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RESEARCH ARTICLE

Quality characteristics and ultra structural changes of restructured buffalo meat slices with flaxseed flour as binder: A novel value added technology

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ABSTRACT

In India, spent buffaloes are the main contributors of meat, which is tough and low priced and poor demand in the market. In this context, a new technological intervention is needed for restructuring of meat to enhance value-added meat production. Accordingly, an investigation was carried out to analyze the efficacy of different levels of flaxseed flour (C, T1, T2, and T3) as a novel binder for making of Restructured Buffalo Meat Slices (RBMS). The results revealed that T3 (RBMS added with 6% flaxseed flour) demonstrated significantly (P<0.01) higher cooking yield, water-holding capacity (WHC), batter stability and lower diameter shrinkage than control and RBMS incorporated with 2 % and 4 % flax seed flour. None of the treatments had significant influence on pH, total protein extractability, collagen content and solubility of RBMS. Moreover, T3 revealed significantly (P<0.01) higher moisture, total protein, total fat and total ash content than RBMS formulated with other levels of flax seed flour. Conversely, significantly lower in hardness, springiness, chewiness and higher in cohesiveness than other treatments. However, T3 influenced the colour scores, though at different degrees. Ultra structural images of cooked samples displayed the protein matrix between myofibrils which indicates gel network of flaxseed gum and proteins, subsequently caused more cohesiveness. This implies that the flaxseed flour acted as excellent novel binder for binding meat pieces. T3 has proven significantly greater sensory scores than other treatments. Thus, it is clearly evident that T3 has improved most of the quality attributes of buffalo meat and can be recommended as a novel value added technology for meat industry.

Keywords: Buffalo meat; Flaxseed flour; Quality characteristics; Restructuring; Ultra structure

INTRODUCTION

Generally in Indian market, spent buffaloes are major meat contributors and spent buffalo meat is generally tough and also deprived of demand for consumption. Better utilization of such meat entails technological interventions. Restructuring implies binding or holding of meat pieces together by using naturally occurring proteins to form a meat product for consumers with many of the properties of steak and roast meat. During manufacturing of restructured meats, different non meat additives importantly ingredients rich in protein per cent and polysaccharides are used to improve binding efficiency between meat chunks. Different polysaccharides like alginates, carrageenans and fibrimex are commonly using for binding of large pieces of meat chunks during restructuring process (Boles and Shand, 1999). Many researchers studied capability of different binding agents during restructured meat products and reported accordingly. Gudapureddy et al., (2013) concluded that addition 1.5 % calcium alginate had significantly (P<0.5) better quality characteristics in restructured mutton slices than control samples. Similar to these lines, Bhaskar Reddy et al., (2015) revealed that addition of 1.5 % carrageenan had superior quality characteristics than control and lower per cent added carrageenan in restructured mutton slices. Several commercial binding systems are developed by various manufacturers for restructured meat products that will work in different ways and influence differently to change in ingredients and quality of the finished product.

In order to overcome above limitations, it is imperative to develop a novel binder to bind meat pieces during restructuring processing without alteration of finished products textural attributes. To develop various novel binders, meat technologists are always exploring the abilities of different natural and novel food ingredients

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and their suitability in various meat products. Among several food crops, flaxseed (*Linum usitatissimmum L.*) is one of the important functional food ingredient from several reported scientific literature due to desirable nutritional and binding enhancement properties. In addition, flaxseed has considered as "functional food" due to its healthy nutritional profile, potential to lower risk of cardiovascular diseases and carcinomas (Fuchs et al., 2013). The important constituents of flaxseed flour are total protein (21%), total fat (41%) and dietary fiber (28%). The ability of water holding and also emulsifying properties of flaxseed mucilage with different components of food matrix may be having beneficial effect during meat processing (Singer et al., 2011). Hence, the present investigation was conducted to determine the binding efficacy of various levels of flaxseed flour to act as novel binder on various physico-chemical, proximate, textural, sensory, and ultra structural characteristics of restructured buffalo meat slices (RBMS).

MATERIALS AND METHODS

Meat and other ingredients

Buffalo meat (Cara beef) was collected from adult male buffalo slaughtered by Halal method from local market of Tirupati, Andhra Pradesh, India. Non meat ingredients were procured from retail stores of Tirupati. Food additives and chemicals used in different formulations of RBMS were procured from standard companies for quality analysis.

Preparation of restructured buffalo meat slices (RBMS)

The formulation for RBMS were presented in Table 1 and processing and preparation of RBMS was followed the protocol of Bhaskar Reddy et al., (2013) and briefly as follows: thawed the frozen buffalo meat then minced the buffalo meat chunks by using 20 mm size plate, then cured the meat chunks with curing ingredients about 12 hr at 4±1°C then all samples vacuum tumbled for 1 hour then added with rest of non meat ingredients then massaged for 15 min till meat formed tacky exudate. Later, the meat batter was made into 4 equal parts and one part was kept as control and remaining 3 parts mixed with 2%, 4% and 6% of flaxseed flour then massaged meat batter separately for 15 min till uniform mixing of flaxseed flour with meat batter accomplished.

The meat batters were filled separately in steel moulds and then cooked for 45 min (up to 75±1°C) then the product immediately chilled to 4±1°C. After chilling, the cooked loaf was made into slices of 6 mm thickness with the help of meat slicer and packaged in LDPE pouches for further quality and sensory analysis.

ANALYTICAL PROCEDURES

Physico-chemical characteristics

The weight difference of samples before and after cooking was calculated as per cent cooking yield. For estimating batter stability, batter was taken into LDPE bags and boiled in water bath at 80°C for 20 min and the exudate was removed and the cooked meat was weighed and expressed as batter stability in per cent. Water holding capacity (WHC) of RBMS was estimated as per Wardlaw et al., (1973). The pH of both batter and cooked product was determined by micro coupled digital pH meter (Systronics). Total protein extractability was estimated as per Joo et al., (1999). Collagen content was measured by the procedure of Nueman and Logan, (1950) with few modifications. The per cent collagen solubility was estimated by the method described by Mahendrakar et al., (1989) and calculated as described below.

$$\% \text{ Collagen solubility} = 7.14 \text{ x } \% \text{ HP solubilized}$$

Proximate Composition (moisture, protein and fat content) of RBMS was determined as per AOAC (2002). Texture

Table 1: Formulations of RBMS manufactured by using various levels of flaxseed flour

S. No	Name of the ingredients	Level of flaxseed flour addition			
		Control	T1 (2%)	T2 (4%)	T3 (6%)
1.	Buffalo meat (Cara beef)	84.535	82.535	80.535	78.535
2.	Flaxseed flour	0	2	4	6
3.	Salt	1.5	1.5	1.5	1.5
4.	Sodium tripolyphosphate (STPP)	0.4	0.4	0.4	0.4
5.	Sodium nitrite	0.015	0.015	0.015	0.015
6.	Sodium ascorbate	0.05	0.05	0.05	0.05
7.	Sugar	1.0	1.0	1.0	1.0
8.	Ice flakes	6.0	6.0	6.0	6.0
9.	Spice mix	1.5	1.5	1.5	1.5
10.	Maida	3.0	3.0	3.0	3.0
11.	Onion garlic paste	2.0	2.0	2.0	2.0
	Total	100.00	100.00	100.00	100.00

profile attributes of RBMS was recorded as per the protocols outlined by Bourne (1978) using Texturometer (Tinius Olsen, Model H1KF, England).

Instrumental color values

Instrumental color scores were recorded by the Commission Internationale de l'Eclairage L*a*b* system using a Hunter Lab Mini Scan EZ 45º/0º Spectrometer (Model: 4500L, Serial No: MSEZ2341, Hunter Associates Laboratory Inc, USA). The pre calibrated instrument was used and results were displayed digitally in terms of lightness (*L**), redness (*a**), and yellowness (*b**), on LCD screen of color reader and each sample was measured 3 times at various locations and the average value was taken.

Ultra structural studies

The processed samples were scanned under scanning electron microscope (SEM, Model: JOEL-JSM 5600) as per the standard procedures outlined by Bozzola and Russell, (1998) and under transmission electron microscope (TEM, Model: Hitachi, H-7500, Japan) as per the standard procedures (Bozzola and Russell, 1998).

Sensory evaluation

The RBMS were evaluated for sensory attributes using a 8-point hedonic scale as per Keeton (1983). The sensory attributes such as flavor, appearance, cohesiveness, juiciness, chewiness, mouth coating etc are considered for evaluation along with an overall acceptability by the panelists.

STATISTICAL ANALYSIS

All the experiments were repeated for four times and the data generated for the different quality attributes was taken as duplicates for each experiment. The generated data were subjected to ANOVA (analysis of variance) as per Snedecor and Cochran (1995).

RESULTS AND DISCUSSION

Physico-chemical characteristics

The impact of flaxseed flour on physico-chemical attributes of RBMS has been presented in Table 2. The RBMS added with 6% flaxseed flour (T3) had significantly ($P<0.01$) more cooking yield than Control and RBMS with other levels of flax seed flour. As the The level of incorporation of flaxseed flour enhanced from 0 to 6 per cent, cooking yield was gradually increased from 83.39% to 88.57%. This increment in cooking yield might be due to hydrophilic nature of flaxseed starch and also gelatinizing property of flax seed mucilage and also higher retention capacity of both fat and water by flaxseed flour in food matrix. Similar to these results, Naveena et al., (2006) concluded that ragi millet improved cooking yield in chicken patties than control patties and Bhaskar Reddy et al., (2018) observed similar improvement in cooking yield of chicken meat nuggets added with flaxseed flour.

Batter stability (per cent) and water holding capacity (WHC) of both batter and product RBMS was significantly ($P<0.01$) influenced by various levels of flaxseed flour. RBMS added with 6 % flaxseed flour (T3) had significantly ($P<0.01$) higher batter stability and WHC compared to other formulations. This might be due to possible interaction between soluble meat and flour proteins which increases the fat agglomeration while improving stability during restructuring process (Salahuddin et al., 1991). Further, higher WHC in RBMS added with 6% flaxseed flour due to more protein-flaxseed gum-water interactions with muscle proteins, resulted more intricate protein network structure where more quantity of the water retained (Moreno et al., 2010). Generally vegetable flours reported to stabilize the batter through fat encapsulating properties and gel forming ability of starch components. Similar to these results,

Table 2: Mean±S.E values of physico-chemical characteristics of RBMS as influenced by different levels of flaxseed flour

Physicochemical Characteristics	C	T1	T2	T3
Batter				
WHC (%)	29.89^{a}±0.67	31.54^{b}±0.19	33.39^{c}±0.27	39.27d±0.61
Batter stability (%)	86.47^{a}±0.83	88.14^{b}±0.22	90.03^{c}±0.41	91.50d±0.30
pH	5.81^{a}±0.20	6.08^{b}±0.17	6.10^{b}±0.13	6.05^{b}±0.23
Product				
Cooking Yield (%)	83.39^{a}±0.47	84.93^{b}±0.31	86.27^{c}±0.29	88.57d±0.47
WHC (%)	41.19^{a}±0.13	43.55^{b}±0.25	44.31^{c}±0.29	46.10d±0.37
pH	6.12±0.17	6.09±0.20	6.11±0.17	6.13±0.11
Diameter shrinkage (%)	7.11^{c}±0.12	6.56^{b}±0.29	6.60 ^{b}±0.19	5.14^{a}±0.43
Total Protein Extractability (mg/g tissue)	197.12±0.59	196.89±1.20	197.09±0.85	196.97±1.53
Collagen Content (%)	1.36±0.13	1.39±0.09	1.35±0.21	1.41±0.16
Collagen Solubility (%)	32.07±0.20	31.98±0.14	32.11±0.34	31.88±0.27

^{abcd}Means showing different superscripts in a row differ significantly ($P<0.01$); (n=8).
RBMS manufactured without flaxseed flour (C); RBMS with 2% (T1), 4% (T2) and 6% (T3) flaxseed flour

Chen et al., (2006) found higher interaction between meat proteins and flaxseed gum.

The minor variations in pH values of both batter and cooked RBMS might be due to the protein extractability variations in their respective treatments. The pH of the cooked RBMS was higher than batter due to protein denaturation during cooking. Similar results found by Bilek and Turhan (2009) in raw and cooked beef patties made with flax seed flour and Sharma et al., (2014) in restructured mutton chops extended with flaxseed flour. RBMS added with 6% flaxseed flour had lower diameter shrinkage than other treatments. A significant (P<0.01) reduction in diameter shrinkage from 7.11% in control RBMS to 5.14% in T3 samples were found. The plausible reasons for this were addition of flaxseed flour improved the water uptake by myofibrils during restructuring process, thus stabilized the meat batter and subsequent loss of less water during cooking (Bhaskar Reddy et al., 2015).

Incorporation of flax seed flour not (P>0.01) influenced total protein extractability (mg/g of tissue), collagen content and collagen solubility of RBMS. During restructuring process, the impact of both vacuum tumbling and massaging had similar effect on protein extractability values of RBMS. In restructuring process, the major forces responsible for muscle protein and flax gum interactions were electrostatic in nature (Bernal et al., 1987) which causes formation of more protein and gum mixture gels. The minor variations in per cent collagen content and collagen solubility were found between RBMS incorporated with various levels of flaxseed flour, but these differences were not statistically significant. Non-significant difference between RBMS added with flaxseed flour might be due to same source of meat which had same collagen content without much variation.

Proximate composition

Incorporation of flax seed flour significantly (P<0.01) influenced proximate composition of RBMS (Table 3). The higher level of moisture in 6% added RBMS was due to the protein net work formed with flax seed mucilage which retained more quantity of water during cooking (Chen et al., 2006) of product.

By adding flaxseed flour, the per cent protein increment was from 17.71 to 20.86 per cent in raw RBMS and from 19.10 to 23.16% in cooked RBMS. The higher protein content in flaxseed flour added RBMS due to comparatively more protein of flaxseed flour (24-26%) than cara beef. Addition of flaxseed flour increased the fat content of both raw and cooked RBMS significantly (P<0.05) which caused by more fat content of flaxseed flour (37.67%). Total ash content of RBMS added with flaxseed flour increased significantly (P<0.05) due to total dry matter content of flaxseed flour which replaced the lean meat during formulation. Sharma et al., (2014) also found similar results in restructured mutton chops.

Textural characteristics

The textural attributes of RBMS was influenced by flax seed flour (Fig. 2). RBMS added with 6% flaxseed flour significantly (P<0.05) lower hardness and an increased level of flaxseed flour addition, hardness values gradually decreased from 64.29 N to 30.47 N. In cooked meat systems, due to heat, flax seed gums acquire the gelling nature which may leads more bind enhancing between meat chunks (Andres et al., 2006). Springiness values (mm) decreased significantly (P<0.05) by incorporating flaxseed flour in RBMS formulations. Cohesiveness values increased with per cent flaxseed flour level increased from 2 to 6. RBMS added with 6 per cent flaxseed flour significantly (P<0.05) more cohesive than other formulations. Due to formation of gels with muscle proteins with flax seed gums leads to higher cohesiveness of the finished product during restructuring process. Gumminess (N) and chewiness (N mm) values significantly (P<0.01) reduced with flaxseed flour levels increased. The range of reduction in gumminess values was from 30.84 to 21.96 N and chewiness values was from 38.55 to 18.79

Table 3: Mean±S.E values of proximate composition of RBMS as influenced by different levels of flaxseed flour

Proximate Composition	C	T1	T2	T3
Batter				
Moisture (%)	68.27±0.11^{a}	69.49±0.13^{b}	71.68±0.28^{c}	73.51±0.56^{d}
Protein (%)	17.71^{a}±0.10	18.98^{b}±0.18	19.11^{b}±0.45	20.86^{c}±0.17
Fat (%)	1.93^{a}±0.34	2.68^{b}±0.22	3.29^{c}±0.17	3.98^{bc}±0.30
Total Ash (%)	0.39^{a}±0.20	1.67^{b}±0.37	2.58^{c}±0.19	3.20^{d}±0.23
Product				
Moisture (%)	65.13±0.19^{a}	66.25±0.27^{b}	67.17±0.39^{c}	69.13±0.31^{d}
Protein (%)	19.10^{a}±0.25	21.04^{b}±0.17	21.81^{c}±0.64	23.16^{d}±0.15
Fat (%)	2.71^{a}±0.20	3.69^{b}±0.41	4.47^{c}±0.24	4.51^{c}±0.56
Total Ash (%)	0.67^{a}±0.13	1.98^{b}±0.25	3.05^{c}±0.31	3.97^{d}±0.26

^{abcd}Means showing different superscripts in a row differ significantly (P<0.01); (n=8).
RBMS manufactured without flaxseed flour (C); RBMS with 2% (T1), 4% (T2) and 6% (T3) flaxseed flour

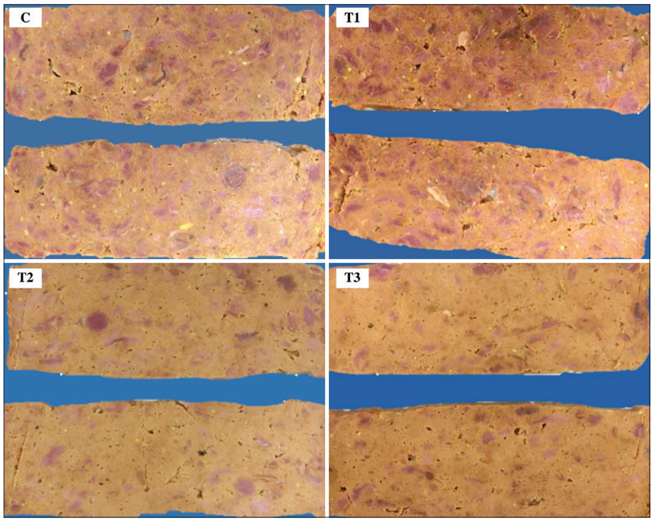

Fig 1. Photographs of RBMS developed by addition of different levels of flax seed flour. (RBMS without flaxseed flour (C); RBMS with 2 % (T1), 4 % (T2) and 6 % (T3) flaxseed flour)

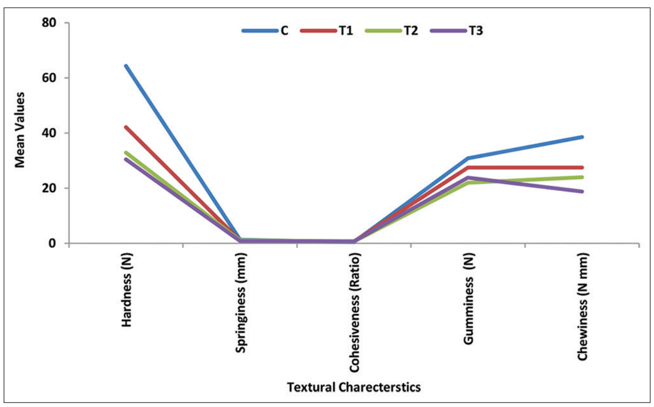

Fig 2. Textural Characteristics of RBMS as influenced by different levels of flax seed flour.

N mm from control to 6% flaxseed added RBMS. The reduction of gumminess and chewiness values by addition of flaxseed flour might be due to more soluble nature of hydrocolloid gel during heating (Holmes et al., 1986). The variations in the textural characteristics of RBMS might be due to interactions between meat protein and flaxseed gums during restructuring process (Chen et al., 2004). In addition to this, amount and type of fibre available in flours also influenced the textural properties of meat products. Insoluble fibre was capable of increasing the consistency of meat products by forming an three dimensional network which makes batter more stable. These above observations revealed that the flaxseed flour acted as best binding agent between meat chunks and it acted as a novel binder during restructuring process. The obtained results were in congruent with Andres et al., (2006) in chicken sausages made with whey protein concentrates and Sharma et al., (2014) in restructured mutton chops extended with flaxseed flour.

Instrumental colour scores

A significant (P<0.01) influence of flaxseed flour on instrumental colour (*CIE-L *, a*, b*, chroma* and *hue values*) scores of RBMS was found (Fig. 3). Control RBMS had more lightness (*L**) and redness (*a**) scores than RBMS added with flaxseed flour. A significant (P<0.01) lower lightness (*L**) and redness (*a**) scores of RBMS was found by incorporating flax seed flour. No significant (P>0.01) differences were found for yellowness (*b**) values of RBMS with flaxseed flour. Chroma values also reduced from 21.55 (control RBMS) to 15.35 (T3) by incorporating flaxseed flour but hue scores significantly (P<0.01) raised as the concentration of flaxseed flour addition raised from 0 to 6 per cent. The differences between instrumental colour values also might be due to the normal biological variability of the meat and flaxseed flour used. Reduced lightness (*L**) and redness (*a**) values in RBMS may be due to addition of flaxseed flour during restructuring process (Colmenero et al., 2003) and also the dark colour of flaxseed flour which attributed to the dark colour to the finished product. Similar to these results, Bilek and Turhan, (2009) reported that decreased *L** values in beef patties added with flaxseed flour and also found that *a** (redness) score was lower in uncooked control beef patties than flaxseed flour added beef patties.

Ultra structure

By careful examination of SEM and TEM images, the pattern of binding between the meat chunks during restructuring process was confirmed with textural attributes of RBMS. The ultra structure photographs of SEM showed a clear adhesion line between the meat surfaces. In uncooked control RBMS (Fig. 4a), less visible binding structure was noticed between myofibrils and also found little interactions with gums. In addition, less total protein exudates between the muscle chunks and the proteins were fragmented and did not have the solid gel network. In uncooked flaxseed flour added RBMS (Fig. 4b to 4d); the zone of binding between meat chunks showed a well structured matrix with more interactions of thick strands and this might be due to binding force improved with increased concentration of flaxseed flour.

Cooked samples of RBMS (Fig. 4e to 4h), the protein matrix between myofibrils indicated the gel network of flaxseed gum and proteins subsequently caused more cohesiveness. There were several minute particles with a globular or elongated shape, which might represented the homogenous structure of fat emulsion or the fat globules confined locally within the protein matrix. The level of batter formation depended on the several conditions of fat and protein components, temperature of restructuring process and equipments used for meat restructuring

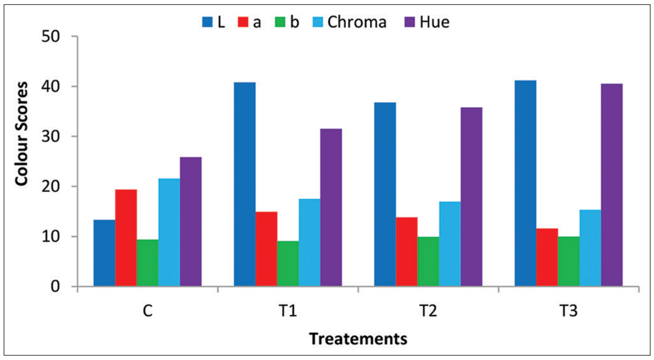

Fig 3. Colour scores of RBMS as influenced by different levels of flax seed flour.

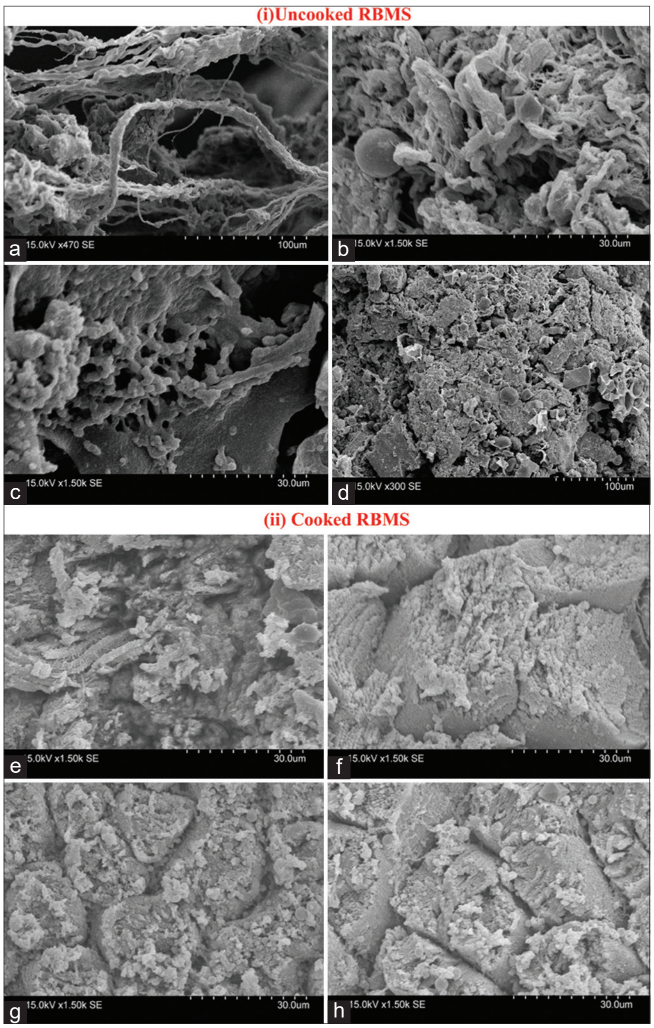

Fig 4. Scanning electron microscope (SEM) micrographs (X1500 magnification) of raw RBMS without flax seed flour (a), with 2 % flax seed flour (b), with 4 % flax seed flour (c) and with 6 % flax seed flour (d) and cooked RBMS without flax seed flour (e), with 2 % flax seed flour (f), with 4 % flax seed flour (g) and with 6 % flax seed flour (h).

process (Zayas, 1985). Sample containing flaxseed flour as binder in batter formation with protein and fat particles were uniformly distributed in the batter structure. Zheng et al., (2017) revealed that the net work formation of three dimensional structures in batter made with flaxseed gum where samples with flaxseed flour were more compact. Furthermore, the products made with flaxseed flour had bigger and more complete gel clusters than control samples. These protein exudates that were favored with solubilization action of salt, STPP and mechanical treatments (Vacuum tumbling and massaging), having higher binding capacity (Katsaras and Peetz, 1989). The addition of flaxseed flour changed the ultra structure of the RBMS; the meat chunks were coated by the flaxseed flour components, so that there was higher fragmentation of chunks were occurred. Meanwhile, the protein net work structures became less continuous and more dispersed. The structural changes of RBMS containing different levels of flaxseed flour were intermediate between the control and the RBMS with flaxseed flour. These results were in accordance with Montero et al (2000) and Perez-Mateos et al., (2002) who reported that the alginate network was connected with muscular protein net.

Transmission electron microscopy of RBMS added with flaxseed flour (Fig. 5a to 5d) viewed as aggregation of protein clusters which was separated by void spaces with irregular shapes in different places. The empty places are had retained more water by the restructured batter matrix. In all images, it is showing that aggregated protein clusters connected with fine threads depicting as a distinct network which is vital in binding of meat chunks. The presence of an extended protein network explained the role of flaxseed gum when used as a novel binder in the meat system. In the present study, the void spaces and crevices diminished and the structure became more compact and dense as the concentration of flaxseed flour increased (Fig. 5d). The TEM images showing the micro structure of the myofibrils is much amorphous, shrink and the Z-disks became diffused, thickened and disrupted throughout the filaments. At RBMS added with 6% flaxseed flour (Figure 5d), flaxseed distributed on equal basis, occupying the intracellular and some of the intercellular spaces and the flaxseed gum appeared as a continuous network embedding with water. It was possible that at this concentration of flaxseed flour may act as filler or as a cementing agent.

Sensory evaluation

The influence of incorporation of various concentrations of flaxseed flour on sensory characteristics of RBMS was presented in Table 4. A significant ($P<0.01$) higher appearance values found in RBMS added with 6% flaxseed flour. The ranges of appearance scores were from 6.88 to 7.42 and gradual increase of appearance scores were found in higher concentrations of flaxseed flour added RBMS. The appearance of flaxseed flour added RBMS ranged from light red to dark red. Schwartz and Mandigo (1976) reported that increased protein binding quality with

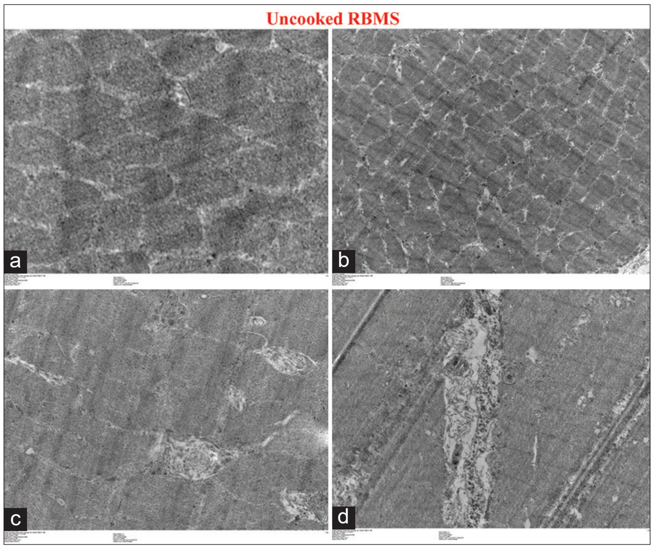

Fig 5. Transmission electron microscope (TEM) micrographs [X 5000 magnification] of raw RBMS added without flax seed flour (a), with 2 % flax seed flour (b), with 4 % flax seed flour (c), with 3 % flax seed flour (d).

Table 4: Mean±S.E values of sensory characteristics of RBMS as influenced by different levels of flaxseed flour

Sensory Characteristics	C	T1	T2	T3
Appearance	6.88^{a}±0.31	7.05^{b}±0.22	7.18^{b}±0.13	7.42^{c}±0.28
Flavour	7.03±0.14	7.14±0.09	7.12±0.21	7.10±0.15
Chewiness	7.06±0.11	7.03±0.33	7.04±0.12	7.08±0.14
Cohesiveness	6.83^{a}±0.15	7.15^{b}±0.17	7.23^{b}±0.26	7.26^{b}±0.13
Juiciness	6.63^{a}±0.21	7.08^{b}±0.12	7.39^{c}±0.19	7.41^{c}±0.12
Mouth coating	6.12±0.23	6.61^{b}±0.11	6.65^{b}±0.23	6.68^{b}±0.12
Overall palatability	6.56^{a}±0.10	6.81^{b}±0.09	6.89^{b}±0.11	7.17^{c}±0.23

abcdMeans showing different superscripts in a row differ significantly (P<0.01); (n=8).
RBMS manufactured without flaxseed flour (C); RBMS with 2% (T1), 4% (T2) and 6% (T3) flaxseed flour

higher WHC improved the colour scores of restructured beef roasts. Flavor and chewiness scores of RBMS were not significantly (P>0.01) affected by addition of various levels of flaxseed flour. RBMS added with 6% flaxseed flour had significantly (P<0.05) more cohesive than other treatments. Addition of flaxseed flour enhanced the cohesiveness values from 6.83 to 7.26 and this might be due to the significant increase in binding due to increased viscosity of samples by addition of flaxseed flour during restructuring process caused more cohesive of the product (Wang et al., 2011). RBMS added with 6% flaxseed flour had significantly (P<0.01) higher juiciness scores than other treatments. Juiciness scores of RBMS increased as the level of flaxseed flour enhanced from 0 to 6%. Higher juiciness scores in flaxseed flour added samples also could be a consequence of greater protein-flaxseed gum-water interactions in RBMS samples resulted in a more stable protein network structure where the water was retained. In addition, the composition of the flaxseed flour enriched formulation might be the reason for reduced moisture loss during cooking than the control RBMS.

Addition of flaxseed flour in RBMS had significantly (P<0.01) higher mouth coating scores than control RBMS. There was no significant (P>0.01) difference found between RBMS added with different levels of flaxseed flour. The higher mouth coating in RBMS added with flaxseed samples might be due to gelled form of flaxseed gum and their interaction with meat proteins. A significant (P<0.01) higher overall palatability scores were found in RBMS added with 6% flaxseed flour (T3) than control and remaining formulations. There was no significant (P>0.01) difference between RBMS added with 2 (T1) and 4 (T2) per cent flaxseed flour. The higher overall acceptability scores might be due to RBMS with flax seed flour had higher appearance, cohesiveness and mouth coating scores than control RBMS. Similar to these results, Bilek and Turhan (2009) concluded that 6% flaxseed flour was considered optimum for use as an enhancer to the nutritive value in beef patties. Sharma et al., (2014) also found no significant difference in flavor scores of restructured mutton chops extended with flaxseed flour as a excellent binding agent. These results were in congruent with Reddy et al., (2018) in chicken meat nuggets extended with different levels of flaxseed flour.

CONCLUSIONS

By obtaining the present results, it may be concluded that incorporation of flaxseed flour in the preparation of RBMS significantly increased most of the quality attributes. In addition, the nutritive values of the RBMS also prominently enhanced with the incorporation of flaxseed flour in conjunction with enhanced textural quality and colour scores. The flaxseed gum has made excellent gel network during restructuring process which was very obviously demonstrated in both scanning and transmission electron micrographs. This indicates that flaxseed acts as excellent binder during restructuring process and RBMS added with flaxseed flour rated superior sensory scores than other treatments. Therefore, it is clearly evident that the incorporation of flaxseed flour has improved quality attributes of buffalo meat, which can be recommended as a novel value added technology for meat industry.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors are do not having any conflicts of interest related to the manuscript.

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Authors' contributions

G.V. Bhaskar Reddy: concept generation, execution of research work, methodology, supervision, visualization, writing original draft.

K. Viswanatha Reddy: formal analysis, methodology, visualization.

P. Amaravathi: methodology, writing, ultra structural studies and review of original draft.

REFERENCES

Andres, S., N. Zaritzky and A. Califano. 2006. The effect of whey protein concentrates and hydrocolloids on the texture and colour characteristics of chicken sausages. Int. J. Food Sci. Technol. 41: 954-961.

AOAC. 2002. Official Method of Analysis. Revision 1. 17th ed. Association of Official Analytical Chemists Inc, Arlington, Virginia.

Bernal, V. M., C. H. Smajda, J. L. Smith and D. W. Stanley. 1987. Interactions in protein/polysaccharide/calcium gels. J. Food Sci. 52: 1121-1125, 1136.

Bhaskar Reddy, G. V., A. R. Sen, P. N. Nair, K. S. Reddy, K. K. Reddy and N. Kondaiah. 2013. Effects of grape seed extract on the oxidative and microbial stability of restructured mutton slices. Meat Sci. 95: 288-294.

Bhaskar Reddy, G. V., A. R. Sen, K. S. Reddy, K. K. Reddy, T. M. Rao and N. Kondaiah. 2015. Quality characteristics of restructured mutton slices developed by cold-set binding system. Indian J. Small Rumin. 21: 300-305.

Bilek, A. E. and S. Turhan. 2009. Enhancement of the nutritional status of beef patties by adding flaxseed flour. Meat Sci. 82: 472-477.

Boles, J. A. and P. J. Shand. 1999. Effects of raw binder system, meat cut and prior freezing on restructured beef. Meat Sci. 53: 233-239.

Bourne, M. C. 1978. Texture profile analysis. Food Technol. 32: 62-67, 72.

Bozzola, J. J., and L. D. Russell. 1998. Electron Microscopy: Principles and Techniques for Biologists. Jones and Bartlett Publishers, Sudbury, Massachusetts, pp. 19-67.

Chen, H. H., S. Y. Xu and Z. Wang. 2004. Separation and purification of acidic polysaccharides and neutral polysaccharides in flaxseed gum. J. Food Ferment Ind. 1: 96-100.

Chen, H. H., S. Y. Xu and Z. Wang. 2006. Gelation properties of flaxseed gum. J. Food Eng. 77: 295-303.

Colmenero, J. F., A. Serrano, J. Ayo, M. T. Solas, S. Cofrades and J. Carballo. 2003. Physicochemical and sensory characteristics of restructured beef steak with added walnuts. Meat Sci. 65: 1391-1397.

Fuchs, R. H. B., R. P. Ribeiro, M. Matsushita, A. A. C. Tanamati, E. Bona and A. H. P.de Souza. 2013. Enhancement of the nutritional status of Nile tilapia (*Oreochromis niloticus*) croquettes by adding flaxseed flour. LWT Food Sci. Technol. 54: 440-446.

Gudapureddy, V. R., A. R. Sen, P. N. Nair, K. K. Reddy and K. S. Reddy. 2013. Quality characteristics of restructured mutton slices developed by cold-set binding system. FleischWirtschaft Int. 3: 65-70.

Joo, S. T., R. G. Kauffman, B. C. Kim and G. B. Park. 1999. The relationship of sarcoplasmic and myofibrillar protein solubility to colour and water-holding capacity in porcine longissimus muscle. Meat Sci. 52: 291-297.

Katsaras, K. and P. Peetz. 1989. Scanning electron-microscopy in meat research. Alimenta. 28: 119-122.

Keeton, J. T. 1983. Effect of fat and NaCl/phosphate levels on the chemical and sensory properties of pork patties. J. Food Sci. 48: 878-881, 885.

Mahendrakar, N. S., N. P. Dani, B. S. Ramesh and B. L. Amla. 1989. Studies on influence of age of sheep and post-mortem carcass conditioning treatments on muscular collagen content and its thermoliability. J. Food Sci. Technol. 26: 102-105.

Salahuddin, M., N. Kondaiah and A. S. R. Anjaneyulu. 1991. Effect of maida, potato and texturized soy as binders on the quality of chicken and mutton kababs. J. Food Sci. Tech. 28: 301-303.

Montero, P., J. L. Hurtado and M. Perez-Mateos. 2000. Microstructural behaviour and gelling characteristics of myosystem protein gels interacting with hydrocolloids. Food Hydrocoll. 14: 455-461.

Moreno, H. M., J. Carballo and A. J. Borderías. 2010. Use of microbial transglutaminase and sodium alginate in the preparation of restructured fish models using cold gelation: Effect of frozen storage. Innov. Food Sci. Emerg. Technol. 11: 394-400.

Naveena, B. M., M. Muthukumar, .A. R. Sen,. Y. Babji and T. R. K. Murthy. 2006. Quality characteristics and storage stability of chicken patties formulated with finger millet flour (*Eleusine coracana*). J. Muscle Foods. 17: 92-104.

Nueman, R. E. and M. A. Logan. 1950. The determination of hydroxyproline. J. Biol. Chem. 184: 299-306.

Perez-Mateos, M., T. Solas and P. Montero. 2002. Carrageenans and alginate effects on properties of combined pressure and temperature in fish mince gels. Food Hydrocoll. 16: 225-233.

Reddy, G. V. B., B. O. Reddy, J. Indumathi and A. Ravi. 2018. Quality characteristics of functional chicken meat nuggets extended with flax seed flour. Indian J. Poult. Sci. 53: 219-224.

Schwartz, W. C., and R. W. Mandigo. 1976. Effect of salt, sodium tripolyphosphate and storage on restructured pork. J. Food Sci. 41: 1266-1269.

Sharma, H., B. D. Sharma. S. K. Mendiratta, S. Talukder and G. Ramasamy. 2004. Efficacy of flaxseed flour as bind enhancing agent on the quality of extended restructured mutton chops. Asian Aust. J. Anim. Sci. 27: 247-255.

Singer, F. A. W., F. S. Taha, S. S. Mohamed, A. Gibriel and M. E. Nawawy. 2011. Preparation of protein/mucilage products from flaxseed. Am. J. Food. Technol. 6: 260-278.

Snedecor, G. W. and W. G. Cochran. 1995. Statistical Methods. 8th ed.

Oxford and IBH Publishing Co., New Delhi.

Wang, Y., D. Li, L. J. Wang and B. Adhikari. 2011. The effect of addition of flaxseed gum on the emulsion properties of soybean protein isolates (SPI). J. Food Eng. 104: 56-62.

Wardlaw, F. B., L. H. Mccaskill and J. C. Acton. 1973. Effect of postmortem muscle changes in poultry meat loaf properties. J. Food Sci. 38:421-423.

Zayas, J. F. 1985. Structural and water binding properties of meat emulsions prepared with emulsified and unemulsified fat. J. Food Sci. 50: 689-692.

Zheng, Y., P. Xu, S. Li, X. Zhu, C. Chen and C. Zhou. 2017. Effect of L-lysine/L-arginine on the physicochemical properties and quality of sodium-reduced and phosphate-free pork sausage. Int. J. Nutr. Food Sci. 6: 12-18.