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Research Paper

Utilization of Buffalo Hydrolysed Fascia in Buffalo Meat Patties

JagannathaRao. B, Kondal Reddy. K, Basawaraj Awati, Kulkarni. V. V and Madhava Rao. T

Department of Livestock Products Technology, Veterinary College,KVAFSU, Bidar-585401

ABSTRACT

Epimysial fascia was collected from buffalo carcasses under hygienic conditions from organised slaughterhouse, was cut into thin slices, defatted,further subjected to mild drying and finally powdered in a hammer mill. The powdered fascia was subjected to enzymatic hydrolysis by suspending them in mild 0.1 N hydrochloric acid solution containing 0.1% pepsin (w/w of fascia) of1:10,000 strength for 8 hours at 37°C.The concentration of 0.1 % of pepsin used for this study was decided based on degree of hydrolysis. Physico-chemical properties like proximate principles, collagen contentand in- vitro pepsin digestibility of hydrolysed fascia weredetermined. Buffalo meat patties were prepared by replacing lean meat at 0, 10, and 20%. Replacement of lean with hydrolysed fascia did not affect proximate composition of patties except proteins. Amongst processing qualities though pH increased, the emulsion stability, shear force, cooking yield lowered.Nutritional qualities like available lysine,collagen increased significantly whereas calculated PER decreased but remained well above universally accepted index of 2.5.Texture and sensory attributes were also significantly affected but remained good to very good upto a level of 20%. Results from this study indicated that meat could be replaced up to 20% withhydrolysed fascia in patties formulations without affecting sensory qualities along with nutritional gain.

Keywords : *Hydrolysed fascia, Buffalo meat, Patties, Quality*

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INTRODUCTION

After addressing the calorie hunger, there is immense need to supply animal proteins at reduced cost in the diet of common mass to fulfil the dietary requirement of proteins in developing countries like India. To be sustainable, meat industry is under constant pressure to improve the utilization of by-products from its slaughter operations. Though, some of the meat by-productsare used in feed and fertilizer formulations, but profits from these outlets are marginal (Caldironi and Ockerman, 1982).

At the same time, modern consumers are no longer satisfied with the traditional meat products. Rapid urbanization and change in life style have increased demand for more nutritious and ready to eat products. Novel processing technique for meat resulted in the production of meat products which are tasty and convenient with superior sensory qualities. However, high cost of these products makes it difficult for an average consumer in developing countries to use these products regularly in their diet (Malav*et al*. 2013). Therefore development of technology which aims at designing low cost meat products along with utilization of abattoir waste like fascia may solve the problem of environmental pollution also.

In this direction, fascia is one of the under - utilized byproduct from slaughter animals, and has good amount of protein. In most of the slaughterhouses in our country, fascia (epimysial collagen) is discarded as waste and amounts to 3Kg/ buffalo slaughtered as per Kondaiah*et al.* (1983). Fascia is composed of 70 percent water with proteins and proteoglycans. The two proteins of fascia are collagen and elastin. The proteoglycans associated with fascia are glucosamine and chondroitin and are used widely now a days as nutritional supplements for arthritis. The major protein of fascia is collagen. Collagen has been found to be bacteriologically safe for human consumption with bland taste and flavour, presently being used in food, medicine, cosmetics and biomedical materials (Vedamurthy and Rao, 2010). Meat containing raw material added with collagen or its fractions could enhance its technological and rheological properties (Rao and Henrickson, 1983). Fascia recovered from epimysial region of the carcass can serve as potential protein source. Hydrolysed fascia hypothesised to be good in terms of digestibility, lysine availability and protein efficiency ratio.Reports are available on various uses of collagen in different food products including meat products (Ranganayaki 1982; Rao and Henrickson 1983; Charvez*et al.*, 1985).

But literature on utilization of hydrolysed buffalo fascia in meat food products is scanty. Hence, an attempt was made to recover fascia from slaughtered buffalo, subject it to the process of hydrolysis and incorporate in buffalo meat patties.

*Corresponding author E-mail: dr.jagannathrao@gmail.com

MATERIALS AND METHODS

Collection of fascia: Epimysial fascia was collected from buffalo carcasses under hygienic conditions and stored in deep freeze (-18^{0}C) till further use.

Preparation of fascia: Frozen fascia after thawing was cut into thin slices of 1-1.5 mm thickness in a slicer and defatted with ten volumes of ethanol with continous stirring for 6 hours and subjected to mild drying and finally powdered in a hammer mill.

Hydrolysis of fascia: Based on degree of hydrolysis, the powdered fascia was subjected to enzymatic hydrolysis by suspending them in mild 0.1 N hydrochloric acid solution containing 0.1% pepsin (w/w of fascia) of 1:10,000 units strength for 8 hours at 37^{0}C in an incubator.

Degree of hydrolysis: Powdered fascia was subjected to three different concentrations of pepsin (1:10,000) viz. 0.1%, 0.5% and 1% and degree of hydrolysis is ascertained at every 2 hours.Degree of hydrolysis was evaluatedaccording to percent of trichloroacetic acid ratio method as described by Hoyle and Merritt (1994) and Fonkwe and Singh (1995). At every 2 hours of hydrolysis, 20 ml of hydrolysate was added to 20 ml of 20% (w/v) trichloroaceticacid to produce 10% trichloroacetic acid (TCA) soluble material. The mixtures were left to stand for 30 min to allow precipitation followed by centrifugation at 8000 rpm for 15 minutes. The supernatant was analysed for soluble nitrogen by Kjeldhal method (AOAC, 1995). Samples from the hydrolysate was also analysed for protein content. Degree of hydrolysis (DH) was calculated using the formula below:

$$\% \text{ DH} = \frac{\text{Soluble N in TCA 10\% (W/V)}}{\text{Total N in the sample}} \times 100$$ where N= nitrogen.

Recipe of patties: Deboned buffalo meat was packed in clean polyethylene bags and frozen at -20^{0}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 hydrolysed fascia 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 ^{0}C to obtain an internal temperature of about 75^{0}C.Six such trials were conducted for each level of incorporation.

Analysis of sample: Proximate composition was determined according to AOAC (1995) methods. The *in vitro* pepsin digestibility of the hydrolysed fascia was performed as per the standard method (AOAC., 1995) with slight modifications as per (ICONTEC, 1994).

The pH of raw emulsion as well as cooked patties was determined by the method of AOAC (1995) using pH meter. Emulsion stability and percent cooking yield were determined by the method of Baliga and Madiah(1970) with slight modifications. Amount of collagen in hydrolysed fascia and meat patties was calculated by estimating hydroxyproline content according to the procedure of Neuman and Logan (1950).

Available lysine content of patties was determined by the method of Carpenter (1960). Calculated protein efficiency ratio (PER) of patties was calculated by adopting the procedure of Lee *et al.* (1978) who by using varying amounts of collagen in the meat product bioassay, computed the following linear regression equation to estimate the PER of meat product from collagen content,

$$PER = -0.229 (\text{Collagen \%}) + 3.1528.$$

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). Data obtained were analysed statistically as per the method outlined by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Degree of hydrolysis: An attempt has been made to use hydrolysed fascia in meat product by subjecting fascia to a minimum concentration of pepsin over a period of time, so that the product quality and nutritive value is enhanced. For enzymatic hydrolysis, pepsin was used as the proteolytic enzyme to breakdown milled fascia resulting in both soluble and insoluble fraction. From meat processing point of view, the soluble fraction contains the hydrolyzed proteins that can be converted and incorporated with other main components while processing meat products. The degree of hydrolysis (DH) was determined and the results were shown in Table 1. As expected that DH increased with increasing reaction times

(P<0.05). The 8 hours of reaction time gave DH in the range of 25.17 -27.33 % yet there were no significant differences in DH among the enzyme concentrations. Hence, pepsin (1:10000) at lowest possible concentration of 0.1% is used for hydrolysing fascia.

Table 1: Degree of hydrolysis of fascia by pepsin at different concentration and time

	Conc. of pepsin enzyme(1:10000)		
Reaction time in hrs	**0.1%**	**0.5%**	**1.0 %**
2	10.5 ± 0.619[a]	10.60 ± 0.447[a]	15.00 ± 0.447[b]
4	15.30 ± 0.667[a]	16.75 ± 0.833[a]	18.10 ± 0.447[b]
6	20.17 ± 0.543[a]	19.22 ± 0.422[a]	22.00 ± 1.633[a]
8	25.17 ± 1.108[a]	27.33 ± 1.109[a]	26.67 ± 0.843[a]

Physico-chemical composition of hydrolysed fascia: The physico-chemical composition of hydrolysed fascia is presented in Table 2.The similar observations with respect to protein % and collagen content of hydrolysed fascia were made by the authors of the committee on health aspect of collagen as a food ingredient (FDA, 1981), particularly with native hide collagen from slaughtered cattle.

Table 2: Physico-chemical composition of hydrolysed fascia

Proximate	**Composition**	**Hydrolysed fascia**
	Wet basis	Dry basis
Moisture %	67.50 ± 0.33	_____
Protein %	29.94 ± 0.24	89.77 ± 0.175
Fat%	0.61 ± 0.065	_____
Ash%	0.70 ± 0.04	_____
Collagen(mg/g)	89.58 ± 0.068	
pH	4.58 ± 0.085	
Nutritional quality In- vitro pepsin digestibility %	70.00 ± 16.56	

Means with different superscripts in a row differ significantly (P<0.05). (n=6)

Processing quality characteristics of patties:

The processing quality, proximate composition and nutritional characteristics of patties are presented in Table 3. Following are processing qualities.

***Emulsion stability*:** The emulsion stability decreased significantly (P<0.05) with the incorporation of hydrolysed fascia in this study might be attributed to loss of binding characteristics due to enzymatic hydrolysis of the fascia. Enzymatic hydrolysis resulted in the formation of low molecular weight components and peptides which do not possess any emulsification properties as opined by Cronlund and Woychik (1987), Bailey and Light (1988) as evidenced by degree of hydrolysis.

pH: The pH of cooked patties increased significantly(P<0.05) with the increasing addition of hydrolysed fascia in buffalo meat patties.Similar increase in pH values in bologna incorporated with hide collagen was reported by Rao and Herickson (1983). Collagen molecule constitute about 40% polar amino acid residues of which 11% are basic and 9% acidic and about 17% are hydroxyl amino acids as opined by Cassel and Mckenna (1953). This higher proportion of basic amino acids in collagen might be responsible for the observed increase in the pH of patties incorporated with the hydrolysed fascia.

Shear force value: Measuring the force in shearing is one of measure for tenderness. In the present study significantlylower shear values of the patties with hydrolysed fascia might be attributed to the disintegration of structure of the native collagen after enzymatic hydrolysis and similar results are obtained by Reddy *et al.*(1998).

Cooking yield: The results indicated that the cooking yield decreased (P<0.05) significantly when patties were incorporated with hydrolysed fascia that might be due to the higher moisture content of hydrolysate as well as loss of functional properties by way of formation of low molecular weight compounds on hydrolysis.

Proximate composition of patties: Amongst all groups, the moisture content increased significantly(P<0.05) where as the protein content of patties decreased significantly(P<0.05) in patties containing 20 % hydrolysed fascia. The crude fat content of patties did not vary with the incorporation of hydrolysed fascia. No change in moisture and protein contents of patties incorporated with hydrolysed fascia at 10 % level was observed when compared to control. It was in agreement with findings of Rao and Henrickson (1983)and Reddy *et al.*(1998). The decrease in protein content of patties with 20% hydrolysed fascia in the present study might be due to relatively higher moisture content contributed by 0.1 N Hydrochloric acid added for enzymatic hydrolysis during preparation of hydrolysed fascia.

Nutritional characteristics of patties incorporated with fascia

***Collagen*:** It increased significantly (P<0.05) with increase in the incorporation of hydrolysed fascia. In the present study

Table 3: Processing quality, proximate composition and nutritional characteristics of cooked buffalo meat patties incorporated with different levels of hydrolysed fascia.

	Processing quality		
Levels of incorporation (%)	**0**	**10**	**20**
Emulsion stability(%)	93.66 ± 0.45[a]	91.61 ± 0.37 [b]	89.33 ± 0.40 [c]
pH	6.35 ± 0.010[c]	6.47 ± 0.005[b]	6.52 ± 0.006[a]
Shear force value (Kg)	0.76 ± 0.009 [a]	0.62 ± 0.017[b]	0.41 ± 0.018[c]
	Proximate composition		
Moisture(%)	61.04 ± 0.246[b]	60.73 ± 0.147[b]	63.11 ± 0.101[a]
Protein (%)	17.5 ± 0.502 [a]	18.4 ± 0.238[a]	14.08 ± 0.327[b]
Ether extract (%)	17.61 ± 0.218	17.73 ± 0.307	17.68 ± 0.246
	Nutritional characteristics		
Collagen (mg/g)	17.38 ± 0.462 [c]	22.34 ± 0.437 [b]	30.39 ± 0.2 [a]
Protein efficiency ratio	3.11 ± 0.005[a]	3.1 ± 0.001 [b]	3.07 ± 0.001 [c]
Available lysine (%)	1.32 ± 0.013[c]	1.63 ± 0.01[b]	1.79 ± 0.008[a]

Means with different superscripts in a row differ significantly (P<0.05).(n=6)

increased collagen content was observed in treatmentgroups compared to control was in agreement with the findings of Strange and Whiting (1990) and Reddy *et al. (*1998). Strange and Whiting (1990) reported 2.5% of collagen in the restructured beef steaks incorporated with epimysium at 10% level. In the present study also the collagen content was similar to that reported by the above workers.

***Calculated protein efficiency ratio*:** It is evident that the calculated protein efficiency ratio (PER) of patties incorporated with different levels of fascia showed significant decrease (P<0.05) from control. However, the calculated PER of patties with 20 % hydrolysed fascia was significantly lower compared to the groups with 10 % hydrolysed fascia. Protein efficiency ratio values reflect both the level of essential amino acid in a protein or proteinaceous food as well as bioavailability of those amino acids. In USDA (1989), a minimum PER of 2.5 have been specified for most fabricated foods. Lee *et al*. (1978) reported that a PER of 2.5 correspond to 28.5% of collagen in the test product and increasing the collagen content of meat products from 10 to 30% reduced it from 3 to 2.5.Collagen alone is a poor protein but has supplementary nutritional value when added in meat products. Vaughn *et al.*(1979) reported a PER value of 0.6 for pigs ear (70% collagen) while Happich*et al.* (1975) reported a PER value less than zero for cattle hide (80% collagen) increasing to a PER value of 1.1 when only 50% collagen was present. Erbersdobler*et al.* (1970) reported that collagen if used with balanced proteins and within limits, did not decrease the nutritive value of the diet. However, Kofranyi and Jekat (1969) reported improved biological value of meat containing 15-20% collagen.

In the present study the calculated PER values for all the groups of patties ranged from 3.07 to 3.11. These values are more than the minimum PER limit of 2.5 as specified by Lee *et al.* (1978). The collagen content of different patties ranged from 1.73% in control to 3.03 % in patties with 20 % hydrolysed fascia. The patties with 10% fascia corresponded to 2.23% collagen while that with 20 % hydrolysed fascia contained 3.03 % collagen. These observations were almost in accordance with those of Strange and Whiting (1990) who reported 2.5% of collagen content in restructured beef steaks incorporated with 10 % epimysium. The reason for higher PER values recorded in the present study might be attributed to lower contents of collagen of the product than the maximum level i.e. 28.5 % collagen for a minimum PER of 2.5 reported by Lee *et al.* (1978). In this study, patties incorporated with hydrolysed fascia recorded lower PER values than the patties with control and might be correlated with the higher content of collagen. From the above discussion it is evident that incorporation of hydrolysed fascia up to 20 % level did not have any adverse effect on PER, rather it was well above cut off value of 2.5 and had increased availability of lysine.

Available lysine: The available lysine content of patties incorporated with hydrolysed fascia at different levels were

not only different from that of control but also differed significantly (P<0.05) from one another and increased with simultaneous increase in the amount of hydrolysed fascia incorporated. Lysine is one of essential amino acids and its amount in plasma proteins is taken as a criterion of the biological value of food. Lysine and threonine are the limiting amino acids in cereal foods. Collagen has lysine in fair quantity when compared to native muscle and other proteins of food animal. However, meat proteins containing 15 to 20% collagen have a considerable excess of these amino acids as supplementary effect of collagen is well appreciated (Rogov *et al.*, 1992). As the collagen is converted into gelatin on cooking it becomes more digestible and nutritionally more advantageous. In the present study patties incorporated with hydrolysed fascia showed increase in the amount of available lysine. This was in agreement with the findings of Dvorak and Vognarov (1965) who reported increase in the content of available lysine in canned meat due to gelatinization of collagen.

The amino acid lysine is present in the form of hydroxylysine residues are distributed along the peptide chains are known to be involved formation of intermolecular cross links. At cooking temperatures, collagen is solubilised and reduced to small fragments by heat as per Bailey and Light (1988). In the light of above explanation the observed increase in the available lysine content of patties containing hydrolysed fascia might be attributed to the hydrolysis of fascia resulting in the release of bound lysine by cleavage of collagen cross links during cooking. FDA has already approved the use of collagen up to 15% in meat blocks and claims no disadvantages of using it to such an extent.

Sensory evaluation scores of buffalo meat patties with incorporation of hydrolysed fascia at different levels: The mean scores of sensory parameters of buffalo meat patties with different levels of fascia are presented in Table 4.

General appearance: There was a decrease in general appearance scores as compared to control, when hydrolysed fascia was incorporated in patties. The patties with 20% hydrolysed fascia scored significantly lower score amongst all groups. Inspite of significant differences in the general appearance scores, all the groups scored very good on subjective scale.

Flavour: The patties with 20% hydrolysed fascia scored significantly lower score amongst all groups. All the groups scored very good on subjective scale. Lower flavour scores observed for patties incorporated with higher levels of hydrolysed fascia were in agreement with the findings of Jones (1984) and Charvez*et al.*(1985) and might be attributed to the bland flavour of collagen as suggested by the above workers.

Table 4: Sensory scores of buffalo meat patties with incorporation of hydrolysed fascia at different levels.

	Processing quality		
Levels of incorporation (%)	**0**	**10**	**20**
Appearance	6.88 + 0.09[a]	6.78+ 0.07[a]	6.23+ 0.09[b]
Flavour	6.75 + 0.09[a]	6.67+ 0.06[a]	6.09+ 0.04[b]
Juiciness	6.64+ 0.06[c]	7.05+ 0.07[b]	7.19+ 0.07[a]
Texture	6.62 + 0.09 [a]	6.39+ 0.07[b]	5.82+ 0.08[c]
Mouth coating	7.16+ 0.10	7.08+ 0.10	7.27+ 0.06
Overall acceptability	6.50+ 0.07[a]	6.39+ 0.08[a]	6.15+ 0.09[b]

Means with different superscripts in a row differ significantly (P<0.05). (n=6)

Juiciness: There was a gradual increase in juiciness scores compared to control which was significant statistically. Charvez *et al.* (1985) reported increase in juiciness scores as the collagen content increased as evidenced in this study.

Texture: The textural scores gradually decreased with simultaneous increase in the incorporation of fascia. The textural scores decreased as the incorporation levels of fascia increased which was in agreement with the findings of Jones (1984), Charvez *et al.* (1985), Strange and Whiting (1990) and Reddy *et al.* (1998).

Mouth coating: All the groups were scored more or less same.

***Overall acceptability*:** There was a gradual decrease in overall acceptability scores when hydrolysed fascia was incorporated upto 20% level. However, all the groups scored very good on

the subjective scale. The overall acceptability scores decreased as the fascia level increased which was in agreement with the findings of Jones (1984) and Charvez *et al.*(1985).It may be concluded that hydrolysed fascia may very well be used as a substitute of lean meat in buffalo patties with better processing and nutritional advantage.

CONCLUSIONS

Results from this study indicated that meat could be replaced up to 20% with hydrolysed fascia in patties formulations without affecting sensory qualities along with nutritional gain.Thus, utilization of fascia from abattoirs in meat products not only help meat processor to develop least cost formulation for patties but also encourages effective use of byproducts and solve the problem of environmental pollution.

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