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Effect of egg white powder on quality and structural properties of restructured buffalo meat slices

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Abstract

In India, the main source of buffalo meat is old buffaloes, and such spent animal meat is tough, and fetches low demand. Generally, spent animal meat is tough, and has poor binding capacity, thus unable to be consumed fresh, which necessitates different binding technologies for efficient transformation of old and spent buffalo meat into nutrient rich, value-added, restructured meat products. The present work was conducted to develop the restructured buffalo meat slices (RBMS) using egg white powder (EWP) as an innovative novel binder within the meat batter at different levels *i.e.* 0, 1, 2, and 3% (C, T1, T2, and T3, respectively). The developed RBMS were subjected to evaluation on efficacy of different concentrations of egg white powder on different quality characteristics during restructuring process. RBMS prepared with 3% EWP (T3) showed significantly ($p < 0.01$) higher cooking yield, batter stability, and water holding capacity than other treatments. RBMS prepared with 3% EWP (T3) had significantly ($p < 0.01$) lower diameter shrinkage in the product, and improved the moisture and protein contents in both raw batter and cooked product. The addition of EWP significantly ($p < 0.01$) decreased the hardness, and increased the cohesiveness values in RBMS. Significantly ($p < 0.01$) better instrumental colour scores and superior sensory scores were recorded in RBMS added with EWP. Scanning electron microscopy photographs of RBMS batter showed that the proteins appeared to be well formed strands in EWP-treated samples, and the solid gel network was also found strongly binding the meat gels. A well-built matrix with more thick strands was observed and further increased with increasing EWP content in RBMS added with EWP batter gels. In the case of transmission electron microscopy photographs, RBMS added with 3% EWP showed that egg proteins were evenly distributed, filling both the intracellular and some of the intercellular spaces between cells; and the egg proteins formed a continuous network, interwoven with water and muscle proteins. Based on the obtained results, the addition of EWP provided improved physico-chemical characteristics such as better proximate composition, textural quality, and instrumental colour scores; and superior sensorial scores than control samples. Therefore, the addition of 3% EWP to restructured meat batter can be recommended as a novel binder for the development of different value added and restructured meat products using buffalo meat.

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Introduction

Buffalo meat (cara beef) is highly acceptable and known for its low cholesterol and calorific value among red meats (Naveena and Kiran, 2014). Buffalo meat has been gaining so much importance due to its export potential and domestic demand, which also offers cost advantages over lamb meat. The demand for buffalo meat is evident with an average annual growth rate of 13.75%, compared to other meats with an annual growth rate of 11.31% (Bardhan *et al.*, 2014). Buffalo meat majorly contributed to India's total meat export (94%) in 2021 - 2022, which accounted for 18% of the global beef meat export (APEDA, 2023). India being the second largest buffalo meat exporter for the year 2022 shows the potential of buffalo meat in augmenting farmers' income. In Indian markets, the meat of spent buffaloes is a significant source of supply, but tends to be tough, leading to reduced consumer demand.

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Better utilisation of such meats is possible with technological interventions, and one such intervention is meat restructuring, which enables the value addition of underutilised or low-cost meat, meat cuts, and trimmings (Bhaskar Reddy *et al.,* 2023b).

Traditional or conventional methods of restructuring meat products use the thermal binding property of meat proteins that are extracted by different means (Boles and Shand, 1999). However, the main problem associated with the restructured products obtained through thermal binding is the discoloration, which occurs due to salt and oxidative rancidity (Bhaskar Reddy *et al.,* 2013). To overcome such problems, different techniques that use cold-set binding are applied to prepare restructured meat products (Lennon *et al.,* 2010). However, these cold-set binding technologies are very costly and not economically viable. Therefore, there is an urgent need to exploit novel binding systems with lower costs for efficient transformation of old and spent buffalo meat into nutrient-rich, value-added meat products. The inclusion of carrageenan significantly improved quality attributes compared to the control samples in mutton slices produced by restructuring process (Bhaskar Reddy *et al.,* 2015). The inclusion of 6% flax seed flour in buffalo meat slices significantly enhanced their quality attributes, making it a promising and innovative value-added technique for the meat industry (Bhaskar Reddy *et al.,* 2023a).

Egg white powder (EWP) is a good source of functional proteins, which perform the functions like texture enhancement, emulsification, and nutrient enhancement, in different food systems such as confectionary, bakery, and meats (Hsu and Sun, 2006). Additionally, EWP is utilised to enhance the textural qualities, gelling capacity, and emulsification and binding properties in restructured meat products (Pietrasik, 2003; Teye *et al.,* 2012). However, the information on potential of EWP to act as a novel binding agent in restructured meat products is scanty. By considering these facts, a systematic study was conducted to assess the influence of EWP as a novel binder on various quality attributes and ultra-structure of restructured buffalo meat slices.

Materials and methods

Meat and other non-meat ingredients

At regular intervals, buffalo meat (cara beef) was sourced from adult male buffaloes (~450 kg) which were slaughtered by halal method at approximately 4.00 AM in the Municipal Slaughterhouse, Tirupati, Andhra Pradesh, India, and brought to the laboratory at approximately 7.00 AM, followed by conditioning at refrigerated temperature for 24 h. The buffalo meat was collected from a group of typical carcasses. Other non-meat ingredients such as cane sugar, refined wheat flour, spices, and condiment mix were purchased from a local grocery store in Tirupati, Andhra Pradesh, India. EWP and other analytical grade reagents were procured from Himedia Pvt. Ltd., Mumbai, India.

Processing of restructured buffalo meat slices (RBMS)

The formulation (%) for the development of RBMS, including control and treatments, are tabulated in Table 1. The sequential procedure for the manufacturing of RBMS was as followed: The chilled buffalo meat portions at 4 ± 1°C were minced using 20 mm diameter holes in the plate of laboratory mincer (Kumar Industries, Coimbatore, India). The minced meat was then added with curing ingredients (salt, sodium nitrite, sodium ascorbate, sodium tripolyphosphate, and sugar), and allowed to cure at refrigeration temperature for 12 h. The samples were vacuum-tumbled for 1 h, and mixed with ice flakes, refined wheat flour, spice mix, and onion garlic paste, followed by massaging for 15 min to extract the myofibrillar proteins, and mixed with curing ingredients that formed tacky exudates of meat. The batter was maintained at 10 ± 1°C throughout the mechanical processing (vacuum-tumbling and massaging), then removed from massager and separated into four equal parts, out of which, three parts were mixed with EWP at 1, 2, and 3% (w/w) with meat batter, and one part was used as control. This was followed by thorough massaging for 15 min to ensure thorough mixing of EWP with meat batter.

The processed batters were carefully filled into stainless steel moulds (L × W × H of 18 × 12 × 6 cm; and for each batch, four moulds were used), followed by steam cooking until the core temperature reached to 75 ± 1°C (measured by Koch probe thermometer) for approximately 45 min. Steam-cooked product was chilled at a refrigerated temperature (4 ± 1°C) for 12 h (Samsung Model No. RT47B6238UT/TL), sliced into 6 mm thick uniform pieces using a meat slicer (Sirman Mod. No: Auto m, 300 VV, Italy), and the quality and sensory analyses were carried out immediately.

Table 1. Formulations for RBMS prepared with different levels of egg white powder (EWP).

Sample	Ingredient	Egg white powder			
		Control (0%)	T1 (1%)	T2 (2%)	T3 (3%)
1.	Buffalo meat (cara beef)	84.535	83.535	82.535	81.535
2.	Egg white powder	0	1	2	3
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

Quality analysis

Physico-chemical characteristics

Cooking yield

About 100 g of the batter was filled in the stainless-steel round moulds with a diameter of 3 inches, and cooked for 45 min (core temperature of the product reached to 75 ± 1°C, which was measured by Koch probe thermometer). Percent cooking yield was determined by calculating the weight differences of batter before and after cooking.

Batter stability

Batter stability of raw RBMS batter was determined according to Kondaiah *et al.* (1985). About 25 g meat batter was packed in LDPE pouches, and boiled at 80°C for 20 min. The weight difference between uncooked and cooked batter was expressed as percent batter stability.

Water-holding capacity

The water-holding capacity (WHC) of the RBMS was determined according to Wardlaw *et al.* (1973). Briefly, 20 g of batter was taken in a centrifuge tube, and then centrifuged by adding 30 mL of NaCl (0.6 M) in refrigeration temperature (4 ± 1°C) for 15 min at 3,000 rpm. The resulting supernatant was measured, and the water retained by the samples was expressed as WHC in percentage.

pH

The pH of meat homogenate of both batter and cooked products were determined using combined glass electrode of pH meter (Systronics, Model, 361) calibrated against buffer of pH 4, 7, and 10 to record the pH of the suspension.

Protein extractability

The total proteins, both myofibrillar and sarcoplasmic, were extracted according to Joo *et al.* (1999). Briefly, 2 g sample was homogenised with 40 mL of ice-cold 1.1 M potassium iodide in 0.1 M phosphate buffer (pH 7.2), and left to shake overnight at 4°C. Subsequently, the samples were centrifuged for 20 min at 5,000 rpm following the Biuret method to determine the protein concentration in the supernatant.

Collagen content

The batter was analysed for hydroxyproline content according to Nueman and Logan (1950). Briefly, 2 g of the meat samples were mixed with 40 mL of 6 N HCl, and allowed to hydrolyse for 18 h. After hydrolysis, the resulting solution was filtered and adjusted to a final volume of 50 mL using distilled water. The pH of the solution was then brought to 7.0 by adding 40% NaOH to 25 mL of the hydrolysate, and the volume was adjusted to 50 mL with distilled water. Next, 1 mL of aliquot was used for the estimation of hydroxyproline. The measurement of hydroxyproline content was performed using a UV-VIS spectrophotometer (Model: UV-1700 Pharma Spec, SHIMADZU, Japan) at an absorbance of 540 nm. The content of hydroxyproline was determined by referring to a standard graph. The final calculation of collagen content was determined by multiplying the hydroxyproline content by 7.14, and expressed as mg/g tissue.

Collagen solubility

The collagen solubility was determined according to Mahendrakar *et al.* (1989). Briefly, 5 g of batter was placed in a 250 mL glass beaker, and carefully covered with a watch glass lid before immersing it in a water bath, and boiling for about 30 min. Then, the cooked meat was homogenised in a blender for 2 min by adding 50 mL of distilled water at 4 ± 1°C. The extract from the homogenised sample was centrifuged at 4,000 rpm for 30 min, and then it was added with aliquots of cooked out juice, and were hydrolysed at 108°C for 18 h in a hot air oven. Collagen solubility percent was calculated and expressed as percent solubilised hydroxyproline multiplied by 7.14.

Proximate composition

The proximate composition of the developed RBMS was determined following the procedures outlined by the Association of Official Analytical Chemists (AOAC, 2000). The moisture content was determined using hot air oven at 105°C (AOAC method 925.09); the protein content was determined by the Kjeldahl method (AOAC method 955.04); the fat content was determined by the Soxhlet method (AOAC method 920.39); and the ash content was determined using a muffle furnace at 550°C for 6 h (AOAC method 940.26).

Texture profile analysis (TPA)

The texture profile analysis (TPA) of the developed RBMS samples were analysed following the procedure outlined by Bourne (1978). A Texturometer (Tinius Olsen, Model H1KF, England) was employed for this purpose. The samples, which had been refrigerated, were equilibrated to ambient temperature (25°C). Test samples measuring 1.5 cm^3 were taken from the central portion of each block, and used for the analysis.

Instrumental colour scores

The Commission Internationale de l'Eclairage (CIE, 1986) was employed for the evaluation of instrumental colour of the developed RBMS using a Konica Minolta colour reader (Model No: CR 20). The instrument used was pre-calibrated, and the colour meter's aperture was positioned vertically on the surface of the samples. The results were digitally displayed on an LCD screen of the colour reader, indicating lightness (L*), redness (a*), yellowness (b*), hue, and chroma. Each sample underwent three measurements at different locations, and the mean value was calculated.

Ultra-structural studies

The samples for scanning electron microscopy (SEM) were processed following the procedures outlined by Bozzola and Russell (1998). The samples were then scanned under a scanning electron microscope (JOEL-JSM 5600 model) at 1,500× magnification. The processed RBMS batter sections were then examined under a transmission electron microscope (TEM, Model: Hitachi, H-7500, Japan) at a 10,000× magnification.

Sensory evaluation

The sensory attributes of the RBMS were assessed using an 8-point hedonic scale, ranging from 8 (extremely like) to 1 (extremely dislike) (Keeton, 1983). The evaluation included factors such as appearance, flavour, cohesiveness, chewiness, juiciness, mouth coating, and overall acceptability as determined by the panellists. The sensory evaluation was carried out from 3.30 to 4.00 PM in a properly ventilated area. The sensory panel consisted of 12 experienced members who had prior exposure to consuming restructured products. They were provided with pre-heated samples, which had been heated at 40°C for 30 sec. The samples were served on white plates, and evaluated under natural light. Each panellist received four samples from four treatment groups, which means one sample from one treatment group at a time or in a single sitting, and a total of four times or four sittings in the entire experiment were carried out to evaluate the sensory attributes of the developed RBMS. The product was developed in the laboratory, and sensory evaluation done only for testing of basic sensory attributes related to the developed product by trained panellists only. These panellists should not be considered as consumers, and also these panellists were not a representative of a particular consumer population.

Statistical analysis

The data generated for the different quality attributes were duplicated for each of the four replicated experiments, and data were expressed as means ± standard error. Statistical analysis was processed using SPSS-PASW statistics software version 20.0 for Windows, with One-way analysis of variance (ANOVA). Paired *t*-test and Duncan's multiple range tests were used to compare the means, and determine the significant differences ($p < 0.01$) between groups (Snedecor and Cochran, 1989).

Results and discussion

Physico-chemical characteristics

The addition of EWP significantly influenced the physico-chemical quality of RBMS (Table 2). The RBMS containing 3% EWP (T3) had significantly ($p < 0.01$) higher WHC (both batter and cooked samples), batter stability (%) (batter) and cooking yield (%) (cooked product) compared to the control and RBMS added with 1% (T1) and 2% (T2) EWP. The range of cooking yield was between 82.27% (C) and 87.23% (T3), batter stability was between 81.42% (C) and 87.11% (T3), and WHC was between 24.17% (C) and 29.05% (T3). As the level of EWP increased from 0 to 3%, a gradual and significant ($p < 0.01$) increase in cooking yield, batter stability, and WHC were noticed. The higher cooking yield in RBMS added with 3% EWP (T3) was attributed to the increase in protein content due to the addition of EWP that bound to the meat chunks during restructuring process (Bhaskar Reddy *et al.,* 2015). Further, adding EWP during restructuring process resulted in lower cooking loss due to high content of coagulable protein that contributed to the stable gel matrix. The higher batter stability and WHC in EWP added samples was due to the formation of stabilised gel network between egg protein, meat protein, and water (Pietrasik, 2003; Muthia *et al.,* 2012). Thus, EWP improved the WHC by binding the water in the matrix, making the batter more uniform. Further, the coagulation of egg albumin helped in binding the meat pieces during the restructuring process of meat (Xiong, 1997). Adding spray-dried egg white increased the cook yield of gels made of mechanically deboned chicken meat, mutton, chicken products, and cooked chicken eggs (Boles and Shand, 1999; Bhaskar Reddy *et al.,* 2017). Further, higher cooking yield and water holding capacity in EWP-added RBMS might have been due to the increased gelation of extracted meat proteins with albumin and other egg proteins, which absorbed higher water within myofibrils. Additionally, EWP significantly increased the restructured meat batter stability because of their higher hydrophilic, as well as lipophilic binding sites, and also myofibrillar and albuminous interactions, which attributed to higher fat and water retention by the egg proteins with its excellent surface-active properties, as well as water-fat-protein interactions (Bhaskar Reddy *et al.,* 2023b).

Table 2. Physico-chemical characteristics of RBMS prepared with different levels of egg white powder (EWP) (n = 8/group).

Physico-chemical characteristic	Egg white powder			
	Control (0%)	**T1 (1%)**	**T2 (2%)**	**T3 (3%)**
Uncooked batter				
WHC (%)	24.17 ± 0.26^a	26.87 ± 0.57^b	27.12 ± 0.41^c	29.05 ± 0.34^d
Batter stability (%)	81.41 ± 0.42^a	84.13 ± 0.27^b	85.97 ± 0.48^c	87.11 ± 0.19^d
pH	5.59 ± 0.17^a	5.95 ± 0.19^b	6.03 ± 0.24^b	5.98 ± 0.10^b
Cooked product				
Cooking yield (%)	82.27 ± 0.25^a	85.14 ± 0.41^b	85.16 ± 0.13^b	87.23 ± 0.29^c
WHC (%)	27.75 ± 0.22^a	29.44 ± 0.17^b	32.33 ± 0.54^c	35.17 ± 0.15^d
pH	6.09 ± 0.10	6.11 ± 0.24	6.05 ± 0.11	6.17 ± 0.19
Diameter shrinkage (%)	9.94 ± 0.31^c	7.51 ± 0.30^b	7.43 ± 0.25^b	6.02 ± 0.19^a
Total protein extractability (mg/g tissue)	184.79 ± 1.15	183.81 ± 0.84	185.03 ± 1.08	184.56 ± 1.24
Collagen content (%)	1.42 ± 0.20	1.38 ± 0.28	1.40 ± 0.09	1.43 ± 0.15
Collagen solubility (%)	31.20 ± 0.11	31.18 ± 0.57	32.17 ± 0.35	31.62 ± 0.53

Values are mean ± S.E. Means followed by different lowercase superscripts in similar row differ significantly ($p < 0.01$). C: restructured buffalo meat slices (RBMS) prepared without egg white powder (EWP); T1: RBMS prepared with 1% egg white powder; T2: RBMS prepared with 2% egg white powder; and T3: RBMS prepared with 3% egg white powder.

The addition of EWP significantly ($p < 0.01$) increased the pH of RBMS batter than control samples, but it did not result in any treatment differences in the pH of cooked RBMS. The higher pH values in restructured buffalo meat batter added with EWP were attributed to the pH values of EWP. Similar to these results, increasing EWP levels in buffalo meat cutlets and sausages increased its pH values as well as yields (Ahamed *et al.,* 2007). On contrary to these results, Teye *et al.* (2012) reported lower pH values of frankfurters added with fresh or dried egg albumin than control samples. RBMS containing 3% EWP (T3) had significantly ($p < 0.01$) lower percent diameter shrinkage compared to remaining formulations. Adding EWP decreased shrinkage from 9.94% in control to 6.02% in T3 samples. This might have been due to the higher water retention capacity of EWP during restructuring process by forming protein-protein and protein-water bonds, thus releasing less amount of water during cooking (Bhaskar Reddy *et al.,* 2017). There was a negative correlation between diameter shrinkage and cooking yield as evidently reported by different researchers (El-Magoli *et al.,* 1996; Bhaskar Reddy *et al.,* 2015).

The addition of EWP did not significantly ($p > 0.01$) influence the total protein extractability (mg/g of tissue), collagen content, and collagen solubility of RBMS. Both vacuum-tumbling and massaging had a comparable impact on the protein extractability values of RBMS during the restructuring process. The main forces involved in the interaction between meat protein and egg albumen are predominantly of an electrostatic nature (Bernal *et al.,* 1987). Minor differences between collagen content and solubility of control and treated RBMS were found; however, these differences were not statistically significant. The lack of significant variation between RBMS samples with EWP may be attributed to the fact that they were sourced from the same meat, which exhibited consistent collagen content without notable variation (Bhaskar Reddy *et al.,* 2015).

Proximate composition

The addition of different levels of EWP significantly ($p < 0.01$) influenced the proximate composition of RBMS (Table 3). A significant ($p < 0.01$) higher percent moisture, protein, and total ash contents were found in both restructured buffalo meat batter and cooked product added with 3% EWP (T3). Adding EWP to RMBS did not influence ($p > 0.01$) its fat content in both restructured buffalo meat batter and cooked RBMS. The range of percent moisture content in batter RBMS was between 71.63 and 76.67%, and cooked RBMS was between 67.76 and 72.17%, and the percent protein content range was between 17.14 and 23.19%, and between 19.87 and 24.98% in restructured buffalo meat batter and

Table 3. Proximate composition of RBMS prepared with different levels of egg white powder (EWP) (n = 8/group).

Proximate composition	Egg white powder			
	Control (0%)	**T1 (1%)**	**T2 (2%)**	**T3 (3%)**
Uncooked batter				
Moisture (%)	71.63 ± 0.51^{a}	74.98 ± 0.28^{b}	75.13 ± 0.64^{c}	76.67 ± 0.71^{d}
Protein (%)	18.14 ± 0.13^{a}	20.91 ± 0.22^{b}	22.10 ± 0.45^{c}	23.19 ± 0.25^{d}
Fat (%)	2.09 ± 0.10	2.13 ± 0.17	1.99 ± 0.35	2.08 ± 0.15
Total Ash (%)	0.43 ± 0.36^{a}	0.39 ± 0.13^{a}	0.69 ± 0.33^{ab}	0.93 ± 0.41^{c}
Cooked product				
Moisture (%)	67.76 ± 0.27^{a}	69.34 ± 0.18^{b}	72.22 ± 0.52^{c}	72.17 ± 0.40^{c}
Protein (%)	19.87 ± 0.14^{a}	22.30 ± 0.34^{b}	24.08 ± 0.27^{c}	24.98 ± 0.71^{d}
Fat (%)	2.44 ± 0.29	2.29 ± 0.18	2.59 ± 0.37	2.98 ± 0.43
Total Ash (%)	0.59 ± 0.24^{a}	0.61 ± 0.12^{a}	1.21 ± 0.29^{b}	1.89 ± 0.53^{c}

Values are mean ± S.E. Means followed by different lowercase superscripts in similar row differ significantly ($p < 0.01$). C: restructured buffalo meat slices (RBMS) prepared without egg white powder (EWP); T1: RBMS prepared with 1% egg white powder; T2: RBMS prepared with 2% egg white powder; and T3: RBMS prepared with 3% egg white powder.

cooked RBMS, respectively. Increasing the content of EWP in restructured buffalo meat batter and cooked RBMS from 0 to 3% caused a gradual increase in its protein, moisture, and ash contents. The higher moisture content in RBMS added with EWP might have been due to more water retention capacity of EWP during batter formation than control samples. The differences in the proximate components could have been due to the composition of EWP. Binders from non-meat origin have good potential for nutritional and textural enhancement of meat products. EWP could be one of such ingredients with a very good ability of binding and textural improvement. The findings reported in the present work agreed with Teye *et al.* (2012) and Bhaskar Reddy *et al.,* (2023b).

Textural characteristics

The textural characteristics of RBMS were significantly ($p < 0.01$) influenced by the addition of EWP (Table 4). RBMS added with 3% EWP (T3) had significantly ($p < 0.01$) lower hardness, springiness, and chewiness, and higher cohesiveness values and varied gumminess values than control and remaining formulations. With the addition of increased levels of EWP, hardness values gradually decreased from 64.20 to 29.75 N. Generally, EWP acts as a good gelling agent due to the denaturation and unfolding of its proteins during the heat treatment. Adding EWP during restructuring process in the meat system weakened the protein-protein network, resulting in reduced gel strength and associated firmness of the product; however, the ratio of protein-to-protein bonds were the major factor that influence the extent of hybrid complexes formations between the myofibrillar and egg protein (Pietrasik, 2003). Springiness values (mm) varied significantly ($p < 0.01$) with the addition of EWP in RBMS formulations. Increasing the level of EWP in RBMS from 0 to 3% caused a significant ($p < 0.01$) increase in its cohesiveness values, which was attributed to the binding properties of EWP. The decrease in chewiness values by the addition of EWP might have been due to variations in gel forming strength by different concentration of EWP. The variations in the textural quality might have been due to subjecting EWP to heating which would expose a greater number of hydrophobic functional groups that would

Table 4. Textural and sensorial characteristics (8 = extremely liking and 1 = extremely not liking) of RBMS prepared with different levels of egg white powder (EWP) (n = 8/group for textural characteristics, and n = 48/group for sensorial characteristics).

Quality parameter	Egg white powder			
	Control (0%)	**T1 (1%)**	**T2 (2%)**	**T3 (3%)**
Textural characteristic				
Hardness (N)	64.20 ± 4.79^d	35.94 ± 3.44^c	33.30 ± 5.46^b	29.75 ± 5.47^a
Springiness (mm)	1.25 ± 0.08^b	0.82 ± 0.10^a	1.12 ± 0.27^b	0.71 ± 0.23^a
Cohesiveness (Ratio)	0.65 ± 0.19^a	1.01 ± 0.07^b	1.07 ± 0.34^b	1.35 ± 0.12^c
Chewiness (N mm)	51.85 ± 2.47^d	29.76 ± 2.25^b	39.93 ± 1.17^c	28.53 ± 1.60^a
Gumminess (N)	41.49 ± 1.80^d	36.28 ± 1.84^b	35.64 ± 1.13^a	40.17 ± 1.33^c
Sensorial characteristic				
Appearance	6.54 ± 0.27^a	6.91 ± 0.51^b	6.88 ± 0.19^b	7.15 ± 0.23^c
Flavour	6.64 ± 0.37^a	6.89 ± 0.21^b	7.06 ± 0.17^c	7.11 ± 0.43^c
Chewiness	6.95 ± 0.20	7.02 ± 0.31	6.98 ± 0.28	7.08 ± 0.15
Cohesiveness	6.68 ± 0.46^a	7.07 ± 0.10^b	7.29 ± 0.42^c	7.33 ± 0.25^c
Juiciness	6.51 ± 0.27^a	6.89 ± 0.17^b	7.12 ± 0.53^c	7.52 ± 0.37^d
Mouth coating	6.35 ± 0.11^a	6.53 ± 0.23^b	6.56 ± 0.29^b	6.60 ± 0.17^b
Overall palatability	6.63 ± 0.18^a	6.75 ± 0.29^b	7.09 ± 0.42^c	7.29 ± 0.56^d

Values are mean ± S.E. Means followed by different lowercase superscripts in similar row differ significantly ($p < 0.01$). C: restructured buffalo meat slices (RBMS) prepared without egg white powder (EWP); T1: RBMS prepared with 1% egg white powder; T2: RBMS prepared with 2% egg white powder; and T3: RBMS prepared with 3% egg white powder.

involve in more protein-protein interactions with the help of hydrophobic interactions and hydrogen bonding. Egg white improved the textural properties of surimi gels and Bologna sausages (Chen *et al.,* 1993; Carballo *et al.,* 1995). These results agreed with Ahamed *et al.* (2007) on their studies on buffalo meat cutlets, and Bhaskar Reddy *et al.* (2023a) on RBMS added with flax seed flour.

Instrumental colour scores

Adding EWP to RBMS showed a significant ($p < 0.01$) effect on its instrumental colour scores (Figure 1). RBMS added with 3% EWP (T3) had significantly ($p < 0.01$) higher lightness (L*), yellowness (b*), and hue (degrees) values, and lower redness (a*) and chroma values than control sample and RBMS added with 1% (T1) and 2% (T2) EWP. The addition of EWP increased the L* values from 43.33 to 48.97, b* values from 6.41 to 9.66, and hue values from 23.86 to 38.41, and decreased a* values from 19.46 to 12.35, and chroma values from 21.55 to 15.87. The variations in instrumental colour values could be attributed to the inherent biological variability of the meat, and the composition and nature of EWP. Higher lightness (L*) and yellowness (b*) values with the addition of EWP could have been due to the replacement of lean meat with yellow-coloured EWP during restructuring process. The colour characteristics of cooked RBMS were influenced by the pigmentation of the meat used (Bhaskar Reddy *et al.,* 2023b). Generally, cooking or heating increase lightness, and decrease yellowness and redness in meat products (Lee *et al.,* 1998).

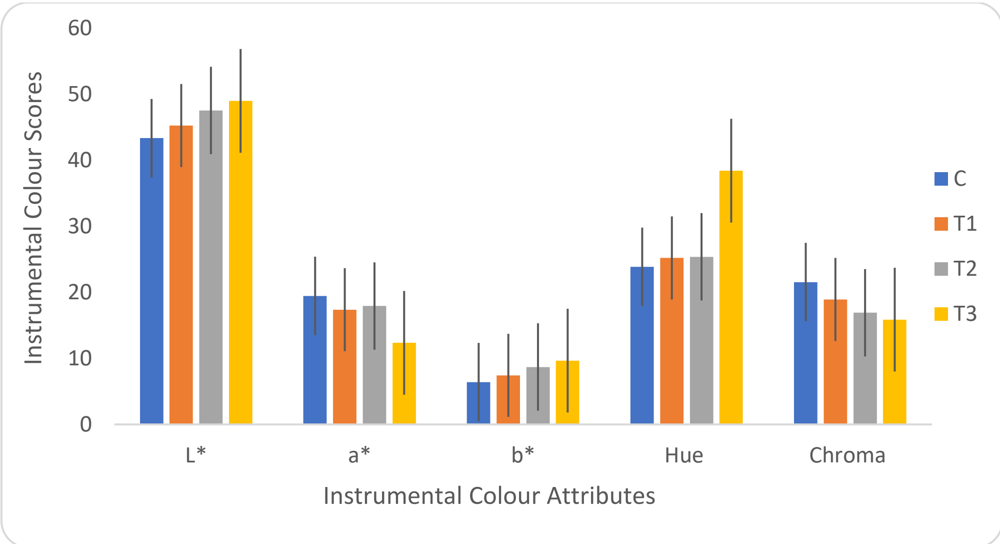

Figure 1. Instrumental colour scores of RBMS prepared with different levels of egg white powder (EWP) ($n = 8$). L*: Lightness; a*: redness, and b*: yellowness. C: restructured buffalo meat slices (RBMS) prepared without egg white powder (EWP); T1: RBMS prepared with 1% egg white powder; T2: RBMS prepared with 2% egg white powder; and T3: RBMS prepared with 3% egg white powder.

Ultra-structural studies

The ultra-structural changes with the addition of EWP in RBMS are depicted in Figure 2. In scanning electron microscopy (SEM), uncooked control RBMS gels (Figure 2a) showed weak binding between myofibrils, and large spaces were also found between muscle cells with few interactions. In addition, the protein exudates were very scanty between the muscle chunks, and the proteins appeared to be fragmented, did not have the solid gel network, and also had more breaks in meat gels. A well-built matrix with thicker strands was observed, and further increased with increasing EWP content in RBMS added with EWP batter gels (Figures 2b - 2d). Ultra-structural changes in cooked products of RBMS added with EWP (Figures 2e - 2h) examined under SEM revealed a strong gel formation between egg proteins, myofibrillar proteins, salt, and polyphosphate, which formed as a mixed gel, and subsequent continuous gelation and network formation happened with the aid of cooking, which caused more binding between meat chunks during restructuring process.

Figure 2. Scanning electron microscope (SEM) micrographs (1.5× K magnification) of uncooked restructured buffalo meat batter without EWP **(2a)**, with 1% EWP **(2b)**, with 2% EWP **(2c)**, and with 3% EWP **(2d)**; and cooked RBMS without EWP **(2e)**, with 1% EWP **(2f)**, with 2% EWP **(2g)**, and with 3% EWP **(2h)**.

The protein matrix had several small particles that represent the homogenous structure of fat globules or emulsion. The factors that influence the batter formation were properties of protein and fat components, temperature of restructuring process, and equipment for batter formation (Gorbatov and Zayas, 1973; Chanyongvorakul *et al.,* 1995). At the centre, a dense area was formed due to the aggregation of several globules that resembled small fat globules surrounded by a thin film, and uniformly distributed protein and fat droplets, and this homogenous structure favoured the interaction between water, fat, and protein which was found in the SEM examination of EWP-added batter samples.

This phenomenon was supported by higher protein solubility, due to which the protein participated well in the interaction with fat and water. During heating process, the myofibrillar and egg proteins decoiled, and the sulphur-containing amino acids formed di-sulphide bonds thus establishing a stable three-dimensional structure, like in the case of T3 samples (Figure 2h). During heating, the adhesion of meat protein with egg protein was apparent (Figures 2f - 2h). Chanyongvorakul *et al.* (1995) also found that the formation of iso-peptide bonds contributed to strong protein-protein interaction that stabilised the network. The protein exudates, which were facilitated by the combined effects of salt, STPP (sodium tripolyphosphate), and mechanical treatments such as vacuum-tumbling and massaging, exhibited excellent binding properties (Katsaras and Peetz, 1989).

Transmission electron microscopy (TEM) images of RBMS added with EWP (Figures 3a - 3d) showed water retention in the meat batter system that was indicated by the protein clusters separated by irregular void spaces. These clusters were connected by fine threads, forming a distinct network. However, the results indicated that the structure became more compact as increasing levels of EWP content would further decrease the void spaces and crevices (Figure 3d). In the case of RBMS added with 3% EWP (T3) (Figure 3d), egg proteins were evenly distributed, filling both the intracellular and some of the intercellular spaces between cells. The egg proteins formed a continuous network, interwoven with water and muscle proteins. At this particular concentration, it was likely that the EWP served as a bonding agent, holding the meat chunks together. These results agreed with Bhaskar Reddy *et al.* (2023a) in RBMS added with different levels of flax seed flour, Chanyongvorakul *et al.* (1995) in globulin gels formed by transglutaminase reaction, and Montero *et al.* (2000) and Perez-Mateos *et al.* (2002) who reported that the alginate network was connected with muscular protein net.

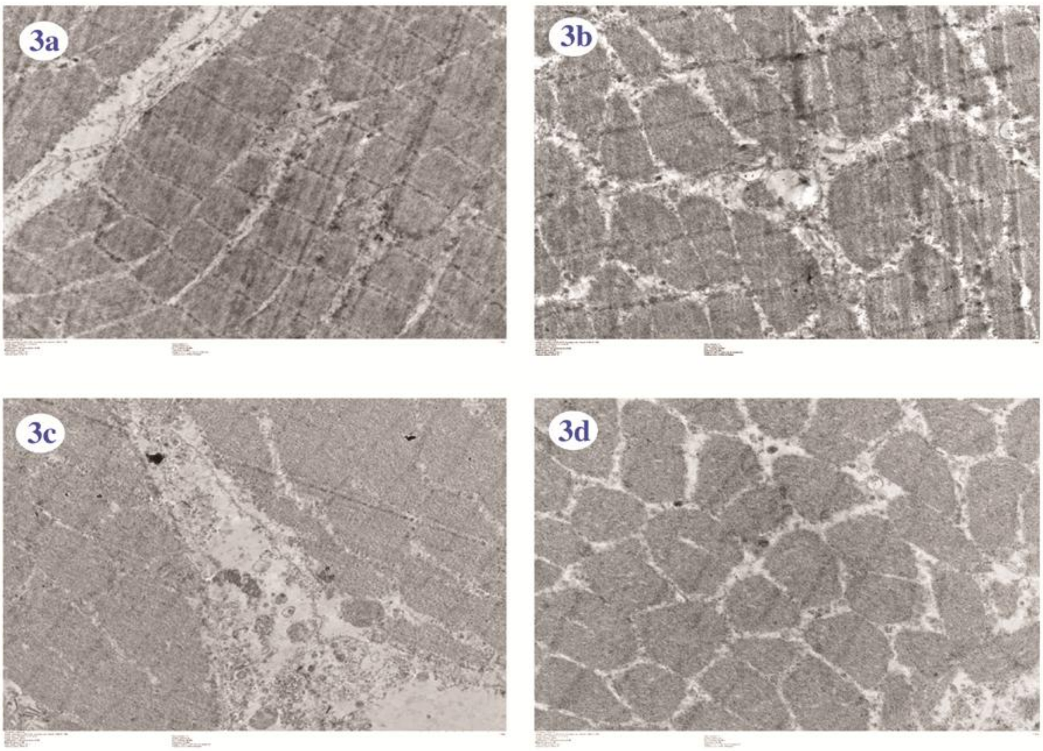

Figure 3. Transmission electron microscope (TEM) micrographs (10× K magnification) of uncooked restructured buffalo meat batter added without EWP **(3a)**, with 1% EWP **(3b)**, with 2% EWP **(3c)**, and with 3% EWP **(3d)**.

Sensory evaluation

The addition of different levels of EWP to RBMS showed significant ($p < 0.01$) effect on its sensory characteristics (Table 4). The range of sensory scores of RBMS were appearance (6.54 - 7.15), flavour (6.64 - 7.11), chewiness (6.95 - 7.08), cohesiveness (6.68 - 7.33), juiciness (6.51 - 7.52), mouth coating (6.35 - 6.60), and overall palatability (6.63 - 7.29). The addition of EWP significantly ($p < 0.01$) increased the sensory scores like appearance, flavour, cohesiveness, juiciness, and overall palatability scores of RBMS. When the level of EWP increased from 0 to 3%, a gradual and significant ($p < 0.01$) increase in the sensory scores were recorded, but it did not show any effect on chewiness scores. RBMS added with EWP had better sensory scores which might have been due to the higher moisture retention in the product. Adding egg white in the product may result in an egg flavour, which was also observed with increased EWP concentrations, and it was suggested not to use higher levels (more than 10 g of dry albumen powder/kg meat) of EWP in meat products to avoid negative changes in flavour (Teye *et al.,* 2012). Adding EWP to RBMS increased its water retention capacity during restructuring process and cooking, thus improved its juiciness. The addition of EWP has the potential to increase the mouth coating of RBMS as well as many sensory attributes. However, the cohesiveness of RBMS prepared using EWP was significantly ($p < 0.01$) firmer than the control RBMS. During heating, the protein coagulates, forming cream-like structures that bind together the batter structural units (Barbut, 1995; Xiong, 1997). Similarly, Kalaikannan *et al.* (2007) noticed higher fat and moisture retention in chicken patties that were added with egg proteins, which also improved its overall sensory attributes.

Conclusion

Based on these results, it was found that physico-chemical quality of RBMS was significantly influenced by the addition of EWP. Moreover, the addition of EWP not only enhanced the nutritive value of RBMS, but also improved its textural quality and instrumental colour attributes. During the restructuring process, the egg proteins formed a remarkable gel network, as evident from both scanning and transmission electron microscopy. Sensory evaluations showed that RBMS added with 3% EWP received higher ratings than other treatments. Overall, these findings clearly indicated that the addition of EWP has positively impacted the quality of buffalo meat batter during restructuring process, and acted as an excellent novel binder.

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