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## Utilization of recovered protein isolates from rumen of slaughtered buffalo in buffalo meat patties

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### Abstract

Protein isolates were recovered from the minced rumen tissue by improved alkaline extraction followed by isoelectrical precipitation using acid. Physico-chemical properties, proximate principles, collagen content, *in vitro* pepsin digestibility and microbial profile of rumen and rumen protein isolates were determined. Microbial qualities of both rumen and derived isolates are studied. Buffalo meat patties were prepared by replacing lean meat at 0, 10 and 20% by rumen protein 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 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 protein isolate were given higher scores and rated as very good to excellent.

**Keywords:** Buffalo 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 rumen of slaughtered buffalo, subject it to the process of hydrolysis and incorporate in buffalo meat patties.

## Materials and methods

### Collection of rumen

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 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 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 rumen protein isolates

(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 rumen protein isolates (BRPI) and patties as well. The *in vitro* pepsin digestibility of the buffalo rumen protein isolates (BRPI) was performed as per the standard method AOAC (1995) [1] with slight modifications as per ICONTEC (1994) [13]. The microbiological quality of raw rumen and their 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 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 rumen is presented in Table 1. The percent recoveries from rumen were in accordance with Swingler and Lawrie (1979) [25], Darine Selmane *et al.* (2010) [8] and Babu *et al.* (1993) [3]. They also used the same method of alkaline extraction to recover proteins from bovine rumen and from ovine rumen respectively.

### Physico-chemical composition of protein isolates from buffalo rumen

The proximate composition of rumen and derived protein isolates including physico chemical characteristics, collagen, pH, *in-vitro* digestibility and microbial load are presented in Table 2. This proximate composition of rumen values are in

agreement with Gault and Lawrie (1980) <sup>[11]</sup> for bovine rumen. The pH of BRPI and collagen content was comparatively less in isolates than raw tissues. They are in agreement with the values reported by Swingler and Lawrie (1979) <sup>[25]</sup> for protein isolates from bovine rumen. In the present study, rumen protein isolate found to have  $0.12 \pm 0.005$  percent collagen which was similar to the values reported for bovine rumen protein isolates reported by Swingler and Lawrie (1979) <sup>[25]</sup>.

In-vitro digestibility % of BRPI was found to be  $(79.30 \pm 16.0)$  and these values are in agreement with Song *et al.* (1984) <sup>[24]</sup> who reported similar in-vitro pepsin digestibility values for protein isolate from swine lung and stomach.

### Microbial quality

The microbial profiles of rumen and protein isolates are presented in Table 2. The protein isolates i.e. BRPI (Buffalo Rumen Protein Isolate) recorded significantly ( $p < 0.05$ ) lower Standard Plate Count (SPC) and Psychrotrophic counts (PTC) than that of raw tissues as presented. 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 and these results were in close agreement with the findings of Swingler *et al.* (1979) <sup>[25]</sup> and Song *et al.* (1984) <sup>[24]</sup> for bovine rumen and Babu *et al.* (1993) <sup>[3]</sup> for ovine 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 were are in agreement with the values reported by Swingler *et al.* (1979) <sup>[25]</sup> for bovine rumen and Babu *et al.* (1993) <sup>[3]</sup> for ovine rumen. The higher microbial load of raw rumen 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 BRPI at different levels

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

#### 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) <sup>[6]</sup>, Fogg and Harrison (1975) <sup>[9]</sup> in beef, Kesava Rao and Kowale (1988) <sup>[17]</sup> in buffalo meat patties, Babu *et al.* (1993) <sup>[3]</sup> in mutton patties incorporated with by-products, Jelen *et al.* (1982) <sup>[15]</sup> in luncheon meat incorporated with alkali extracted chicken protein, Boles *et al.* (2000) <sup>[5]</sup> 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) <sup>[12]</sup> and Bouton *et al.* (1971) <sup>[6]</sup>.

No significant difference was recorded in pH of emulsions with different levels of incorporation of BRPI. The mean pH of cooked buffalo meat patties increased significantly ( $p < 0.05$ ) higher than those for meat emulsion at all incorporation levels.

### Cooking yield

Cooking yield of all patties incorporated with different levels of incorporation of BRPI increased significantly ( $P < 0.05$ ) than control. 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) <sup>[26]</sup>, Perera and Anglemier (1980) <sup>[22]</sup> and Darine Selmane *et al.*, ((2010) <sup>[8]</sup>) 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 ( $P < 0.05$ ) 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) <sup>[3]</sup> while working with incorporation by-products in chevon patties. The shear force values increased significantly ( $p < 0.05$ ) with increased levels of bovine rumen protein isolate (BRPI) leading to better emulsion stability and texture of patties.

### Nutrient composition of buffalo meat patties with incorporation of BRPI at different levels

Nutrient composition of buffalo meat patties with the incorporation of 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) <sup>[18]</sup>, Babu *et al.* (1993) <sup>[3]</sup>, Jelen *et al.* (1982) <sup>[15]</sup> who incorporated alkali extracted by-product proteins in emulsion based meat products.

### Available lysine

Incorporation of BRPI at 10, 20 and 30% levels recorded significantly higher level of lysine ( $p < 0.05$ ) when compared to control. There was an increasing trend in availability of lysine when incorporation of 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% as reported by Swingler *et al.* (1978) [25]. Hence, it may be concluded that patties containing BRPI are better source of available lysine.

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

The mean scores of sensory attributes of buffalo meat patties with different levels of BLPI and BRPI are presented in Table 4.

### General appearance

The general appearance of patties with 30% level BRPI incorporation was significantly ( $p < 0.05$ ) lower than that of other levels. The patties with 30% incorporation levels of BRPI recorded significantly ( $p < 0.05$ ) lower scores as compared to control. BRPI when incorporated up to 20% levels, there was a gradual, though not statistically significant decrease in scores as compared to control.

### Flavour

The 30% incorporation levels of BRPI recorded significantly ( $p < 0.05$ ) lower scores than the control. BRPI when incorporated upto 20% level, there was a gradual decrease in the flavour scores when compared to control, but was not significant statistically.

### Texture

However, 30% incorporation levels of BRPI recorded significantly lower scores ( $p < 0.05$ ) compared to control. There was a gradual, though not statistically significant decrease in the textural scores than control, when BRPI was incorporated upto 20% level.

### Juiciness

BRPI when incorporated upto 20% level, there was a gradual decrease in juiciness scores as compared to control which was not significant statistically. However, 30% incorporation levels of BRPI recorded significantly ( $p < 0.05$ ) lower scores when compared to control.

### Mouth coating

BRPI when incorporated upto 20% level, there was a gradual decrease in mouth coating scores when compared to control which was not significant statistically. However, 30%

incorporation levels of BRPI recorded significantly ( $p < 0.05$ ) lower scores when compared to control.

### Overall acceptability

BRPI when incorporated upto 20% level, there was a gradual decrease in overall acceptability scores which was not significant statistically. However, patties with 30% level of BRPI recorded significantly ( $p < 0.05$ ) lower scores when compared to control.

The cooked meat patties incorporated with different levels of rumen protein isolate along with control were subjected to sensory evaluation by the semi-trained taste panel members.

The sensory evaluation scores for general appearance, flavour, texture, mouth coating, juiciness and overall acceptability of patties incorporated upto 20% level of 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 panelists preferred patties incorporated upto 20% level of protein isolates. 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.	Rumen	
	Yield obtained (g)	Protein recovery in %
1	155	31.00
2	161	32.20
3	162	32.40
4	157	31.40
5	159	31.80
6	166	33.20
Mean±S.E	160 ± 1.592	32.00 ± 0.318

Values are Mean ± SE of six replicates.

**Table 2:** Proximate composition, physicochemical and microbial quality of buffalo rumen and Rumen Protein isolate

Proximate Composition	Rumen	BRPI
Moisture %	82.50 ± 0.25	80.47 ± 0.368
Protein %	14.57 ± 0.08	14.32 ± 0.379
Fat%	2.32 ± 0.02	0.57 ± 0.026
Ash%	0.43 ± 0.01	0.56 ± 0.028
Collagen%	2.75 ± 0.02	0.12 ± 0.005
Physico-chemical quality		
pH	7.12 ± 0.01	4.45 ± 0.033
Microbial Quality Characteristics		
Standard plate counts (log/g)	6.38 ± 0.018 <sup>a</sup>	4.59 ± 0.029 <sup>b</sup>
Psychrotrophic counts (log/g)	4.79 ± 0.02 <sup>a</sup>	2.69 ± 0.026 <sup>b</sup>
Total Coliforms count	Nil	Nil
Total Staphylococcus count	Nil	Nil

Values are Mean ± SE of six replicates.

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

BRPI=Buffalo rumen protein isolate.



**Table 3:** Processing quality characteristics of buffalo meat patties with incorporation of BLPI and BRPI at different levels

Levels of incorporation (%)				
Processing quality characteristics				
Parameters	Control	BRPI 10	BRPI 20	BRPI 30
pH of meat emulsion	5.72 ± 0.025	5.64 ± 0.046	5.62 ± 0.031	5.61 ± 0.088
*pH of cooked patties	6.33 ± 0.013	6.28 ± 0.038	6.28 ± 0.038	6.26 ± 0.041
Cooking yield %	87.46 ± 0.022 <sup>a</sup>	88.79 ± 0.112 <sup>b</sup>	89.8 ± 0.309 <sup>b</sup>	89.87 ± 0.467 <sup>b</sup>
Shear force value in kg	0.78 ± 0.02 <sup>a</sup>	0.89 ± 0.02 <sup>b</sup>	0.99 ± 0.03 <sup>c</sup>	1.06 ± 0.02 <sup>d</sup>
Nutrient-composition				
Moisture %	63.05 ± 0.009 <sup>a</sup>	63.73 ± 0.027 <sup>b</sup>	63.95 ± 0.01 <sup>c</sup>	64.95 ± 0.004 <sup>d</sup>
Protein %	18.81 ± 0.014 <sup>b</sup>	18.72 ± 0.144 <sup>b</sup>	18.56 ± 0.009 <sup>b</sup>	17.79 ± 0.066 <sup>a</sup>
Ether extract %	13.61 ± 0.078 <sup>c</sup>	13.52 ± 0.007 <sup>bc</sup>	13.38 ± 0.01 <sup>b</sup>	12.83 ± 0.025 <sup>a</sup>
Total ash %	2.16 ± 0.10 <sup>a</sup>	2.07 ± 0.12 <sup>a</sup>	2.01 ± 0.10 <sup>a</sup>	1.98 ± 0.08 <sup>a</sup>
Available Lysine %	1.32 ± 0.026 <sup>a</sup>	1.5 ± 0.081 <sup>b</sup>	1.65 ± 0.025 <sup>c</sup>	1.87 ± 0.055 <sup>d</sup>
Essential amino acid				
Available Lysine %	1.32 ± 0.026 <sup>a</sup>	1.5 ± 0.081 <sup>b</sup>	1.65 ± 0.025 <sup>c</sup>	1.87 ± 0.055 <sup>d</sup>

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 ( $p < 0.05$ ), BRPI=Buffalo rumen protein isolate.

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

Sensory Evaluation Parameters	Level of incorporation (%)			
	Control	BRPI10	BRPI20	BRPI30
General appearance	7.05 ± 0.258 <sup>b</sup>	6.83 ± 0.307 <sup>b</sup>	7.00 ± 0.258 <sup>b</sup>	5.85 ± 0.342 <sup>a</sup>
Flavour	7.17 ± 0.167 <sup>b</sup>	7.03 ± 0.258 <sup>b</sup>	7.01 ± 0.447 <sup>b</sup>	5.5 ± 0.224 <sup>a</sup>
Texture	7.18 ± 0.165 <sup>b</sup>	6.83 ± 0.654 <sup>b</sup>	7.08 ± 0.258 <sup>b</sup>	5.17 ± 0.307 <sup>a</sup>
Juiciness	7.19 ± 0.167 <sup>b</sup>	6.83 ± 0.401 <sup>b</sup>	7.12 ± 0.30 <sup>b</sup>	5.69 ± 0.333 <sup>a</sup>
Mouth coating	7.17 ± 0.167 <sup>b</sup>	7 ± 0.258 <sup>b</sup>	6.83 ± 0.401 <sup>b</sup>	5.5 ± 0.224 <sup>a</sup>
Overall acceptability	7.19 ± 0.167 <sup>b</sup>	6.83 ± 0.307 <sup>b</sup>	7 ± 0.258 <sup>b</sup>	5.71 ± 0.211 <sup>a</sup>

Values are Mean ± SE of six replicates; Means with different superscripts (row-wise) differ significantly ( $p < 0.05$ ). 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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