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Comparative study on utilization of recovered protein isolates from rumen and lung of slaughtered buffalo in buffalo meat patties

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Abstract

Protein isolates were recovered from the minced lung and rumen tissue by improved alkaline extraction followed by isoelectric precipitation using acid. Physico-chemical properties, proximate principles, collagen content, *in-vitro* pepsin digestibility and microbial profile of both rumen and lung protein isolates were determined. Microbial qualities of both rumen and lung derived isolates are studied. Buffalo meat patties were prepared by replacing lean meat at 0, 10, and 20% by both rumen and lung derived isolates. The mean pH values of cooked patties in all incorporation levels of protein isolates was significantly higher than the pH of raw emulsion. Cooking yield increased significantly when compared to control. Shear force value increased significantly as the incorporation level increased. The moisture content of patties gradually increased significantly while the protein and fat decreased with increase in incorporation levels of rumen protein isolates. In the present study, the rumen protein isolate has shown superior digestibility and buffalo meat patties containing rumen or lung protein isolate at 20% incorporation level have shown significant increased available lysine in comparison to control and the sensory evaluation scores for patties incorporated upto 20% level of rumen or lung protein isolate were given higher scores and rated as very good to excellent. Meat patties with 20% level of rumen protein isolates are nutritionally better in terms of digestibility and available lysine.

Keywords: Buffalo rumen lung/rumen protein isolate, utilization, buffalo meat, patties, quality

Introduction

Protein hunger is one of the prime areas of food science research, as India has already addressed calorie hunger through food revolution after the independence. Presently, much of the research is directed to design low protein rich food for the use of common man. The severe shortage of protein of high biological value in developing countries and the high cost of meat has fostered great interest in the possibility of fabricating protein rich foods from alternative sources like proteins from vegetable and bacterial sources called meat analogues. In view of the frequent drawbacks of low acceptability, absence of organoleptic quality and high cost of meat analogues prefabricated from vegetable or bacterial proteins, it would be highly desirable to reassess the potential for making edible and attractive low cost foods from the substantial amounts of proteins present in abattoir byproducts which are currently wasted.

Apart from the traditional usage, as animal feeds and as organic fertilizers, by products from animal processing are widely converted to protein isolates and hydrolysates Gbogouri *et al.* (2004) [10]. This method of utilization is different from other conventional methods because the chemical components of the byproducts (protein) are first recovered and then utilized rather than direct use of byproducts. In the production of protein isolates, the proteins are first extracted from the organic material using water, alkali or acid extraction depending on the pH and temperature at which the protein is soluble. The soluble protein can then be recovered from the clarified solution through precipitation and dried in order to obtain the protein isolate. To overcome shortage of proteins of high biological value simple, safe and economic way of recovery of proteins with high nutritive value from meat byproducts in the form of isolates and hydrolysates has become the mandate. Great emphasis has been placed on developing 'enzymatic' hydrolysis methods for production of protein hydrolysates as they are proved to be bioactive with many health benefits. However, as the process does not remove pro-oxidants and pigments, the hydrolysates are often rancid and dark coloured.

Also, peptide formation yields bitterness and the high temperature used in the enzyme inactivation process denatures the sensitive proteins which destroy their functionality Lanier (1994) [19]. Today, few commercially available enzymatically produced protein concentrates have found limited use due to poor product quality, lack of functionality and a rancid odour/taste. Hence, better method will be to produce isolates by the pH adjustment or pH shift technology at room temperature Omana and Betti (2012)[21] rather than use of enzymes and membranes for concentrating proteins as the former is costly and the latter pose the problem of soiling. Hence an attempt was made to recover proteins from lung of slaughtered buffalo, subject it to the process of hydrolysis and incorporate in buffalo meat patties.

Materials and Methods

Collection of rumen and lung

Buffaloes that were selected from nearby villages were brought to organized abattoir. These animals were withheld feed for about twenty four hours but had free excess to drinking water. They were subjected to ante-mortem examination and those found to be free from diseases or any abnormalities were slaughtered. These animals were stunned by mild electrical stunning before exsanguinations and thorough bleeding was ensured. They were dressed by adopting the standard slaughter procedure and subjected to post mortem examination.

Preparation of lung

The lungs were collected from slaughtered animals. Trachea removed and thoroughly cleaned with running tap water. Further, it was cut into small pieces and washed with distilled water several times to ensure maximum removal of blood and minced through 8 mm and further with 4 mm plate in meat mincer (Advanced laboratories, Chennai).

Preparation of rumen

The rumen was evacuated of the ruminal contents by cutting obliquely and inverting the ruminal wall. The black mucous membrane was removed after scalding at 60 °C for 2 min and also by mechanical scrapping under running tap water. It was cut into small pieces cleaned with distilled water and minced in meat mincer by passing through 8 mm followed by 4 mm plate.

Recovery of Proteins

The method of protein recovery from slaughterhouse byproducts as suggested by Darine Selmane *et al.* (2010) [8] was standardized in Laboratory. Five hundred grams of minced tissue samples was homogenised in Food mincer for 5 minutes and suspended in 10 litres of water (minced offal/solvent ratio of 1:20 w/v). The mixture was stirred with a magnetic stirrer for 10 min and the pH of the slurry was adjusted to pH 10 with 10M sodium hydroxide and left at room temperature and the extraction was allowed to continue for 2 hours with constant stirring while the pH is kept constant. The slurry was centrifuged at 4000 rpm for 20 min. The residues were re-extracted with the same solvent under similar conditions. The supernatants were combined and proteins precipitated by adjusting the pH to 4.5 (pH of minimum solubility) with 1M HCl, followed by separation by centrifugation at 4000 rpm for 20 min. The resulting protein isolate was in the form of semi solid paste, which was filtered

through muslin cloth to drain out excess moisture. The recovered buffalo lung protein isolates (BLPI) and rumen protein isolates (BRPI) were recorded for their yield and stored at -18 °C for further use.

Recipe of patties

Deboned buffalo meat was packed in clean polyethylene bags and frozen at -20 °C until use. The standardized recipe contained 85 parts buffalo meat with 15 parts of sun flower oil and green condiments 5%, table salt 2%, dry spices mix 1%, sugar 1%, phosphate 0.5%, sodium nitrite 0.02% and ice water 12%. Buffalo meat patties for the present study prepared by incorporating buffalo lung and rumen protein isolates (BLPI/ BRPI) at 0, 10, 20 percent levels by replacing lean meat.

Preparation of patties

Meat emulsion was made utilizing above mentioned ingredients. Sixty grams of meat emulsion was moulded in aluminium circular mould and placed on perforated trays and cooked for 18 minutes in a preheated oven at 180 °C to obtain an internal temperature of about 75 °C. Six such trials were conducted for each level of incorporation.

Analysis of sample

Proximate/ Nutrient composition was determined according to AOAC (1995) [1] methods for both buffalo lung protein isolates (BLPI) and rumen protein isolates (BRPI) and formed patties as well. The *in vitro* pepsin digestibility of both buffalo lung protein isolates (BLPI) and rumen protein isolates (BRPI) were performed as per the standard method AOAC (1995) [1] with slight modifications as per ICONTEC (1994) [13]. The microbiological quality of raw lung / rumen and their derived protein isolates was evaluated by estimating standard plate count (SPC), psychrotrophic plate count (PPC), total coliform, total staphylococcal count as per the standard procedure of APHA (2001) [2].

The pH of raw emulsion as well as cooked patties was determined by the method of AOAC (1995) [1] using pH meter. Emulsion stability and percent cooking yield were determined by the method of Baliga and Madiah (1970) [4] with slight modifications. Amount of collagen in buffalo lung protein isolates (BLPI)/rumen protein isolates (BRPI) and meat patties was calculated by estimating hydroxyproline content according to the procedure of Neuman and Logan (1950) [20]. Available lysine content of patties was determined by the method of Carpenter (1960) [7].

Objective texture/ shear force value of the patties was recorded using a Warner-Bratzler shear device. Each patty was made into small piece of 1.5 cm and the force required to shear the patties was recorded. The sensory attributes of the product were evaluated by six semi trained panelists, using an 8 point Hedonic scale as per Keeton (1983) [16].

Statistical analysis

Data obtained were analyzed statistically as per the method outlined by Snedecor and Cochran (1980) [23]. The results were demonstrated as mean \pm SE. The results were considered statistically significant when ($p < 0.05$).

Results and Discussion

Protein recovery

Yield of protein isolates from buffalo lung and rumen is

presented are in Table 1. Protein was recovered by improved alkaline extraction method originally suggested by Swingler and Lawrie (1979) [25] and modified by Darine Selmane *et al.* (2010) [8]. In the present study also, conditions reported by these earlier workers in terms of time, strength of chemicals required for solubilization and in turn yield of protein were found to be optimum for the best recovery of protein from lungs and rumen. The yield of proteins from 500 g tissues recorded were 186.33 ± 1.892 g for lungs and 160 ± 1.59 g for rumen. In terms of percent protein recovery it was $37.27 \pm 0.37\%$ for lungs and $32.0 \pm 0.318\%$ for rumen. However, on the basis of dry matter there was no difference in the yield of protein from lungs (35.85 g) and rumen (35.80g). The percent recoveries from lungs as well as rumen were in accordance with Swingler and Lawrie (1979) [25], Darine Selmane *et al.* (2010) [8], Babu *et al.* (1993) [3] they also used the same method of alkaline extraction to recover proteins from bovine lungs and rumen and from ovine lungs and rumen, respectively.

Physico-chemical composition of protein isolates from buffalo rumen and lung

The proximate composition of lung, rumen and derived protein isolates including physico-chemical characteristics, collagen, pH, *in-vitro* digestibility and microbial load are presented in Table 2. The average crude protein content of buffalo raw lung is higher than the rumen whereas the collagen contents of both raw tissues are similar and the pH of rumen higher than lung tissue. The fat, ash and collagen contents of both the tissues are nearly equal. The values are in accordance with the Gault and Lawrie (1980) [11] for bovine lung and rumen. The percent composition of lung protein isolate and rumen protein isolate was as under. Protein $12.8 \pm 0.19\%$ and $14.32 \pm 0.37\%$; Moisture $84.16 \pm 0.172\%$ and $80.47 \pm 0.368\%$; Fat $1.01 \pm 0.01\%$ and $0.57 \pm 0.026\%$; Total ash $0.58 \pm 0.017\%$ and $0.56 \pm 0.028\%$. They are in agreement with the values reported by Swingler and Lawrie (1979) [25] for protein isolates from bovine lungs and rumen. In the present study lung protein isolate contained 0.22 ± 0.012 and rumen protein isolate 0.12 ± 0.005 percent collagen which was similar to the values reported for bovine lung and rumen protein isolates by Swingler and Lawrie (1979) [25]. In spite of the equal amount of collagen in raw tissues viz. lungs (2.47 ± 0.03) and rumen (2.75 ± 0.023), the lung protein isolate contained more collagen than rumen protein isolate indicating that lung tissue contained more soluble collagen as compared to rumen. *In vitro* pepsin digestibility of BLPI was 63.90 ± 16.2 and BRPI is 79.30 ± 16.0 percent, indicating that BRPI is a better protein isolate. These values are in agreement with Song *et al.* (1984) [24] who reported similar *in-vitro* pepsin digestibility values for protein isolate from swine lung and rumen.

Microbial quality

The major problem associated with raw by-products is their high microbial population, originating from gastro intestinal tract and their consequent susceptibility to deteriorative changes. The alkaline extraction method significantly lowered the microbial population of protein isolates. In the present study the standard plate counts and psychrotrophic plate counts of lung protein isolate (4.12 ± 0.01 and 2.27 ± 0.037 log units/g) and rumen protein isolate (4.59 ± 0.029 and 2.69 ± 0.026 log units/g) recorded were lower than the values for raw lung (5.13 ± 0.008 and 3.54 ± 0.014 log units/g) and rumen

(6.38 ± 0.018 and 4.79 ± 0.02 log units/g) which were in close agreement with the findings of Swingler *et al.* (1979) [25] and Song *et al.* (1984) [24] for bovine lungs and rumen and Babu *et al.* (1993) [3] for ovine lungs and rumen. These authors have used alkaline method of extraction for isolating protein. The lower microbial counts of isolates recorded in the present study might be attributed to the alkaline extracted method followed by acid precipitation and low pH of the isolates.

The standard plate counts and psychrotrophic plate counts values recorded for raw rumen (6.38 ± 0.018 and 4.79 ± 0.02 log units/g) were higher than those for raw lungs (5.13 ± 0.008 and 3.54 ± 0.014 log units/g) which are in agreement with the values reported by Swingler *et al.* (1979) [25] for bovine lungs and rumen and Babu *et al.* (1993) [3] for ovine lungs and rumen. The higher microbial load of raw rumen when compared to raw lungs might be due to contribution of microbial population from ruminal contents. Nil counts were observed for coliforms and staphylococcus in both the raw tissues and derived protein isolates.

Processing quality characteristics of buffalo meat patties with the incorporation of BLPI/BRPI at different levels

Processing quality characteristics of buffalo meat patties and nutrient composition are presented in Table 3.

Buffalo meat patties were prepared incorporating lung protein isolate and rumen protein isolate at different levels (0, 10, 20, 30%) replacing lean. However, no published literature is available to validate the results observed in respect of quality characteristics of buffalo meat patties incorporated with different levels of isolates.

pH: There was a gradual decrease in pH of raw emulsion with increase in incorporation levels of protein isolates from 0 to 30%. This decreasing trend might be due to low pH of protein isolates (4.2/4.5). On cooking, the pH of all patties with or without incorporation of protein isolates increased by 0.5 to 0.9 units. The increase in pH of cooked patties was in agreement with the findings of Bouton *et al.* (1971) [6] and Fogg and Harrison (1975) [9] in beef, Kesava Rao and Kowale (1988) [17] in buffalo meat., Babu *et al.* (1993) [3] in mutton patties incorporated with by-products, Jelen *et al.* (1982) [15] in luncheon meat incorporated with alkali extracted chicken protein, Boles *et al.* (2000) [5] in beef sausages incorporated with beef bone extracted protein, on cooking. The increase in the pH recorded in the present study was attributed to change in the protein charge as well as cooking loss as suggested by Hamm and Deatherage (1960) [12] and Bouton *et al.* (1971) [6].

Cooking yield: There was a gradual increase in cooking yield with increase in incorporation levels of protein isolates. From the results of decreased pH values with increased incorporation of isolates in raw/cooked patties, there should have been a decrease in cooking yield. It was observed by Young and Lawrie (1974) [26], Perera and Anglemier (1980) [22] and Darine Selmane *et al.* (2010) [8] that the protein isolated from slaughterhouse by-products by alkaline extraction method retained their functional characteristics and nutritional quality. The findings of the present study also indicated that the protein isolates may have some role in improving the water holding capacity as well as emulsion stability of the meat on cooking.

Shear force value: Shear force value increased significantly with increase in incorporation levels of protein isolates in

buffalo meat patties. This might be due to better emulsion stability leading to better texture of patties. This view was supported by Babu *et al.* (1993) [3] while working with incorporation by-products in chevon patties.

Proximate/Nutrient composition of buffalo meat patties with incorporation of BLPI and BRPI at different levels

Nutrient composition of buffalo meat patties with the incorporation of BLPI and BRPI at different levels are presented in Table 3. The moisture content of patties gradually increased while protein content decreased with increase in incorporation levels of lung/rumen protein isolate from 0 to 30%. The ether extract content also decreased gradually with increase in incorporation levels of isolates. This might be due to higher moisture and lower ether extract contents of protein isolates incorporated. The increase in moisture content and decrease in ether extract content recorded in this study is in close agreement with the findings of Krokha and Shtulboi (1978) [18], Babu *et al.* (1993) [3], Jelen *et al.* (1982) [15] who incorporated alkali extracted by-product proteins in emulsion based meat products.

Available lysine: There was an increasing trend in availability of lysine when incorporation of BLPI and BRPI at different level was undertaken as compared to control. As a class, meat proteins are rich sources of lysine and methionine. The proteins in the organs meat/ offals are similar in composition and differ from those in muscle tissue in being poorer in lysine, tryptophan and tyrosine and richer in proline, hydroxyproline and glycine (Jayathilakan, 2012) [14]. As most of Indian diet is predominantly cereal/ legume based, meat products and by-products play an important role in supplying essential amino acids like lysine and methionine.

In the present study, the BRPI has shown superior digestibility and buffalo meat patties containing BRPI at 20% incorporation level have shown increased available lysine ($P < 0.05$) to rest of the patties (except patties with 30% BRPI) containing isolates at different incorporation levels. This may be due to increased available lysine in BRPI i.e. 9.95% when compared BLPI having 7.77% as reported by Swingler *et al.* (1978). Hence, it may be concluded that patties containing BRPI are better source of available lysine when compared to patties containing BLPI. Meat and patties containing with 20% level of BLPI are nutritionally better in terms of

digestibility and available lysine.

Sensory evaluation scores of buffalo meat patties with incorporation of BLPI and BRPI at different levels

The cooked meat patties incorporated with different levels of lung protein isolate and rumen protein isolate along with control were subjected to sensory evaluation by the semi-trained taste panel members and are presented in Table 4. The sensory evaluation scores for general appearance, flavour, texture, mouth coating, juiciness and overall acceptability of patties incorporated upto 20% level of lung protein isolate and rumen protein isolate were given higher scores and rated as very good to excellent. The patties with 30% levels of protein isolates were given lower scores and rated as fair to good. It was observed that panellists preferred patties incorporated upto 20% level of protein isolates. In addition to it, members have not shown any preference for patties with different levels of lung protein isolate and rumen protein isolates. However, the patties with higher incorporation level i.e 30% of lung protein isolates have got lower scores than patties with rumen protein isolate of same levels in terms of overall acceptability which is supported by general appearance. It may be attributed to the higher amount of residual haem pigment of lung protein isolate incorporated patties resulting in dark brown appearance and lower sensory scores. It may be concluded that buffalo meat patties can be incorporated with these isolates upto 20% replacing lean meat and patties containing with 20% level of BRPI are nutritionally better in terms of digestibility and available lysine.

Table 1: Yield of protein isolates obtained from 500g of buffalo lung and rumen tissues

Batch No.	Lungs		Rumen	
	Yield obtained (g)	Protein recovery in%	Yield obtained (g)	Protein recovery in%
1	190	38.00	155	31.00
2	186	37.20	161	32.20
3	192	38.40	162	32.40
4	180	36.00	157	31.40
5	182	36.40	159	31.80
6	188	37.60	166	33.20
Mean±S.E	186.33±1.892	37.27±0.378	160±1.592	32.00±0.318

Values are Mean±SE of six replicates.

Table 2: Comparative study of proximate composition, physicochemical and microbial quality of buffalo lung and lung Protein isolate with rumen and rumen protein isolate.

Proximate Composition	Lung	BLPI	Rumen	BRPI
Moisture %	77.69±0.16	84.16±0.172	82.50±0.25	80.47±0.368
Protein %	17.50±0.16	12.80±0.19	14.57±0.08	14.32±0.379
Fat%	2.74±0.08	1.01±0.01	2.32±0.02	0.57±0.026
Ash%	0.48±0.01	0.58±0.017	0.43±0.01	0.56±0.028
Collagen%	2.47±0.03	0.22±0.012	2.75±0.02	0.12±0.005
Physico-chemical quality				
pH	6.60±0.03	4.23±0.017	7.12±0.01	4.45±0.033
<i>In vitro</i> digestibility%	—————	63.90±16.2 ^a	—————	79.30±16.0 ^b
Microbial Quality Characteristics				
Standard plate counts (log/g)	5.13±0.008 ^b	4.12±0.01 ^d	6.38±0.018 ^a	4.59±0.029 ^c
Psychrotrophic counts (log/g)	3.54±0.014 ^b	2.27±0.037 ^d	4.79±0.02 ^a	2.69±0.026 ^c
Total Coliforms count	Nil	Nil	Nil	Nil
Total Staphylococcus count	Nil	Nil	Nil	Nil

Values are Mean±SE of six replicates.

Means with different superscripts (row-wise) differ significantly ($P < 0.05$)

BLPI=Buffalo lung protein isolate, BRPI= Buffalo rumen protein isolate.

Table 3: Comparative study on Processing and Nutritional quality characteristics of buffalo meat patties with incorporation of BLPI and BRPI at different levels

Parameters	Levels of incorporation (%)						
	Control	BLPI 10	BLPI 20	BLPI 30	BRPI 10	BRPI 20	BRPI 30
pH of meat emulsion	5.72±0.025	5.68±0.036	5.67±0.046	5.66±0.08	5.64±0.046	5.62±0.031	5.61±0.088
*pH of cooked patties	6.33±0.013	6.29±0.038	6.27±0.04	6.27±0.049	6.28±0.038	6.28±0.038	6.26±0.041
Cooking yield %	87.46±0.022 ^a	88.79±0.17 ^b	89.76±0.078 ^b	89.89±0.997 ^b	88.79±0.112 ^b	89.8±0.309 ^b	89.87±0.467 ^b
Shear force value in kg	0.78±0.02 ^a	0.88±0.02 ^b	0.97±0.03 ^c	1.03±0.02 ^d	0.89±0.02 ^b	0.99±0.03 ^c	1.06±0.02 ^d
Proximate/ Nutrient composition							
Moisture %	63.05±0.009 ^a	63.67±0.005 ^b	63.93±0.01 ^d	64.94±0.009 ^d	63.73±0.027 ^c	63.95±0.01 ^d	64.95±0.004 ^e
Protein %	18.81±0.014 ^b	18.74±0.149 ^b	18.55±0.1 ^b	17.78±0.148 ^a	18.72±0.144 ^b	18.56±0.009 ^b	17.79±0.066 ^a
Ether extract %	13.61±0.078 ^c	13.5±0.083 ^{bc}	13.39±0.061 ^b	12.85±0.013 ^a	13.52±0.007 ^{bc}	13.38±0.01 ^b	12.83±0.025 ^a
Total ash %	2.16±0.10 ^a	2.08±0.43 ^a	2.00±0.09 ^a	1.99±0.11 ^a	2.07±0.12 ^a	2.01±0.10 ^a	1.98±0.08 ^a
Essential amino acid							
Available Lysine %	1.32±0.026 ^a	1.43±0.015 ^{ab}	1.53±0.009 ^b	1.77±0.028 ^{cd}	1.5±0.081 ^b	1.65±0.025 ^c	1.87±0.055 ^d

Values are Mean±SE of six replicates* The mean pH values for cooked patties were significantly higher than those for meat emulsion at all the incorporation levels. Means with different superscripts (row-wise) differ significantly (P<0.05), BLPI= Buffalo lung protein isolate; BRPI=Buffalo rumen protein isolate.

Table 4: Comparative Sensory evaluation scores of buffalo meat patties with incorporation of BLPI and BRPI at different levels

Sensory evaluation Parameters	Levels of incorporation (%)						
	Control	BLPI 10	BLPI20	BLPI30	BRPI10	BRPI20	BRPI30
General appearance	7.05±0.258 ^c	6.67±0.211 ^c	6.83±0.307 ^c	5.17±0.167 ^a	6.83±0.307 ^c	7.00±0.258 ^c	5.85±0.342 ^b
Flavour	7.17±0.167 ^b	7.17±0.307 ^b	6.83±0.307 ^b	5.33±0.422 ^a	7.03±0.258 ^b	7.01±0.447 ^b	5.5±0.224 ^a
Texture	7.18±0.165 ^b	7±0.258 ^b	7±0.258 ^b	5.17±0.307 ^a	6.83±0.654 ^b	7.08±0.258 ^b	5.17±0.307 ^a
Juiciness	7.19±0.167 ^b	6.83±0.167 ^b	7.17±0.167 ^b	5.67±0.211 ^a	6.83±0.401 ^b	7.12±0.30 ^b	5.69±0.333 ^a
Mouth coating	7.17±0.167 ^b	7.00±0.365 ^b	7.08±0.365 ^b	5.67±0.333 ^a	7±0.258 ^b	6.83±0.401 ^b	5.5±0.224 ^a
Overall acceptability	7.19±0.167 ^c	7.09±0.32 ^c	7.00±0 ^c	5.17±0.307 ^a	6.83±0.307 ^c	7±0.258 ^c	5.71±0.211 ^b

Values are Mean±SE of thirty six replicates ; Means with different superscripts (row-wise) differ significantly (P<0.05). BLPI= Buffalo lung protein isolate ; BRPI=Buffalo rumen protein isolate.

Conclusions

Hence recovery and utilization of byproducts proteins as done in above experiments is economical and creates a new avenue of revenue both to the abattoir operator and processed meat manufacturer who use these proteins in meat products apart from solving environmental issues. It also helps in designing cheap and nutritious meat products in our country where malnutrition still exist. Proteins recovered from byproducts had better *in-vitro* digestibility, microbial quality and good functional properties. Patties prepared by using them showed nutritional advantages like increased availability of lysine, better texture, cooking yield.

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