1	0.55±0.25 ^{Aa}	1.05±0.05 ^{Ab}	1.13±0.09 ^{Bb}	1.20±0.06 ^{ABb}
T2	ND	ND	0.67±0.21 ^{Aa}	0.93±0.20 ^{Aa}
T3	ND	ND	0.50±0.22 ^{Aa}	0.98±0.21 ^{Ab}

Mean±SE values bearing same superscripts row-wise (small alphabets) and column-wise (capital alphabets) do not differ significantly ($P<0.05$) ($n=6$); C, control emulsion without hydrolysate; PC, emulsion containing 0.02% BHT; T1, emulsion containing 0.03% CCHC; T2, emulsion containing 0.06% CCHC; T3, emulsion containing 0.09% CCHC.

observed that L^* values decreased significantly ($P<0.05$) with the advancement of storage period except for T1 and T2. The decrease in lightness value of meat emulsion during storage might be due to lipid oxidation and microbial growth, but in meat emulsions treated with BHT and protein hydrolysates, the decrease in the L^* values were non-significant, which indicated the antioxidant and antimicrobial activity of test ingredients. Nieto *et al.* (2009) also reported that meat emulsions with added hydrolyzed potato protein (HPP) were darker (lower L^*) than those made without HPP.

The redness (a^*) value of goat meat emulsion with or without test ingredients did not differ significantly among groups but on day 6, the differences were significant ($P<0.05$). For C, PC and T1, the decrease in redness values were also significant ($P<0.05$) during storage period. The decrease in redness of goat meat emulsion might be due to lipid oxidation but the slower rate of decrease in redness of T2 and T3 reflects the antioxidant properties of casein hydrolysates. Similar results were reported by Jin *et al.* (2015) for meat sausages incorporated with mechanical deboned chicken meat hydrolysates. Sun and Xiong (2015) also reported that reduction in a^* value was 7.0–16.9% on incorporation of pea protein hydrolysate (PPH) in ground beef. The yellowness (b^*) values of goat meat emulsion incorporated with different level of casein hydrolysates decreased slightly on day 0, however, it was maintained during entire storage period. The decrease in b^* during

Table 6. Changes in the microbial counts in goat meat emulsion incorporated with CCHC powder during microbial challenge test at refrigeration temperature ($4\pm 2^\circ\text{C}$) storage (Mean±SE).

Group	Storage period (days)			
	0	2	4	6
<i>Staphylococcus aureus (log₁₀cfu/g)</i>				
C	4.95±0.06 ^{Ba}	6.07±0.08 ^{Cb}	6.95±0.08 ^{Cc}	7.90±0.04 ^{Dd}
T1	4.62±0.03 ^{Aab}	4.34±0.16 ^{Ba}	4.89±0.15 ^{Bb}	6.34±0.03 ^{Cc}
T2	4.54±0.14 ^{ABb}	4.23±0.06 ^{ABb}	3.81±0.07 ^{Aa}	4.25±0.06 ^{Bb}
T3	4.44±0.15 ^{Ab}	3.90±0.12 ^{Aa}	3.82±0.06 ^{Aa}	3.96±0.09 ^{Aa}
<i>Listeria monocytogenes (log₁₀cfu/g)</i>				
C	4.82±0.05 ^{Ba}	6.03±0.05 ^{Cb}	6.89±0.09 ^{Bc}	7.87±0.03 ^{Bd}
T1	4.49±0.17 ^{ABb}	4.37±0.15 ^{Bab}	3.90±0.11 ^{Aa}	4.17±0.03 ^{Aa}
T2	4.37±0.16 ^{Ab}	4.09±0.07 ^{Aa}	3.92±0.06 ^{Aa}	4.11±0.07 ^{Aa}
T3	4.27±0.09 ^{Ab}	3.98±0.08 ^{Aab}	3.79±0.05 ^{Aa}	4.03±0.09 ^{Aab}
<i>Bacillus cereus (log₁₀cfu/g)</i>				
C	4.95±0.06 ^{Ba}	6.07±0.07 ^{Cb}	6.95±0.08 ^{Cc}	7.90±0.05 ^{Dd}
T1	4.62±0.03 ^{ABb}	4.54±0.04 ^{Ba}	5.06±0.03 ^{Bc}	6.34±0.03 ^{Cd}
T2	4.47±0.06 ^{Ac}	4.23±0.06 ^{ABb}	3.81±0.07 ^{Aa}	4.25±0.06 ^{Bb}
T3	4.44±0.03 ^{Ab}	3.90±0.12 ^{Aa}	3.82±0.06 ^{Aa}	3.96±0.09 ^{Aa}
<i>Escherichia coli (log₁₀cfu/g)</i>				
C	4.92±0.05 ^{Ba}	6.03±0.05 ^{Db}	6.89±0.09 ^{Cc}	7.87±0.03 ^{Dd}
T1	4.62±0.03 ^{Aa}	4.74±0.04 ^{Cab}	5.16±0.03 ^{Bab}	6.34±0.02 ^{Cb}
T2	4.59±0.04 ^{Ac}	4.23±0.06 ^{Bb}	3.81±0.07 ^{Aa}	4.25±0.06 ^{Bb}
T3	4.54±0.06 ^{Ab}	3.90±0.12 ^{Aa}	3.82±0.06 ^{Aa}	3.96±0.09 ^{Aa}

Mean±SE values bearing same superscripts row-wise (small alphabets) and column-wise (capital alphabets) do not differ significantly ($P<0.05$) ($n=6$); C, control emulsion without hydrolysate; PC, emulsion containing 0.02% BHT; T1, emulsion containing 0.03% CCHC; T2, emulsion containing 0.06% CCHC; T3, emulsion containing 0.09% CCHC.

storage period was significant ($P<0.05$) from day 0 to day 6 for all the groups except T3. At the end of storage, the b^* value of the control was lower compared to treated emulsions and recorded highest for T3. This indicates that although, the treated emulsions had slightly lower b^* value on initial days but it was maintained during course of time, which might be due to the antioxidant properties of the added protein hydrolysates. These results are in concurrence with the reports of Nieto *et al.* (2009).

Hue angle, which indicated visual assessment of meat discolouration and the differences in mean of different groups and during storage did not differ significantly. Similarly, Chroma (saturation) values also did not vary significantly among groups during entire storage period but it decreased significantly ($P<0.05$) with the advancement of storage period. The decrease in chroma value from 0 day to 6th day were significant for all the groups except for T3.

Microbiological quality parameters: Although the incorporation of peptide did not result in significant reduction in log value on day 0 but on day 2, significant ($P<0.05$) reduction in SPC was observed in treated emulsions as compared to C and PC (Table 5). This reflected that these hydrolysates had both bactericidal and bacteriostatic properties, suppressing/inhibiting growth of bacteria in meat emulsion. On day 6, T2 and T3 had

significantly lower SPC count (4.37–4.65 log₁₀cfu/g) than other groups.

The coliform counts of C, PC and treated emulsion did not differ significantly on day 0, but it was significantly (P<0.05) lower in treated emulsions during further storage. On day 2, a significant reduction in coliform counts was observed in treated emulsion in dose dependent manner and again increased steadily upto 6th day, but the treated emulsion maintained lower coliform count during entire storage period.

Yeast and moulds were not detected in T2 and T3 on day 0 and 2, however, it was significantly lower in PC and T1 than that of C. On day 4 and 6, a significantly (P<0.05) lower yeast and mould counts were detected in T2 and T3 than C and PC. Reduction in yeast and mold counts in meat emulsion incorporated with CCP hydrolysate could be attribute to the anti-fungal properties of the protein hydrolysate. Correa *et al.* (2011) also reported inhibitory effect of ovine casein hydrolysates against *Penicillium expansum* and *Aspergillus fumigatus*.

Microbial challenging test in goat meat emulsion for pathogenic/food spoilage microorganism against CCHC: The microbial challenging test (MCT) of CCHC in goat meat emulsion are presented in Table 6. Effect of different concentrations of the CC incorporated in goat meat emulsion and inoculated with microorganism to be tested was studied and observed that on day 0 itself, the bacterial counts (log₁₀cfu/g) decreased significantly (P<0.05) in casein hydrolysates incorporated emulsion. During further storage upto 6th day at refrigeration, the counts for all the tested bacteria decreased upto 4th day (except in some cases), whereas, on day 6, the counts again increased. At the end of storage, the protein hydrolysates incorporated emulsions had significantly (P<0.05) lower counts than control. It was also observed that the emulsion having higher concentration of casein hydrolysate (T3: 900µg/g) had lowest counts at the end of storage. Reduction in log values upto 3.00 CFU/g was observed in this study. This indicates the protein hydrolysates has not only bactericidal effect but it also acted as bacteriostatic agent and maintained lower counts in treated emulsions. However, as the activity of protein hydrolysate depends various factors, therefore, variation in activity of each hydrolysate against each organism was obvious. Similar results were also reported by the Wang (2003) for *S. aureus* and *E. coli* in comminuted meats, Demers-Mathieu *et al.* (2013) against *L. monocytogenes* in Cheddar cheese by an anionic peptides-enriched extract from whey proteins and Osés *et al.* (2015) against *E. coli* and *L. monocytogenes* in lamb meat with protective culture.

Although, a substantial and consistent higher antimicrobial activity was observed in *in vitro* conditions, but in meat model system, the hydrolysate exhibited lower antimicrobial activity. This might be due to interaction of highly charged peptides present in hydrolysates to different food component and thus resulted in lower activity. Shelef *et al.* (1984) also reported lower antimicrobial activity of spice is in food systems than in microbiological media.

Camel milk casein hydrolyzed by α-chymotrypsin could be used as valuable ingredient for enhancing the quality and storage stability of meat emulsions. α-chymotrypsinolyzed camel milk casein hydrolysates could be incorporated into goat meat emulsion upto 0.06% (w/w) without any adverse effects on its quality. Its incorporation into meat emulsion not only improved the physico-chemical properties but it also protected against oxidative and microbial damage to the emulsion when stored under refrigerated (4±1°C) condition.

ACKNOWLEDGEMENT

The authors sincerely acknowledge Director, National Research Centre on Camel, Bikaner, Rajasthan, India and Dean PGS, GADVASU, Ludhiana for providing facilities for this research work.

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