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Utilization of recovered protein isolates from lung of slaughtered buffalo in buffalo meat patties

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Abstract
Protein isolates were recovered from the minced lung 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 lung and lung protein isolates were determined. Microbial qualities of both lung and derived isolates are studied. Buffalo meat patties were prepared by replacing lean meat at 0, 10, and 20% by lung 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 lung protein isolate has shown superior digestibility and buffalo meat patties containing 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 lung protein isolate were given higher scores and rated as very good to excellent.

Keywords: Buffalo lung 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 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).

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) 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 mix1%, 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 protein isolates (BLPI) 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 patties as well. The *in vitro* pepsin digestibility of the buffalo lung protein isolates (BLPI) was performed as per the standard method AOAC (1995) [1] with slight modifications as per ICONTEC (1994) [13]. The microbiological quality of raw lung 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 lung protein isolates (BLPI) 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 is presented are in Table1. The percent recoveries from lung 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 lung and from ovine lung respectively.

Physico-chemical composition of protein isolates from buffalo rumen

The proximate composition of lung 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) [11] for bovine lung. The pH of BLPI and collagen content was comparatively less in isolates than raw tissues. They are in agreement with the values reported by Swingler and Lawrie (1979) [25] for protein isolates from bovine lung. In the present study, lung protein isolate found to have 0.22±0.012 percent collagen which was similar to the values reported for bovine lung protein isolates reported by Swingler and Lawrie (1979 [25] and Babu *et al.* (1993) [3].

In-vitro digestibility % of BLPI was found to be (63.90±16.2) and 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 stomach.

Microbial quality

The microbial profiles of rumen and protein isolates are presented in Table 2. The protein isolates i.e BLPI (Buffalo Lung 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 respiratory 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) [25] and Song *et al.* (1984) [24] for bovine lung and Babu *et al.* (1993) [3] 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 lung were are in agreement with the values reported by Swingler *et al.* (1979) [25] for bovine lung and Babu *et al.* (1993) [3] for ovine lung. The higher microbial load of raw rumen might be due to contribution of microbial population from lung 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 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) [6], Fogg and Harrison (1975) [9] in beef, Kesava Rao and Kowale (1988) [17] in buffalo meat patties, 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].

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) [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 ($P<0.05$) 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) [3] while working with incorporation by-products in chevon patties. The shear force values increased significantly ($P<0.05$) with increased levels of bovine lung protein isolate (BLPI) leading to better emulsion stability and texture of patties.

Nutrient composition of buffalo meat patties with incorporation of BLPI at different levels.

Nutrient composition of buffalo meat patties with the incorporation of BLPI at different levels are presented in Table 3.The moisture content of patties gradually increased significantly while protein content and ether extract decreased with increase in incorporation levels of lung protein isolate from 0 to 30%, non significantly upto 20% level and significantly at 30% level in comparison to control. 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

Incorporation of BLPI 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 BLPI 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 BLPI has shown superior digestibility and buffalo meat patties containing BLPI 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 BLPI i.e. 9.85% as

reported by Swingler *et al.* (1978) [25]. Hence, it may be concluded that patties containing BLPI are better source of available lysine.

Sensory evaluation scores of buffalo meat patties with incorporation of BLPI 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 BLPI incorporation was significantly ($P<0.05$) lower than that of other levels. The patties with 30% incorporation levels of BLPI recorded significantly ($P<0.05$) lower scores as compared to control. BLPI 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 BLPI recorded significantly ($P<0.05$) lower scores than the control. BLPI 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 BLPI 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 BLPI was incorporated upto 20% level.

Juiciness

BLPI 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 BLPI recorded significantly ($P<0.05$) lower scores when compared to control.

Mouth coating

BLPI 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 BLPI recorded significantly ($P<0.05$) lower scores when compared to control.

Overall acceptability

BLPI 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 BLPI recorded significantly ($P<0.05$) lower scores when compared to control.

The cooked meat patties incorporated with different levels of lung 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 lung 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 BLPI are nutritionally better in terms of digestibility and available lysine.

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.

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

	Lung	
Batch No.	**Yield obtained (g)**	**Protein recovery in%**
1	190	38.00
2	186	37.20
3	192	38.40
4	180	36.00
5	182	36.40
6	188	37.60
Mean± S.E	186.33±1.892	37.27±0.378

Values are Mean±SE of six replicates.

Table 2: Proximate composition, physicochemical and microbial quality of buffalo lung and lung Protein isolate

Proximate Composition	**Lung**	**BLPI**
Moisture %	77.69±0.16	84.16±0.172
Protein %	17.50±0.16	12.80±0.19
Fat%	2.74±0.08	1.01±0.01
Ash%	0.48±0.01	0.58±0.017
Collagen%	2.47±0.03	0.22±0.012
Physico-chemical quality		
pH	6.60±0.03	4.23±0.017
In vitro digestibility%	_____	63.90±16.2
Microbial Quality Characteristics		
Standard plate counts (log/g)	5.13 ± 0.008^{a}	4.12 ± 0.01^{b}
Psychrotrophic counts (log/g)	3.54 ± 0.014^{a}	2.27 ± 0.037^{b}
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 ($P<0.05$)

BLPI=Buffalo lung protein isolate.

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

Levels of incorporation (%)				
Processing quality characteristics				
Parameters	Control	BLPI 10	BLPI 20	BLPI 30
pH of meat emulsion	5.72±0.025	5.68±0.036	5.67±0.046	5.66±0.08
*pH of cooked patties	6.33±0.013	6.29±0.038	6.27±0.04	6.27±0.049
Cooking yield %	87.46±0.022 ^{a}	88.79±0.17 ^{b}	89.76±0.078^{b}	89.89±0.997^{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}
Nutrient- composition				
Moisture %	63.05±0.009 ^{a}	63.67±0.005^{b}	63.93±0.01^{c}	64.94±0.009^{d}
Protein %	18.81±0.014 ^{b}	18.74±0.149 ^{b}	18.55±0.1 ^{b}	17.78±0.148 ^{a}
Ether extract %	13.61±0.078 ^{c}	13.5±0.083 ^{bc}	13.39±0.061 ^{b}	12.85±0.013^{a}
Total ash %	2.16±0.10 ^{a}	2.08±0.43 ^{a}	2.00±0.09 ^{a}	1.99±0.11 ^{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 ^{c}

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.

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

Sensory Evaluation Parameters	Level of incorporation (%)			
	Control	**BLPI10**	**BLPI20**	**BLPI30**
General appearance	7.05±0.258 ^{b}	6.77±0.211 ^{c}	6.83±0.307^{bc}	5.17±0.167 ^{a}
Flavour	7.17±0.167 ^{b}	7.17±0.307 ^{b}	6.83±0.307 ^{b}	5.33±0.422 ^{a}
Texture	7.18±0.165 ^{b}	7.00±0.258 ^{b}	7.11±0.348 ^{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}
Mouth coating	7.17±0.167 ^{b}	7.00±0.365 ^{b}	7.08±0.365 ^{b}	5.67±0.333 ^{a}
Overall acceptability	7.15±0.167 ^{b}	7.11±0.32 ^{b}	7.09±0.03 ^{b}	5.17±0.307 ^{a}

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

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