



ISSN Print: 2664-844X
ISSN Online: 2664-8458
NAAS Rating (2026): 4.97
IJAFS 2026; 8(8): 172-189
www.agriculturaljournals.com
Received: 07-06-2026
Accepted: 10-07-2026

Irshad A

Meat Technology Unit,
Department of Livestock
Products Technology and
CHRD-MST, College of
Veterinary and Animal
Sciences, Kerala Veterinary
and Animal Sciences
University, Mannuthy,
Thrissur, Kerala, India

M Muthulakshmi

Department of Livestock
Products Technology (Meat
Science), Veterinary College
and Research Institute,
Namakkal, Tamil Nadu, India

R Rajkumar

Department of Livestock
Products Technology (Meat
Science), Veterinary College
and Research Institute,
Namakkal, Tamil Nadu, India

BD Sharma

Division of Livestock Products
Technology, ICAR-Indian
Veterinary Research Institute,
Izatnagar, Bareilly, Uttar
Pradesh, India

Corresponding Author:

Irshad A

Meat Technology Unit,
Department of Livestock
Products Technology and
CHRD-MST, College of
Veterinary and Animal
Sciences, Kerala Veterinary
and Animal Sciences
University, Mannuthy,
Thrissur, Kerala, India

Combined effects of vacuum packaging and green bell pepper (*Capsicum annuum* L.) paste on the refrigerated stability of restructured buffalo meat steaks

Irshad A, M Muthulakshmi, R Rajkumar and BD Sharma

DOI: <https://www.doi.org/10.33545/2664844X.2026.v8.i8c.1769>

Abstract

This study evaluated the combined effects of vacuum packaging and green bell pepper (*Capsicum annuum* L.) paste on the refrigerated stability of restructured buffalo meat steaks. Five treatment conditions were compared: aerobic packaging without pepper paste, vacuum packaging without pepper paste and vacuum packaging with 5%, 10% or 15% pepper paste. Six matched manufacturing batches were stored at $4\pm 1^\circ\text{C}$ for 20 days and analysed on days 0, 5, 10, 15 and 20. Physicochemical, oxidative, colour, microbial, textural and sensory properties were determined. Treatment condition, storage day and their interaction significantly affected all 29 analysable variables ($P < 0.01$). Vacuum packaging alone reduced day-20 thiobarbituric acid reactive substances from 1.90 to 0.843 mg malondialdehyde/kg, metmyoglobin from 56.10% to 23.22% and total plate count from 7.33 to 5.33 $\log_{10}$ colony-forming units/g compared with aerobic packaging. Overall acceptability correspondingly increased from 3.04 to 5.00. Pepper-paste incorporation provided additional concentration-dependent protection. The vacuum-packaged formulation containing 15% paste recorded the lowest day-20 thiobarbituric acid reactive substances, protein carbonyl content, metmyoglobin and total plate count at 0.375 mg malondialdehyde/kg, 1.70 nmol/mg protein, 11.20% and 3.94 $\log_{10}$ colony-forming units/g respectively. However, the 10% formulation achieved the highest day-20 overall acceptability of 6.12 compared with 5.99 for the 15% formulation. Vacuum packaging substantially delayed refrigerated deterioration while green bell pepper paste provided complementary antioxidant and antimicrobial protection. Incorporation at 15% maximised preservation whereas 10% provided the best balance between stability, texture and sensory acceptability.

Keywords: Buffalo meat, green bell pepper, vacuum packaging, lipid oxidation, microbial stability, sensory quality

1. Introduction

Buffalo meat can be effectively converted into convenient value-added products such as nuggets, rolls and restructured steaks [1, 2]. However, comminution, mixing and restructuring disrupt muscle integrity and increase the exposure of lipids, proteins and pigments to oxygen, thereby increasing susceptibility to oxidative, microbial and sensory deterioration during refrigerated storage [3, 4]. Previous studies on buffalo meat products have demonstrated progressive increases in lipid oxidation, microbial populations and physicochemical spoilage indicators together with declining sensory acceptability during storage [5, 6].

Packaging atmosphere is a major determinant of refrigerated meat stability. Vacuum packaging reduces oxygen availability around the product and can consequently retard lipid and protein oxidation, pigment deterioration and the growth of aerobic spoilage microorganisms. Studies on buffalo meat and buffalo meat products have reported better oxidative, microbiological and sensory stability under vacuum packaging than under oxygen-permeable or conventional aerobic packaging [5-8]. Nevertheless, vacuum packaging does not completely prevent deterioration and may favour the growth of facultative or anaerobic microorganisms during prolonged storage [6]. Combining vacuum packaging with a natural preservative ingredient may therefore provide greater protection than either approach alone. Green bell pepper (*Capsicum annuum* L.) contains phenolic acids, flavonoids, ascorbic acid, carotenoids and other phytochemicals with antioxidant activity [9-11]. These constituents may

retard lipid, protein and pigment oxidation through free-radical scavenging, electron donation, metal chelation and suppression of reactive oxygen species [12, 15, 19]. Phenolic compounds, organic acids and other bioactive constituents may also contribute to microbial inhibition [15, 16, 19]. However, increasing the quantity of vegetable paste in a restructured meat product may dilute meat proteins and alter moisture retention, colour, texture and sensory quality.

Vacuum packaging and plant-derived antioxidants may provide complementary preservation mechanisms. Reduced oxygen availability limits the initiation and propagation of oxidative reactions, while pepper-derived compounds may neutralise reactive species remaining within the product matrix [14-16, 19]. Earlier research demonstrated that combining vacuum packaging with antioxidant ingredients improved the refrigerated stability of buffalo meat nuggets [6]. However, the effects of graded green bell pepper-paste incorporation under vacuum packaging on the oxidative, microbial, textural and sensory stability of restructured buffalo meat steaks have not been established.

The present study therefore compared an aerobically packaged control, a vacuum-packaged control and vacuum-packaged steaks containing 5%, 10% or 15% green bell pepper paste during storage at $4\pm1^\circ\text{C}$ for 20 days. Physicochemical properties, lipid and protein oxidation, residual antioxidant capacity, instrumental colour, metmyoglobin formation, microbial populations, texture profile and sensory quality were evaluated at five-day intervals.

It was hypothesised that vacuum packaging would retard refrigerated deterioration compared with aerobic packaging and that green bell pepper paste would provide additional concentration-dependent antioxidant and antimicrobial protection under vacuum. It was further hypothesised that excessive paste incorporation might compromise the structural and sensory properties of the restructured meat product despite providing greater preservation.

2. Materials and methods

2.1 Experimental design

The experiment evaluated five packaging - formulation conditions during refrigerated storage:

- AP0: aerobic packaging with 0% green bell pepper paste
- VP0: vacuum packaging with 0% green bell pepper paste
- VP5: vacuum packaging with 5% green bell pepper paste
- VP10: vacuum packaging with 10% green bell pepper paste

- VP15: vacuum packaging with 15% green bell pepper paste

Six independent manufacturing batches were prepared. Each batch contained the 0%, 5%, 10% and 15% green bell pepper-paste formulations. The 0% formulation was divided between aerobic and vacuum packaging to provide AP0 and VP0 respectively. Separate packages were allocated to days 0, 5, 10, 15 and 20 of storage at $4\pm1^\circ\text{C}$.

The experiment followed a 5×5 repeated-measures arrangement comprising five treatment conditions and five storage periods. Treatment condition and storage day were considered fixed effects while manufacturing batch was considered a random subject. Because pepper-containing steaks were evaluated only under vacuum packaging, packaging method and pepper level were not analysed as completely crossed factorial effects.

2.2 Source and preparation of buffalo meat

Buffalo meat was obtained from the round portion of commercially slaughtered spent adult buffalo carcasses within approximately 5 to 6 h of slaughter from a licensed meat outlet in Bareilly, Uttar Pradesh, India. Visible external fat, fascia and connective tissue were removed. The trimmed meat was divided into approximately 500 g portions, packed in low-density polyethylene pouches and conditioned at $4\pm1^\circ\text{C}$ for approximately 24 h. It was subsequently stored at $-18\pm2^\circ\text{C}$ until product preparation.

Before processing, the frozen meat was thawed for approximately 16 h at $4\pm1^\circ\text{C}$. The partially thawed meat was sliced across the muscle fibres into approximately 1-cm-thick slices and cut into cubes of approximately 1 cm^3 . Meat temperature was maintained below 10°C throughout processing.

2.3 Preparation of green bell pepper paste

Fresh, mature and undamaged green bell peppers (*Capsicum annum* L.) were procured from a local vegetable market in Bareilly. The peppers were washed under potable running water and allowed to drain. Pedicels, seeds and non-fleshy internal portions were removed. The edible portions were cut into small pieces and homogenised for approximately 5 min using a mixer-grinder until a uniform paste was obtained. Freshly prepared paste was used immediately.

2.4 Product formulation

Green bell pepper paste replaced an equivalent quantity of lean buffalo meat while the quantities of refined wheat flour, curing solution, spice mixture and condiment mixture remained constant.

Table 1: Formulation of restructured buffalo meat steaks

Ingredient	0% formulation	5% formulation	10% formulation	15% formulation
Lean buffalo meat (g)	75	70	65	60
Green bell pepper paste (g)	0	5	10	15
Refined wheat flour (g)	3	3	3	3
Curing solution (ml)	15	15	15	15
Spice mixture (g)	2	2	2	2
Condiment mixture (g)	5	5	5	5
Total	100	100	100	100

Green bell pepper paste replaced an equivalent quantity of lean buffalo meat. The 0% formulation was divided between aerobic packaging (AP0) and vacuum packaging (VP0).

2.5 Preparation of restructured buffalo meat steaks

Partially thawed meat cubes were placed in a paddle mixer (Hobart, Model N50G, USA). Mixing was initiated at low speed with gradual addition of the curing solution. The meat was massaged for 12 min to extract salt-soluble myofibrillar proteins and develop a tacky protein exudate.

Refined wheat flour, spice mixture, condiment paste and the assigned quantity of green bell pepper paste were then added. Mixing was continued for 3 min at medium speed. The total massaging period was 15 min.

The mixture was transferred into stainless-steel moulds and compressed using a wooden press to remove entrapped air. The moulds were closed and steam-cooked without pressure for approximately 40 min until the internal product temperature reached approximately 85°C. The cooked blocks were cooled and cut into steaks of approximately 10-mm thickness.

2.6 Packaging and refrigerated storage

The AP0 steaks were individually packaged under aerobic conditions in heat-sealed 250-gauge low-density polyethylene pouches. The VP0, VP5, VP10 and VP15 steaks were individually placed in 250-gauge low-density polyethylene pouches, evacuated using a vacuum-packaging machine and heat-sealed.

All packages were stored in a temperature-controlled refrigerated cabinet at 4±1°C for 20 days. Separate packages from each treatment and manufacturing batch were allocated to days 0, 5, 10, 15 and 20 and withdrawn at the designated intervals for physicochemical, oxidative, colour, microbiological, textural and sensory analyses.

2.7 Physicochemical analyses

2.7.1 pH and water activity

For pH determination, 10 g of sample was homogenised with 50 mL of distilled water. The pH of the homogenate was measured using a calibrated digital pH meter fitted with a combined glass electrode (EL 68, ELICO Pvt. Ltd., Model LI-120, India).

Water activity was determined using a calibrated water-activity meter (LabSwift, Novasina AG, Switzerland). A representative sample was placed in the sample cup and the value was recorded after the instrument reading had stabilised.

2.7.2 Storage loss

Each packaged sample was weighed before storage and again at its assigned storage interval. Storage loss was calculated as follows:

$$\text{Storage loss (\%)} = \frac{[(\text{initial sample weight} - \text{stored sample weight}) / \text{initial sample weight}] \times 100}{}$$

2.7.3 Total volatile basic nitrogen

Total volatile basic nitrogen was determined using the Conway microdiffusion method [21]. Ten grams of sample was homogenised with 100 mL of distilled water, allowed to stand for 30 min and filtered. One millilitre of the filtrate and 1 mL of saturated potassium carbonate solution were placed in the outer chamber of a Conway diffusion dish. The inner chamber contained 1 mL of 2% boric acid solution with methyl red and bromocresol green mixed indicator.

The dish was sealed and incubated at 37°C for 90-120 min. The volatile bases absorbed by the boric acid solution were

titrated against 0.01 N hydrochloric acid. The results were expressed as mg N/100 g of product.

2.8 Lipid oxidation and residual antioxidant capacity

2.8.1 Peroxide value

Peroxide value was determined using the ferric thiocyanate method [22]. Lipids were extracted using chloroform at a ratio of 2:1 (v/v). An aliquot of the lipid extract was reacted with ferrous chloride and ammonium thiocyanate solutions. The resulting ferric thiocyanate complex was measured spectrophotometrically at 500 nm. The results were expressed as meq O₂/kg fat.

2.8.2 Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances were determined using the distillation method [23]. The sample distillate was reacted with 2-thiobarbituric acid in glacial acetic acid and heated in a boiling water bath for 30 min. Absorbance was measured at 538 nm and the results were expressed as mg malondialdehyde/kg of product.

2.8.3 Residual antioxidant capacity

Residual antioxidant capacity was determined by measuring 2, 2-diphenyl-1-picrylhydrazyl radical-scavenging activity [24]. The antioxidant fraction was extracted using 80% aqueous methanol at a sample-to-solvent ratio of 1:10 (w/v) for 2 h at 25±2°C with continuous shaking. Absorbance was measured at 517 nm.

The activity measured on day 0 was designated as 100% for each treatment and residual antioxidant capacity was calculated as follows:

$$\text{Residual antioxidant capacity (\%)} = (\text{DPPH activity on day } n / \text{DPPH activity on day 0}) \times 100$$

2.9 Protein oxidation

2.9.1 Protein carbonyl content

Protein carbonyl content was determined by 2, 4-dinitrophenylhydrazine derivatisation [25-27]. Extracted myofibrillar proteins were reacted with 10 mM 2, 4-dinitrophenylhydrazine prepared in 2 M hydrochloric acid. Proteins were precipitated using 20% trichloroacetic acid, washed repeatedly with ethanol acetate at a ratio of 1:1 (v/v) and dissolved in 6 M guanidine hydrochloride.

Absorbance was measured at 370 nm and carbonyl content was expressed as nmol carbonyl/mg protein.

2.9.2 Total sulfhydryl groups

Total sulfhydryl groups were determined using 5, 5'-dithiobis-(2-nitrobenzoic acid), according to Ellman [29], with modifications for myofibrillar protein systems [28]. The protein extract was reacted with the reagent in Tris-glycine buffer at pH 8.0 containing 8 M urea. The reaction mixture was incubated at room temperature for 30 min and absorbance was measured at 412 nm.

The results were expressed as nmol sulfhydryl groups/mg protein.

2.10 Instrumental colour and metmyoglobin

Instrumental colour was measured on freshly cut steak surfaces using a calibrated HunterLab MiniScan XE Plus colorimeter (Hunter Associates Laboratory Inc., USA) with illuminant D65 and a 10° standard observer. The instrument

was calibrated using standard black and white reference tiles before measurement.

The CIELAB coordinates L^* , a^* and b^* were recorded at three randomly selected positions on each freshly cut surface. The mean of the three measurements was used as the batch observation.

Metmyoglobin percentage was determined spectrophotometrically according to Krzywicki [30], as modified by Tang *et al.* [31]. A representative sample was homogenised in 0.04 M phosphate buffer at pH 6.8. The homogenate was centrifuged and filtered and absorbance was measured at 503, 557, 582 and 700 nm. The results were expressed as metmyoglobin percentage.

2.11 Microbiological analyses

Twenty-five grams of sample was aseptically transferred into a sterile stomacher bag containing 225 mL of sterile 0.1% peptone water. The sample was homogenised for 60 s to obtain the initial 10^{-1} dilution. Further ten-fold serial dilutions were prepared using sterile 0.1% peptone water and appropriate dilutions were plated in duplicate.

Total plate count was determined on Plate Count Agar after incubation at 37°C for 24–48 h [32]. Psychrotrophic microorganisms were enumerated on Plate Count Agar after incubation at 7°C for 10 days. Lactic acid bacteria were enumerated on de Man, Rogosa and Sharpe agar following anaerobic incubation at 30°C for 48–72 h [33]. Coliforms were enumerated on Violet Red Bile Agar after incubation at 37°C for 24 h. Yeasts and moulds were enumerated on Sabouraud Dextrose Agar after incubation at 25–27°C for 3–5 days [34].

Microbial counts were expressed as $\log_{10}$ CFU/g. Coliforms were not detected in any treatment during storage and were therefore reported descriptively and excluded from inferential statistical analysis.

2.12 Texture-profile analysis

Texture-profile analysis was performed using an EZ-SX Texture Analyser (Shimadzu Corporation, Kyoto, Japan) equipped with TrapeziumX software and fitted with a cylindrical compression probe [35]. Uniform samples measuring $2 \times 2 \times 2$ cm were cut from the cooked product blocks and subjected to two consecutive compression cycles to 70% of their original height.

The pre-test, test and post-test speeds were 2.0, 1.0 and 5.0 mm/s respectively. The interval between the two compression cycles was 5 s. Hardness, cohesiveness, springiness, chewiness and resilience were calculated from the force-time curves. Hardness and chewiness were expressed in newtons while cohesiveness, springiness and resilience were expressed as dimensionless ratios.

2.13 Sensory evaluation

Sensory evaluation was conducted at each storage interval by a semi-trained panel comprising seven scientists and postgraduate students from the Division of Livestock Products Technology. Before evaluation, the samples were reheated to a standardised serving temperature and cut into uniform portions. Samples were presented in odour-free disposable containers identified with randomly assigned three-digit codes. The serving order was randomised and drinking water was provided for palate cleansing between samples.

Panellists evaluated general appearance, flavour, juiciness, texture, binding, saltiness and overall acceptability using an eight-point descriptive scale, where 8 represented extremely desirable and 1 represented extremely undesirable. Scores were recorded individually on paper evaluation forms.

The mean panel score for each independent manufacturing batch was treated as one experimental observation. Thus, six batch-level sensory observations were available for every treatment condition $\times$ storage-day combination.

2.14 Statistical analysis

Data were analysed using a two-way repeated-measures analysis of variance. Treatment condition, storage day and their interaction were included as fixed effects while manufacturing batch was treated as the matched experimental subject or block:

$$Y_{ijk} = \mu + T_i + D_j + (T \times D)_{ij} + B_k + \epsilon_{ijk}$$

where Y_{ijk} was the observed response, μ was the overall mean, T_i was the fixed effect of treatment condition, D_j was the fixed effect of storage day, $(T \times D)_{ij}$ was the treatment condition $\times$ storage-day interaction, B_k was the effect of manufacturing batch and ϵ_{ijk} was the residual error.

Microbial counts were $\log_{10}$ -transformed before statistical analysis. Model residuals were assessed for normality and homogeneity of variance. When significant effects were detected, treatment means within each storage day and storage-day means within each treatment were compared using Tukey-adjusted pairwise comparisons.

The F statistics were reported for the treatment-condition effect [F(4, 20)], storage-day effect [F(4, 20)] and treatment condition $\times$ storage-day interaction [F(16, 80)]. Results were expressed as mean $\pm$ Standard error and statistical significance was declared at $P < 0.05$.

Prespecified within-batch contrasts at day 20 compared AP0 with VP0, VP0 with each pepper-containing treatment and VP10 with VP15. The resulting P values were adjusted using the Holm procedure to control the familywise error rate. A planned linear contrast across VP0, VP5, VP10 and VP15 was used to evaluate the concentration-dependent effect of green bell pepper paste under vacuum packaging.

Six independent manufacturing batches were available for each treatment condition $\times$ storage-day combination ($n = 6$). For sensory variables, $n = 6$ represented the six batch-level mean panel scores. Statistical analyses were performed using SPSS version 24 (IBM Corp., Armonk, NY, USA).

3. Results

3.1 Physicochemical stability

Treatment condition, storage day and their interaction significantly affected pH, water activity, storage loss and total volatile basic nitrogen (TVB-N) ($P < 0.01$; Table 2).

3.1.1 pH

The pH initially declined and subsequently increased in all treatments. AP0 decreased from 6.163 on day 0 to 6.012 on day 10 and increased to 6.350 on day 20. The corresponding day-20 value for VP0 was lower at 6.267, indicating that vacuum packaging moderated the late-storage increase.

Green bell pepper paste further reduced pH under vacuum packaging. Day-20 values decreased progressively from 6.267 in VP0 to 6.192, 6.112 and 6.025 in VP5, VP10 and

VP15 respectively. All five treatments differed significantly on day 20.

3.1.2 Water activity

Water activity declined during storage in all treatment conditions. AP0 showed the greatest reduction, from 0.995 on day 0 to 0.971 on day 20. Vacuum packaging improved water-activity retention, with VP0 recording 0.983 on day 20.

The day-20 values for VP5, VP10 and VP15 were 0.981, 0.978 and 0.973 respectively. Thus, water activity tended to decline with increasing pepper-paste concentration, although some adjacent vacuum-packaged treatments did not differ significantly.

3.1.3 Storage loss

Storage loss increased progressively throughout storage. AP0 recorded the greatest day-20 loss of 6.55%, whereas VP0 recorded 3.61%. Vacuum packaging therefore reduced day-20 storage loss by approximately 44.9% compared with aerobic packaging.

Among the vacuum-packaged treatments, storage loss increased with pepper-paste concentration. Day-20 values were 3.61%, 3.81%, 4.14% and 4.57% in VP0, VP5, VP10 and VP15 respectively. VP0 and VP5 were comparable, while VP10 and VP15 showed significantly greater losses.

3.1.4 Total volatile basic nitrogen

TVB-N increased significantly in all treatments but the rate of accumulation differed markedly. AP0 increased from 9.47 mg N/100 g on day 0 to 32.47 mg N/100 g on day 20. Vacuum packaging alone reduced the day-20 value to 22.14 mg N/100 g, representing a reduction of approximately 31.8% relative to AP0.

Pepper paste produced an additional concentration-dependent reduction in TVB-N under vacuum packaging. Day-20 values were 18.94, 16.97 and 15.14 mg N/100 g in VP5, VP10 and VP15 respectively. All treatments differed significantly at the end of storage, with VP15 showing the least accumulation.

3.2 Lipid oxidation and residual antioxidant capacity

Peroxide value, thiobarbituric acid reactive substances and residual antioxidant capacity were significantly affected by treatment condition, storage day and their interaction ($P < 0.001$; Table 3).

3.2.1 Peroxide value

Peroxide value increased continuously during storage. AP0 increased from 1.18 meq O₂/kg fat on day 0 to 9.34 meq O₂/kg fat on day 20. The day-20 value of VP0 was significantly lower at 4.45 meq O₂/kg fat, corresponding to a reduction of approximately 52.3% compared with AP0.

Further reductions occurred with increasing pepper-paste concentration. On day 20, peroxide values were 3.46, 2.75 and 2.16 meq O₂/kg fat in VP5, VP10 and VP15 respectively. All treatments differed significantly at every storage interval after day 0.

3.2.2 Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances increased in all treatments but accumulated most rapidly in AP0. Values increased from 0.275 to 1.900 mg malondialdehyde/kg between days 0 and 20. Vacuum packaging reduced the day-

20 value to 0.843 mg malondialdehyde/kg, a reduction of approximately 55.6%.

Pepper-paste incorporation provided further protection. Day-20 values decreased progressively from 0.843 mg malondialdehyde/kg in VP0 to 0.620, 0.497 and 0.375 mg malondialdehyde/kg in VP5, VP10 and VP15 respectively. VP15 recorded approximately 80.3% less secondary lipid oxidation than AP0 at the end of storage.

3.2.3 Residual antioxidant capacity

Residual antioxidant capacity declined significantly throughout storage. AP0 retained only 24.8% of its initial activity on day 20, whereas VP0 retained 66.3%.

The pepper-containing treatments retained progressively greater antioxidant activity. Day-20 residual capacities were 71.9%, 76.3% and 80.1% in VP5, VP10 and VP15 respectively. Thus, VP15 showed the greatest antioxidant retention, while AP0 showed the most rapid depletion.

3.3 Protein oxidation

Treatment condition, storage day and their interaction significantly affected protein carbonyl and total sulphydryl contents ($P < 0.001$; Table 4).

3.3.1 Protein carbonyl content

Protein carbonyl content increased throughout storage in all treatment conditions. AP0 increased from 1.35 nmol/mg protein on day 0 to 5.59 nmol/mg protein on day 20. Vacuum packaging reduced the day-20 value to 3.48 nmol/mg protein, representing approximately 37.8% less carbonyl accumulation.

Pepper paste further limited protein oxidation in a concentration-dependent manner. Day-20 carbonyl contents were 2.62, 2.12 and 1.70 nmol/mg protein in VP5, VP10 and VP15 respectively. All treatment conditions differed significantly on day 20 and VP15 showed the lowest carbonyl accumulation.

3.3.2 Total sulphydryl groups

Total sulphydryl content declined significantly during storage. AP0 decreased from 79.4 nmol/mg protein on day 0 to 32.1 nmol/mg protein on day 20. VP0 retained a significantly higher day-20 value of 55.7 nmol/mg protein.

Sulphydryl preservation improved progressively with increasing pepper-paste concentration. Day-20 values were 62.3, 67.6 and 71.2 nmol/mg protein in VP5, VP10 and VP15 respectively. VP15 therefore retained approximately 86.8% of its initial sulphydryl content compared with 40.4% in AP0 and 70.2% in VP0.

3.4 Instrumental colour and pigment stability

Treatment condition, storage day and their interaction significantly affected L*, a*, b* and metmyoglobin percentage ($P < 0.001$; Table 5).

3.4.1 Lightness

Lightness increased during storage in all treatment conditions. AP0 showed the greatest increase, from 42.58 on day 0 to 45.12 on day 20. Vacuum packaging moderated this change, with VP0 reaching 43.78 on day 20.

Green bell pepper paste progressively reduced L* because of formulation-related colour differences. Day-20 values were 42.18, 40.37 and 38.73 in VP5, VP10 and VP15

respectively. All five treatments differed significantly on day 20.

3.4.2 Redness

Redness declined significantly throughout storage. AP0 showed the greatest reduction, decreasing from 9.26 on day 0 to 2.28 on day 20. VP0 retained substantially greater redness and recorded a day-20 a^* value of 7.52.

Initial redness decreased with increasing pepper-paste concentration, with day-0 values of 6.72, 4.06 and 1.53 in VP5, VP10 and VP15 respectively. Nevertheless, the decline during storage was slower in the pepper-containing treatments. Their respective day-20 values were 5.75, 3.48 and 1.20. All treatment conditions differed significantly at the end of storage.

3.4.3 Yellowness

Yellowness increased during storage and with increasing pepper-paste concentration. AP0 increased from 11.48 on day 0 to 14.01 on day 20 while VP0 increased to 12.75.

The pepper-containing treatments had greater initial and final b^* values. On day 20, yellowness values were 13.93, 14.86 and 15.93 in VP5, VP10 and VP15 respectively. VP15 consistently showed the greatest yellowness throughout storage.

3.4.4 Metmyoglobin

Metmyoglobin increased significantly in all treatments but accumulated most rapidly in AP0. Its value increased from 9.18% on day 0 to 56.10% on day 20. Vacuum packaging reduced day-20 metmyoglobin to 23.22%, representing approximately 58.6% less pigment oxidation than AP0.

Green bell pepper paste produced additional concentration-dependent protection. Day-20 metmyoglobin values were 17.35%, 14.57% and 11.20% in VP5, VP10 and VP15 respectively. Relative to AP0, these values represented reductions of approximately 69.1%, 74.0% and 80.0%. All treatments differed significantly on day 20.

3.5 Microbiological quality

Treatment condition, storage day and their interaction significantly affected total plate, psychrotrophic, lactic acid bacteria and yeast and mould counts ($P < 0.01$; Table 6). Coliforms were not detected and were not subjected to inferential analysis.

3.5.1 Total plate count

Total plate count increased throughout storage in all treatments. AP0 increased from 2.88 log₁₀ CFU/g on day 0 to 7.32 log₁₀ CFU/g on day 20. Vacuum packaging reduced the day-20 count to 5.33 log₁₀ CFU/g, a difference of 2.00 log units.

Pepper-paste incorporation further restricted microbial growth. Day-20 counts were 4.81, 4.36 and 3.94 log₁₀ CFU/g in VP5, VP10 and VP15 respectively. All treatment conditions differed significantly at the end of storage and VP15 showed the lowest count.

3.5.2 Psychrotrophic count

Psychrotrophic counts increased progressively and followed a pattern similar to total plate counts. AP0 increased from 2.58 to 7.58 log₁₀ CFU/g between days 0 and 20, whereas VP0 reached 5.31 log₁₀ CFU/g.

Day-20 counts decreased progressively with pepper concentration and were 4.85, 4.36 and 3.91 log₁₀ CFU/g in VP5, VP10 and VP15 respectively. Thus, VP15 recorded approximately 3.67 log units fewer psychrotrophic microorganisms than AP0.

3.5.3 Lactic acid bacteria

Lactic acid bacteria increased significantly during storage. Unlike the other microbial groups, VP0 recorded greater counts than AP0 from day 5 onwards. On day 20, VP0 reached 7.08 log₁₀ CFU/g compared with 6.27 log₁₀ CFU/g in AP0.

Pepper paste reduced lactic acid bacteria in a concentration-dependent manner under vacuum packaging. Day-20 values were 6.27, 5.38 and 4.62 log₁₀ CFU/g in VP5, VP10 and VP15 respectively. AP0 and VP5 were comparable at the end of storage while VP0 recorded the greatest count.

3.5.4 Coliform count

Coliforms were not detected in any treatment at any storage interval. The data were therefore reported descriptively and excluded from statistical analysis.

3.5.5 Yeast and mould count

Yeast and mould counts increased during storage, most markedly in AP0. Its count increased from 1.27 log₁₀ CFU/g on day 0 to 3.83 log₁₀ CFU/g on day 20. VP0 recorded a substantially lower day-20 value of 1.79 log₁₀ CFU/g.

Pepper-paste incorporation provided further inhibition, with day-20 values of 1.53, 1.26 and 1.02 log₁₀ CFU/g in VP5, VP10 and VP15 respectively. All treatment conditions differed significantly on day 20 and VP15 showed the least increase.

3.6 Texture-profile stability

Treatment condition, storage day and their interaction significantly affected hardness, cohesiveness, springiness, chewiness and resilience ($P < 0.001$; Table 7).

3.6.1 Hardness

Hardness declined in all treatments during storage. AP0 decreased from 67.33 N on day 0 to 39.05 N on day 20, representing a reduction of 42.0%. Vacuum packaging improved hardness retention, with VP0 recording 50.38 N on day 20 and a reduction of 25.2%.

Increasing pepper-paste concentration reduced initial hardness but progressively limited storage-related softening. Day-20 values were 48.54, 46.02 and 42.27 N in VP5, VP10 and VP15 respectively. The proportional reductions were 20.3%, 16.3% and 12.1%. Although VP15 remained the softest formulation, it showed the greatest relative preservation.

3.6.2 Cohesiveness and springiness

Cohesiveness decreased from 0.617 to 0.382 in AP0 and to 0.477 in VP0. The day-20 values for VP5, VP10 and VP15 were 0.483, 0.480 and 0.453 respectively. AP0 retained 62.0% of its initial cohesiveness compared with 77.3%, 81.8%, 85.7% and 90.5% in VP0, VP5, VP10 and VP15 respectively.

Springiness followed a similar pattern. AP0 declined from 0.795 to 0.540 while VP0 retained a day-20 value of 0.643. Corresponding values for VP5, VP10 and VP15 were 0.646, 0.639 and 0.617. Relative retention increased from 68.0% in

AP0 to 80.8%, 85.0%, 88.8% and 92.1% in VP0, VP5, VP10 and VP15 respectively.

3.6.3 Chewiness

Chewiness declined markedly because it was derived from hardness, cohesiveness and springiness. AP0 decreased from 33.03 N on day 0 to 8.08 N on day 20, a reduction of 75.6%. VP0 retained a significantly greater day-20 value of 15.45 N.

Day-20 chewiness values were 15.14, 14.13 and 11.82 N in VP5, VP10 and VP15 respectively. VP0 and VP5 were comparable at the end of storage. The proportional decline decreased progressively from 53.2% in VP0 to 44.6%, 36.3% and 26.7% in VP5, VP10 and VP15 respectively.

3.6.4 Resilience

Resilience decreased from 0.380 to 0.209 in AP0 and to 0.277 in VP0. Day-20 values were 0.276, 0.267 and 0.249 in VP5, VP10 and VP15 respectively.

AP0 retained only 55.0% of its initial resilience compared with 72.9%, 79.0%, 83.3% and 89.0% in VP0, VP5, VP10 and VP15 respectively. Thus, vacuum packaging reduced structural deterioration while increasing pepper concentration further improved proportional texture retention despite lowering initial firmness.

3.7 Sensory quality

Treatment condition, storage day and their interaction significantly affected general appearance, flavour, juiciness, texture, binding, saltiness and overall acceptability ($P < 0.001$; Table 8).

3.7.1 General appearance

General-appearance scores declined in all treatments. AP0 decreased from 7.26 on day 0 to 4.00 on day 20 while VP0 retained a significantly higher score of 5.45.

VP10 recorded the highest scores during most of storage and reached 6.24 on day 20. The corresponding values for VP5 and VP15 were 5.90 and 6.14. VP10 and VP15 were comparable at the end of storage. Relative declines were 44.9%, 24.8%, 19.4%, 15.6% and 11.4% in AP0, VP0, VP5, VP10 and VP15 respectively.

3.7.2 Flavour

Flavour declined from 7.14 to 3.49 in AP0 and to 5.13 in VP0. Pepper incorporation significantly improved flavour retention, with day-20 scores of 5.66, 6.14 and 6.31 in VP5, VP10 and VP15 respectively.

VP10 had the highest flavour during the earlier storage intervals, whereas VP15 recorded the highest score on day 20. The proportional decline was greatest in AP0 at 51.1% and lowest in VP15 at 11.8%.

3.7.3 Juiciness

AP0 declined from 6.93 to 4.20 while VP0 retained a day-20 score of 5.72. VP5, VP10 and VP15 recorded final scores of 6.25, 6.47 and 6.10 respectively.

VP10 retained the greatest juiciness throughout most of storage. Although VP15 showed the smallest proportional decline, its score remained below VP10 because of its lower initial juiciness.

3.7.4 Sensory texture

Initial sensory-texture scores were highest in VP5 at 7.41, followed by AP0 and VP0 at 7.17, VP10 at 6.81 and VP15 at 6.47. Scores declined in all treatments but deterioration was greatest in AP0, which reached 4.42 on day 20.

Day-20 scores were 5.65, 6.12, 5.84 and 5.79 in VP0, VP5, VP10 and VP15 respectively. VP5 therefore maintained the highest sensory-texture score, while VP10 and VP15 showed smaller proportional declines from their initial values.

3.7.5 Binding

Binding decreased from 7.26 to 4.16 in AP0 and to 5.57 in VP0. Day-20 scores were 5.94, 6.29 and 6.05 in VP5, VP10 and VP15 respectively.

VP10 retained the highest binding throughout storage. Although VP15 had the lowest initial score, it underwent the smallest proportional decline at 10.8% compared with 42.8% in AP0.

3.7.6 Saltiness

Saltiness declined less markedly than the other sensory attributes. AP0 and VP0 decreased from 6.44 to 5.41 and 5.71 respectively.

Pepper-containing treatments consistently received higher scores. Day-20 values were 6.53, 6.96 and 7.03 in VP5, VP10 and VP15 respectively. VP10 and VP15 were comparable and retained the highest perceived saltiness.

3.7.7 Overall acceptability

Overall acceptability decreased significantly in all treatments. AP0 declined from 6.92 on day 0 to 3.04 on day 20, whereas VP0 retained a score of 5.00. Vacuum packaging therefore substantially slowed the loss of sensory acceptance.

VP10 recorded the highest overall acceptability throughout storage, decreasing from 7.48 to 6.12. Day-20 scores for VP5 and VP15 were 5.68 and 5.99 respectively. The planned day-20 comparison showed that VP10 had slightly but significantly higher overall acceptability than VP15 after Holm adjustment ($P = 0.046$).

Relative declines were 56.1%, 27.8%, 21.3%, 18.2% and 13.9% in AP0, VP0, VP5, VP10 and VP15 respectively. VP15 showed the greatest proportional retention, while VP10 provided the highest absolute acceptability and the best overall sensory balance.

Pearson correlation analysis was performed using the 25 treatment condition $\times$ storage-day means to examine relationships among oxidative, physicochemical, microbial, textural and sensory variables. Principal component analysis was conducted using 28 standardised variables. Coliform count was excluded because coliforms were not detected and chewiness was excluded because it was mathematically derived from hardness, cohesiveness and springiness. Treatment trajectories were evaluated from the first two principal-component scores and two-dimensional displacement between days 0 and 20 was calculated.

3.8 Relationships among quality-deterioration variables

3.8.1 Lipid, protein and pigment oxidation

Peroxide value was strongly positively correlated with thiobarbituric acid reactive substances ($r = 0.991$, $P < 0.001$), indicating close agreement between primary and secondary lipid-oxidation measurements.

Thiobarbituric acid reactive substances were strongly correlated with protein carbonyl content ($r = 0.987$, $P < 0.001$) and metmyoglobin percentage ($r = 0.996$, $P < 0.001$). Lipid, protein and pigment oxidation therefore progressed concurrently during refrigerated storage.

Protein carbonyl content was strongly inversely correlated with total sulfhydryl groups ($r = -0.994$, $P < 0.001$), confirming that increasing carbonyl formation was accompanied by progressive thiol-group depletion.

Because the initial redness of the formulations differed according to pepper-paste concentration, the relationship between a^* and metmyoglobin was evaluated separately within each treatment. Strong inverse correlations were observed in AP0, VP0, VP5, VP10 and VP15, with r values ranging from -0.982 to -0.999 ($P \leq 0.003$). Loss of redness within each formulation was therefore closely associated with metmyoglobin accumulation.

3.8.2 Antioxidant capacity and sensory quality

Residual antioxidant capacity was strongly inversely correlated with thiobarbituric acid reactive substances ($r = -0.972$, $P < 0.001$). Samples retaining greater antioxidant activity accumulated fewer secondary lipid-oxidation products.

Residual antioxidant capacity was also strongly positively correlated with overall acceptability ($r = 0.971$, $P < 0.001$). Greater antioxidant retention was therefore associated with better preservation of sensory quality.

3.8.3 Microbial growth, TVB-N and acceptability

Total plate and psychrotrophic counts were strongly positively correlated with TVB-N, with r values of 0.983 and 0.973 respectively ($P < 0.001$). Increasing microbial populations were therefore closely associated with volatile-base accumulation.

TVB-N was strongly inversely correlated with overall acceptability ($r = -0.979$, $P < 0.001$). Total plate count was similarly associated with declining acceptability ($r = -0.965$, $P < 0.001$). Microbial growth, chemical spoilage and sensory deterioration consequently developed as interconnected processes.

3.8.4 Instrumental and sensory relationships

Storage loss was inversely correlated with sensory juiciness ($r = -0.842$, $P < 0.001$), indicating that greater purge formation was associated with reduced perceived moisture.

Instrumental hardness was positively correlated with sensory texture ($r = 0.813$, $P < 0.001$). Storage-related changes measured through texture-profile analysis were therefore perceptible to the sensory panel.

3.9 Principal component analysis

Principal component analysis was conducted using 28 standardised physicochemical, oxidative, colour, microbiological, textural and sensory variables. The first two principal components explained 93.76% of the total variation. PC1 accounted for 70.97% and PC2 for 22.80%.

PC1 represented the overall refrigerated-deterioration axis. Positive loadings were associated with TVB-N, total plate count, psychrotrophic count, peroxide value, thiobarbituric acid reactive substances, protein carbonyl content, metmyoglobin and storage loss. Negative loadings were associated with residual antioxidant capacity, sulfhydryl

groups, general appearance, flavour, juiciness, binding, sensory texture and overall acceptability.

Movement from negative to positive PC1 scores therefore represented progression from fresh, antioxidant-rich and sensorially acceptable samples towards oxidised, microbiologically deteriorated and less acceptable products. PC2 primarily differentiated the formulations. Positive loadings were associated with b^* , saltiness and storage loss while negative loadings were associated with a^* , L^* , hardness, resilience, water activity, springiness and cohesiveness. Increasing pepper-paste concentration therefore shifted samples along PC2 according to changes in colour, moisture characteristics and initial product structure. AP0 and VP0 had an identical day-0 PC1 score of -4.43 because they originated from the same 0% formulation before packaging. By day 20, AP0 had moved to 14.56 while VP0 reached only 5.34. Corresponding day-20 PC1 scores were 3.05, 1.71 and 1.39 for VP5, VP10 and VP15 respectively.

The two-dimensional displacement between days 0 and 20 was 19.39 units for AP0, 10.05 units for VP0, 8.04 units for VP5, 6.51 units for VP10 and 5.14 units for VP15. Vacuum packaging alone reduced the overall deterioration trajectory by approximately 48.1% relative to aerobic packaging. Incorporation of 5%, 10% and 15% pepper paste under vacuum reduced displacement by approximately 58.5%, 66.4% and 73.5% respectively relative to AP0.

AP0 showed the most rapid movement towards the region characterised by oxidation, microbial growth, volatile-base formation and sensory deterioration. VP15 showed the shortest trajectory and greatest overall preservation, while VP10 followed a similarly protected trajectory with better sensory balance.

3.10 Integrated assessment of refrigerated stability

The combined results demonstrated that vacuum packaging substantially delayed physicochemical, oxidative, microbial, textural and sensory deterioration. Compared with AP0, VP0 showed lower storage loss, TVB-N, lipid and protein oxidation, metmyoglobin, aerobic microbial counts and sensory decline.

Vacuum packaging did not suppress all microbial groups equally. Lactic acid bacteria were greater in VP0 than in AP0 during storage, indicating a shift towards organisms favoured by reduced-oxygen conditions. Green bell pepper paste reduced lactic acid bacteria and the other enumerated microbial groups in a concentration-dependent manner under vacuum packaging.

Pepper-paste incorporation provided additional protection beyond vacuum packaging alone. VP15 consistently recorded the lowest peroxide value, thiobarbituric acid reactive substances, protein carbonyl content, metmyoglobin, TVB-N and microbial counts and retained the greatest residual antioxidant capacity and sulfhydryl content. It also showed the shortest principal-component deterioration trajectory.

However, VP15 had lower initial redness, hardness, binding and some sensory scores because of its greater replacement of lean meat with vegetable paste. VP10 provided a more favourable balance, maintaining the highest overall acceptability, juiciness and binding during most of storage while still providing substantial oxidative and microbial protection.

The results therefore distinguished between maximum preservation and optimum overall product quality. Vacuum packaging with 15% green bell pepper paste maximised refrigerated stability, whereas vacuum packaging with 10% paste provided the best compromise between preservation, structural characteristics and sensory acceptance.

4. Discussion

4.1 Effect of vacuum packaging on refrigerated deterioration

Vacuum packaging substantially improved the refrigerated stability of restructured buffalo meat steaks. Compared with AP0, VP0 showed lower storage loss, TVB-N, lipid and protein oxidation, metmyoglobin formation and aerobic microbial counts together with better retention of textural and sensory quality. These effects were principally associated with reduced oxygen availability, which limited oxygen-dependent oxidative reactions and the growth of aerobic spoilage microorganisms [5-8, 37].

The lower peroxide value and thiobarbituric acid-reactive substances in VP0 demonstrated that vacuum packaging retarded both primary and secondary lipid oxidation. Similar improvements in oxidative and sensory stability have been reported in vacuum-packaged buffalo meat nuggets, buffalo tripe rolls and minced buffalo meat [5-7, 37]. Vacuum packaging has also been shown to reduce colour and oxidative deterioration in ground beef during refrigerated storage [39].

Protein oxidation followed a pattern similar to lipid oxidation. VP0 accumulated fewer protein carbonyls and retained more sulfhydryl groups than AP0. Lipid-derived radicals and secondary oxidation products can initiate or propagate protein oxidation, resulting in carbonyl formation, sulfhydryl depletion, protein aggregation and loss of functionality [40, 41]. The strong associations among thiobarbituric acid-reactive substances, protein carbonyl content and sulfhydryl depletion in the present study support the occurrence of interconnected lipid and protein oxidation. Vacuum packaging also reduced metmyoglobin accumulation and improved redness retention. The close association between lipid oxidation and metmyoglobin formation indicated that limiting lipid-derived reactive compounds contributed to pigment stability. Although vacuum-packaged meat commonly contains a greater proportion of deoxymyoglobin, the slower decline in a^* within VP0 demonstrated improved preservation of the pigment system during storage [7, 37, 39].

4.2 Additional protective effect of green bell pepper paste

Green bell pepper paste provided additional protection beyond that achieved by vacuum packaging alone. Peroxide value, thiobarbituric acid-reactive substances, protein carbonyl content and metmyoglobin percentage decreased progressively from VP0 to VP15, whereas residual antioxidant capacity and sulfhydryl retention increased.

Green bell pepper contains ascorbic acid, phenolic compounds, flavonoids, carotenoids and chlorophyll-related pigments with antioxidant and reducing activities [9-11]. These constituents may interrupt oxidative chain reactions, donate electrons, chelate pro-oxidant metals and suppress reactive oxygen species [15, 19, 45, 46]. The concentration-dependent increase in residual antioxidant capacity

suggested that larger quantities of antioxidant constituents remained available in the meat matrix at higher paste levels. The strong inverse correlation between residual antioxidant capacity and thiobarbituric acid-reactive substances confirmed the functional relevance of the retained antioxidant reserve. Its positive correlation with overall acceptability further indicated that oxidative protection contributed to the preservation of appearance, flavour and overall eating quality.

Plant-derived polyphenols can inhibit heme protein-mediated lipid oxidation and retard myoglobin oxidation [45-47]. The simultaneous reductions in lipid oxidation, protein carbonyl formation and metmyoglobin accumulation therefore suggest that the protective effects of green bell pepper paste involved interconnected oxidative pathways. Similar improvements in oxidative, microbial, textural and sensory stability have been reported following the incorporation of plant-derived antioxidants into buffalo meat sausages and other processed meat products [40, 48-50].

The term complementary effect, rather than synergistic effect, is appropriate because the experiment did not include aerobically packaged formulations containing green bell pepper paste. Consequently, the independent effects of packaging atmosphere and pepper paste and their formal interaction could not be estimated using a completely crossed factorial design.

4.3 Physicochemical and colour changes

The initial decline in pH probably reflected the utilisation of fermentable substrates and the accumulation of acidic metabolites. The subsequent increase, particularly in AP0, was consistent with proteolysis and the formation of ammonia, amines and other alkaline compounds. This interpretation was supported by the concurrent increases in TVB-N and microbial counts. The lower late-storage pH values in the vacuum-packaged treatments were consistent with their slower microbial and biochemical deterioration.

The progressively lower pH values of VP5, VP10 and VP15 were also associated with the intrinsic acidity of green bell pepper paste and the replacement of lean meat with paste. Lower pH may have contributed partly to microbial inhibition and slower volatile-base formation. However, the preservation effect could not be attributed to pH alone because marked concentration-dependent differences were also observed in residual antioxidant capacity and the oxidative indices.

Vacuum packaging reduced storage loss compared with aerobic packaging. This may have been related to reduced oxidative damage to proteins involved in water retention and slower deterioration of the product matrix. Protein oxidation can impair protein functionality through changes in protein solubility, fragmentation and aggregation [40].

In contrast, storage loss increased with pepper-paste concentration under vacuum packaging. Replacement of lean meat with vegetable paste reduced the proportion of salt-soluble myofibrillar proteins available to form the heat-induced binding matrix. The additional moisture and fibre supplied by the paste may also have increased the amount of loosely retained water released during storage. The lower initial hardness and binding values at higher paste levels were consistent with dilution of the meat-protein matrix.

The lower initial L^* and a^* values and higher b^* values at increasing paste concentrations reflected the natural green-yellow pigments of the pepper paste and the dilution of meat

pigments. Absolute redness values among formulations could therefore not be interpreted solely as indicators of pigment stability. Within each treatment, however, the strong inverse association between a^* and metmyoglobin confirmed that progressive redness loss was closely related to pigment oxidation. Plant-derived antioxidants, including tomato- and pepper-derived compounds, have previously been shown to retard myoglobin oxidation and improve meat colour stability [47, 54]. Although VP15 had the lowest absolute redness, it showed the greatest proportional protection against metmyoglobin formation.

4.4 Microbial and chemical spoilage

Vacuum packaging reduced total plate, psychrotrophic and yeast and mould counts compared with aerobic packaging. Reduced oxygen availability restricted the growth of aerobic spoilage microorganisms and delayed the microbial deterioration reflected by TVB-N accumulation. Similar reductions in aerobic microbial populations have been reported in vacuum-packaged buffalo meat products [5-7, 37].

Lactic acid bacteria responded differently from the other microbial groups. VP0 supported greater lactic acid bacteria counts than AP0 during storage, reflecting the selective advantage of organisms capable of growing under reduced-oxygen conditions. Increased lactic acid bacteria populations have similarly been observed in vacuum-packaged buffalo meat and other meat systems [6, 37].

Green bell pepper paste reduced all enumerated microbial groups, including lactic acid bacteria, in a concentration-dependent manner. Phenolic compounds, organic acids and other pepper constituents may alter microbial membrane permeability, disrupt cellular processes and interfere with microbial enzyme systems [55, 56]. The greatest reduction occurred in VP15, suggesting that the antimicrobial constituents of the paste remained at least partly active within the cooked meat matrix.

The strong positive correlations of total plate and psychrotrophic counts with TVB-N demonstrated that microbial growth was closely associated with the degradation of nitrogenous components. Their inverse relationships with overall acceptability further showed that microbial proliferation, volatile-base accumulation and sensory deterioration developed as related spoilage processes.

Coliforms were not detected in any treatment during storage. This finding indicates that no recoverable coliform population was detected using the applied analytical procedure. It should not be interpreted as evidence of a treatment-related antimicrobial effect because no countable coliform population was available for comparison.

4.5 Texture and sensory stability

Vacuum packaging improved the retention of hardness, cohesiveness, springiness, chewiness and resilience. Lower oxidative and microbial deterioration may have reduced protein aggregation, proteolysis and weakening of the restructured meat matrix [40, 58]. The positive correlation between instrumental hardness and sensory texture confirmed that storage-related structural changes measured instrumentally were also perceived by the sensory panel.

Increasing green bell pepper-paste concentration reduced initial instrumental firmness because an increasing proportion of lean meat was replaced with vegetable material. This reduced the quantity of myofibrillar protein

available for binding and increased the relative contribution of moisture and fibre. Nevertheless, the proportional decline in each texture-profile variable became smaller as pepper concentration increased. The antioxidant and antimicrobial protection provided by the paste therefore slowed storage-related textural deterioration despite producing a softer initial product.

The sensory results demonstrated a balance between initial formulation quality and subsequent preservation. VP15 underwent the smallest proportional decline in most attributes and provided the greatest long-term oxidative and microbial protection. However, its lower initial firmness, redness and binding prevented it from achieving the highest overall sensory score.

VP10 maintained the highest overall acceptability throughout storage and recorded a slightly but significantly higher day-20 score than VP15. It also retained favourable juiciness, binding, appearance and flavour. Thus, 10% paste provided substantial antioxidant and antimicrobial protection without causing the greater structural dilution observed at the 15% level. Similar formulation-dependent effects on quality and sensory attributes have been observed in meat products containing pepper-derived ingredients [54, 59].

The higher saltiness scores of the pepper-containing products, despite the use of an unchanged curing solution, suggest that green bell pepper paste and its interaction with the seasoning mixture may have enhanced perceived saltiness or overall flavour intensity. Saltiness remained comparatively stable and was not a principal factor limiting overall acceptability.

4.6 Integrated interpretation and study limitations

Principal component analysis integrated the individual quality measurements into a common deterioration pattern. AP0 showed the greatest displacement towards the region characterised by oxidation, microbial proliferation, TVB-N accumulation and sensory decline. Vacuum packaging reduced this displacement substantially, while increasing pepper-paste concentration progressively shortened the deterioration trajectory.

VP15 produced the smallest multivariate displacement and therefore provided the greatest overall preservation. VP10 followed a similarly protected trajectory but maintained superior sensory and structural characteristics. These findings distinguish the formulation that maximised preservation from the formulation that provided the most favourable overall product balance.

The experimental design had several limitations. Only the 0% formulation was evaluated under both aerobic and vacuum packaging. The study could therefore estimate the effect of vacuum packaging on the control formulation and the concentration-dependent response to pepper paste under vacuum, but it could not estimate a formal packaging atmosphere $\times$ pepper-paste interaction.

Although 250-gauge low-density polyethylene pouches were used, the oxygen-transmission rate of the film, achieved vacuum level and residual headspace oxygen were not determined. These factors can materially influence the oxidative and microbial stability of packaged meat [61, 62]. The sensory evaluation was conducted by a small semi-trained panel and provided controlled comparative measurements rather than direct evidence of consumer acceptance. In addition, a definitive commercial shelf-life

claim would require predefined sensory rejection criteria and applicable microbiological and chemical acceptability limits.

Despite these limitations, the study demonstrated that vacuum packaging and green bell pepper paste provided

complementary preservation mechanisms. Reduced oxygen availability produced the principal improvement over aerobic packaging, while the phytochemical constituents of green bell pepper paste provided additional concentration-dependent antioxidant and antimicrobial protection.

Table 2: Physicochemical changes during refrigerated storage at 4±1°C

pH					
Day	AP0	VP0	VP5	VP10	VP15
0	6.163±0.010 ^{aB}	6.163±0.010 ^{aB}	6.130±0.006 ^{aB}	6.087±0.006 ^{bAB}	6.012±0.008 ^{cAB}
5	6.040±0.009 ^{bC}	6.103±0.010 ^{aC}	6.052±0.009 ^{abCD}	6.023±0.017 ^{bCD}	5.967±0.017 ^{cBC}
10	6.012±0.007 ^{bC}	6.078±0.014 ^{aC}	6.032±0.012 ^{bD}	6.002±0.009 ^{bD}	5.945±0.011 ^{cC}
15	6.145±0.011 ^{aB}	6.175±0.017 ^{aB}	6.082±0.012 ^{bC}	6.058±0.013 ^{bBC}	5.967±0.010 ^{cBC}
20	6.350±0.011 ^{aA}	6.267±0.014 ^{bA}	6.192±0.012 ^{cA}	6.112±0.014 ^{dA}	6.025±0.007 ^{eA}
Water activity (a _w)					
Day	AP0	VP0	VP5	VP10	VP15
0	0.995±0.001 ^{aA}	0.995±0.001 ^{aA}	0.988±0.001 ^{bA}	0.986±0.001 ^{bA}	0.985±0.001 ^{bA}
5	0.992±0.001 ^{abA}	0.996±0.001 ^{aA}	0.989±0.001 ^{bcA}	0.985±0.002 ^{cAB}	0.986±0.003 ^{bcA}
10	0.987±0.001 ^{aB}	0.990±0.003 ^{aAB}	0.981±0.002 ^{aB}	0.984±0.002 ^{aAB}	0.982±0.003 ^{aAB}
15	0.980±0.001 ^{bC}	0.989±0.002 ^{aAB}	0.979±0.001 ^{bB}	0.982±0.002 ^{bAB}	0.978±0.001 ^{bBC}
20	0.971±0.000 ^{cD}	0.983±0.002 ^{aB}	0.981±0.002 ^{abB}	0.978±0.002 ^{abcB}	0.973±0.002 ^{bcC}
Storage Loss (%)					
Day	AP0	VP0	VP5	VP10	VP15
0	0.00±0.00 ^{aE}	0.00±0.00 ^{aE}	0.00±0.00 ^{aE}	0.00±0.00 ^{aE}	0.00±0.00 ^{aE}
5	1.21±0.01 ^{aD}	0.66±0.01 ^{eD}	0.78±0.01 ^{dD}	0.87±0.01 ^{cD}	0.99±0.01 ^{bD}
10	2.58±0.02 ^{aC}	1.42±0.01 ^{eC}	1.59±0.02 ^{dC}	1.75±0.03 ^{cC}	2.04±0.02 ^{bC}
15	4.29±0.07 ^{aB}	2.35±0.04 ^{dB}	2.51±0.02 ^{dB}	2.77±0.04 ^{cB}	3.11±0.04 ^{BB}
20	6.55±0.09 ^{aA}	3.61±0.05 ^{dA}	3.81±0.05 ^{dA}	4.14±0.06 ^{cA}	4.57±0.06 ^{bA}
Total volatile basic nitrogen (mg N/100 g)					
Day	AP0	VP0	VP5	VP10	VP15
0	9.47±0.11 ^{aE}	9.47±0.11 ^{aE}	9.02±0.13 ^{abE}	8.57±0.05 ^{bcE}	8.16±0.11 ^{cE}
5	13.72±0.21 ^{aD}	11.83±0.16 ^{bD}	10.96±0.09 ^{cD}	10.33±0.12 ^{dD}	9.66±0.07 ^{eD}
10	18.69±0.25 ^{aC}	14.52±0.15 ^{bC}	13.04±0.09 ^{cC}	12.09±0.09 ^{dC}	11.28±0.08 ^{cC}
15	24.57±0.39 ^{aB}	17.76±0.18 ^{bB}	15.59±0.16 ^{cB}	14.02±0.14 ^{dB}	12.96±0.08 ^{eB}
20	32.47±0.20 ^{aA}	22.14±0.05 ^{bA}	18.94±0.19 ^{cA}	16.97±0.12 ^{dA}	15.14±0.12 ^{eA}

Values are mean ± SE (n=6). Within a storage day, means with different lowercase superscripts differ significantly. Within a treatment, means with different uppercase superscripts differ significantly ($P < 0.05$). AP0 = aerobic packaging without pepper paste; VP0 = vacuum packaging without pepper paste; VP5, VP10 and VP15 = vacuum packaging with 5%, 10% and 15% green bell pepper paste respectively. Treatment condition, storage day and their interaction were significant for all variables ($P < 0.01$).

Table 3: Lipid oxidation and residual antioxidant capacity during refrigerated storage at 4±1°C

Peroxide value (meq O ₂ /kg fat)					
Day	AP0	VP0	VP5	VP10	VP15
0	1.18±0.01 ^{aE}	1.18±0.01 ^{aE}	0.98±0.01 ^{bE}	0.91±0.01 ^{cE}	0.81±0.01 ^{dE}
5	2.99±0.03 ^{aD}	1.90±0.01 ^{bD}	1.55±0.01 ^{cD}	1.32±0.01 ^{dD}	1.08±0.01 ^{eD}
10	5.42±0.09 ^{aC}	2.87±0.03 ^{bC}	2.26±0.02 ^{cC}	1.84±0.02 ^{dC}	1.43±0.01 ^{eC}
15	7.82±0.13 ^{aB}	3.84±0.06 ^{bB}	2.97±0.05 ^{cB}	2.29±0.02 ^{dB}	1.82±0.01 ^{eB}
20	9.34±0.13 ^{aA}	4.45±0.05 ^{bA}	3.46±0.03 ^{cA}	2.75±0.03 ^{dA}	2.16±0.02 ^{eA}
TBARS (mg malondialdehyde/kg)					
Day	AP0	VP0	VP5	VP10	VP15
0	0.275±0.002 ^{aE}	0.275±0.002 ^{aE}	0.228±0.004 ^{bE}	0.207±0.003 ^{cE}	0.182±0.003 ^{dE}
5	0.528±0.003 ^{aD}	0.365±0.002 ^{bD}	0.295±0.004 ^{cD}	0.258±0.002 ^{dD}	0.217±0.003 ^{eD}
10	0.903±0.014 ^{aC}	0.495±0.006 ^{bC}	0.387±0.004 ^{cC}	0.325±0.006 ^{dC}	0.260±0.004 ^{eC}
15	1.378±0.023 ^{aB}	0.663±0.007 ^{bB}	0.497±0.007 ^{cB}	0.405±0.006 ^{dB}	0.318±0.004 ^{eB}
20	1.900±0.017 ^{aA}	0.843±0.006 ^{bA}	0.620±0.009 ^{cA}	0.497±0.005 ^{dA}	0.375±0.003 ^{eA}
Residual antioxidant capacity (%)					
Day	AP0	VP0	VP5	VP10	VP15
0	100.0±0.0 ^{aA}	100.0±0.0 ^{aA}	100.0±0.0 ^{aA}	100.0±0.0 ^{aA}	100.0±0.0 ^{aA}
5	78.9±0.5 ^{dB}	90.4±0.2 ^{cB}	92.9±0.2 ^{bB}	95.1±0.4 ^{aB}	96.2±0.6 ^{aB}
10	57.2±0.6 ^{dC}	80.7±0.4 ^{cC}	85.9±0.4 ^{bC}	87.7±0.3 ^{bC}	90.9±0.5 ^{aC}
15	39.9±0.2 ^{eD}	73.0±0.1 ^{dD}	78.4±0.3 ^{cD}	82.0±0.3 ^{bD}	85.8±0.5 ^{aD}
20	24.8±0.2 ^{eE}	66.3±0.1 ^{dE}	71.9±0.3 ^{cE}	76.3±0.3 ^{bE}	80.1±0.3 ^{aE}

Values are mean ± SE (n = 6). Within a storage day, means with different lowercase superscripts differ significantly. Within a treatment, means with different uppercase superscripts differ significantly ($P < 0.05$). AP0 = aerobic packaging without pepper paste; VP0 = vacuum packaging without pepper paste; VP5, VP10 and VP15 = vacuum packaging with 5%, 10% and 15% green bell pepper paste respectively. Treatment condition, storage day and their interaction were significant for all variables ($P < 0.01$).

Table 4: Protein oxidation during refrigerated storage at 4±1°C

Protein carbonyl content (nmol/mg protein)					
Day	AP0	VP0	VP5	VP10	VP15
0	1.35±0.02 ^{aE}	1.35±0.02 ^{aE}	1.20±0.01 ^{bE}	1.10±0.02 ^{cE}	1.01±0.01 ^{dE}
5	2.08±0.02 ^{aD}	1.72±0.02 ^{bD}	1.46±0.01 ^{cD}	1.31±0.01 ^{dD}	1.15±0.01 ^{eD}
10	3.00±0.04 ^{aC}	2.17±0.02 ^{bC}	1.84±0.01 ^{cC}	1.52±0.02 ^{dC}	1.30±0.01 ^{eC}
15	4.24±0.06 ^{aB}	2.81±0.03 ^{bB}	2.19±0.03 ^{cB}	1.76±0.02 ^{dB}	1.51±0.01 ^{eB}
20	5.59±0.08 ^{aA}	3.48±0.04 ^{bA}	2.62±0.02 ^{cA}	2.12±0.02 ^{dA}	1.70±0.02 ^{eA}
Total sulfhydryl groups (nmol/mg protein)					
Day	AP0	VP0	VP5	VP10	VP15
0	79.4±0.3 ^{bA}	79.4±0.3 ^{bA}	79.7±0.4 ^{bA}	81.6±0.5 ^{aA}	82.0±0.5 ^{aA}
5	67.8±0.5 ^{dB}	73.6±0.4 ^{cB}	75.8±0.4 ^{bB}	79.2±0.3 ^{aB}	80.2±0.2 ^{aB}
10	56.9±0.4 ^{cC}	68.2±0.2 ^{dC}	71.8±0.4 ^{cC}	75.6±0.6 ^{bC}	77.7±0.5 ^{aC}
15	44.1±0.4 ^{dD}	61.9±0.3 ^{dD}	67.0±0.1 ^{cD}	71.7±0.2 ^{bD}	75.1±0.3 ^{aD}
20	32.1±0.3 ^{eE}	55.7±0.2 ^{dE}	62.3±0.3 ^{cE}	67.6±0.3 ^{bE}	71.2±0.4 ^{aE}

Values are mean ± SE (n = 6). Within a storage day, means with different lowercase superscripts differ significantly. Within a treatment, means with different uppercase superscripts differ significantly ($P < 0.05$). AP0 = aerobic packaging without pepper paste; VP0 = vacuum packaging without pepper paste; VP5, VP10 and VP15 = vacuum packaging with 5%, 10% and 15% green bell pepper paste respectively. Treatment condition, storage day and their interaction were significant for all variables ($P < 0.01$).

Table 5: Instrumental colour and pigment stability during refrigerated storage at 4±1°C

L* (lightness)					
Day	AP0	VP0	VP5	VP10	VP15
0	42.58±0.05 ^{aE}	42.58±0.05 ^{aC}	40.97±0.06 ^{bC}	39.45±0.04 ^{cC}	37.95±0.07 ^{dD}
5	43.07±0.06 ^{aD}	42.75±0.09 ^{bC}	41.07±0.11 ^{cC}	39.75±0.07 ^{dB}	38.12±0.05 ^{eCD}
10	43.62±0.05 ^{aC}	43.13±0.11 ^{bB}	41.37±0.09 ^{cB}	39.85±0.11 ^{dB}	38.28±0.05 ^{eBC}
15	44.33±0.06 ^{aB}	43.42±0.11 ^{bAB}	41.62±0.10 ^{cB}	39.98±0.09 ^{dB}	38.47±0.10 ^{eAB}
20	45.12±0.07 ^{aA}	43.78±0.13 ^{bA}	42.18±0.13 ^{cA}	40.37±0.11 ^{dA}	38.73±0.06 ^{eA}
a* (redness)					
Day	AP0	VP0	VP5	VP10	VP15
0	9.26±0.05 ^{aA}	9.26±0.05 ^{aA}	6.72±0.02 ^{bA}	4.06±0.06 ^{cA}	1.53±0.05 ^{dA}
5	7.61±0.06 ^{bB}	8.84±0.06 ^{aB}	6.47±0.03 ^{cB}	3.91±0.05 ^{dB}	1.41±0.04 ^{eB}
10	5.74±0.05 ^{cC}	8.39±0.05 ^{aC}	6.26±0.02 ^{bC}	3.77±0.04 ^{dC}	1.33±0.04 ^{eC}
15	4.09±0.03 ^{dD}	7.96±0.05 ^{aD}	5.99±0.01 ^{bD}	3.62±0.05 ^{dD}	1.28±0.04 ^{eD}
20	2.28±0.06 ^{dE}	7.52±0.04 ^{aE}	5.75±0.01 ^{bE}	3.48±0.05 ^{cE}	1.20±0.04 ^{eE}
b* (yellowness)					
Day	AP0	VP0	VP5	VP10	VP15
0	11.48±0.02 ^{dE}	11.48±0.02 ^{dE}	12.84±0.03 ^{cE}	13.96±0.05 ^{bD}	15.15±0.05 ^{aD}
5	12.00±0.05 ^{dD}	11.71±0.05 ^{dD}	13.00±0.02 ^{cD}	14.07±0.04 ^{bD}	15.28±0.06 ^{aCD}
10	12.66±0.05 ^{dC}	12.10±0.03 ^{cC}	13.26±0.03 ^{cC}	14.34±0.03 ^{bC}	15.44±0.06 ^{aC}
15	13.36±0.04 ^{cB}	12.39±0.02 ^{dB}	13.50±0.04 ^{cB}	14.57±0.04 ^{bB}	15.65±0.03 ^{aB}
20	14.01±0.03 ^{cA}	12.75±0.03 ^{dA}	13.93±0.02 ^{cA}	14.86±0.04 ^{bA}	15.93±0.05 ^{aA}
Metmyoglobin (%)					
Day	AP0	VP0	VP5	VP10	VP15
0	9.18±0.20 ^{aE}	9.18±0.20 ^{aE}	7.08±0.24 ^{bE}	6.82±0.32 ^{bE}	5.47±0.36 ^{cE}
5	18.15±0.21 ^{dD}	11.87±0.15 ^{bD}	9.37±0.14 ^{cD}	8.42±0.25 ^{dD}	6.73±0.23 ^{eD}
10	29.73±0.34 ^{aC}	15.35±0.14 ^{bC}	11.88±0.21 ^{cC}	10.53±0.22 ^{dC}	8.08±0.26 ^{eC}
15	42.87±0.32 ^{aB}	19.35±0.09 ^{bB}	14.43±0.13 ^{cB}	12.38±0.24 ^{dB}	9.58±0.32 ^{eB}
20	56.10±0.16 ^{aA}	23.22±0.09 ^{bA}	17.35±0.18 ^{cA}	14.57±0.22 ^{dA}	11.20±0.31 ^{eA}

Values are mean ± SE (n = 6). Within a storage day, means with different lowercase superscripts differ significantly. Within a treatment, means with different uppercase superscripts differ significantly ($P < 0.05$). AP0 = aerobic packaging without pepper paste; VP0 = vacuum packaging without pepper paste; VP5, VP10 and VP15 = vacuum packaging with 5%, 10% and 15% green bell pepper paste respectively. Treatment condition, storage day and their interaction were significant for all variables ($P < 0.01$).

Table 6: Microbiological quality during refrigerated storage at 4±1°C

Total plate count (log ₁₀ CFU/g)					
Day	AP0	VP0	VP5	VP10	VP15
0	2.88±0.05 ^{aE}	2.88±0.05 ^{aE}	2.69±0.04 ^{bE}	2.52±0.02 ^{cE}	2.42±0.04 ^{cE}
5	4.23±0.03 ^{aD}	3.62±0.03 ^{bD}	3.31±0.02 ^{cD}	3.10±0.02 ^{dD}	2.86±0.02 ^{eD}
10	5.36±0.02 ^{aC}	4.24±0.02 ^{bC}	3.87±0.01 ^{cC}	3.55±0.01 ^{dC}	3.25±0.03 ^{eC}
15	6.40±0.04 ^{aB}	4.82±0.04 ^{bB}	4.38±0.03 ^{cB}	3.98±0.02 ^{dB}	3.61±0.03 ^{eB}
20	7.32±0.04 ^{aA}	5.33±0.03 ^{bA}	4.81±0.02 ^{cA}	4.36±0.02 ^{dA}	3.94±0.03 ^{eA}
Psychrotrophic count (log ₁₀ CFU/g)					
Day	AP0	VP0	VP5	VP10	VP15
0	2.58±0.05 ^{aE}	2.58±0.05 ^{aE}	2.47±0.04 ^{abE}	2.28±0.06 ^{bcE}	2.21±0.04 ^{cE}
5	4.40±0.04 ^{aD}	3.59±0.03 ^{bD}	3.28±0.04 ^{cD}	3.05±0.04 ^{dD}	2.78±0.03 ^{eD}
10	5.66±0.03 ^{aC}	4.26±0.02 ^{bC}	3.93±0.02 ^{cC}	3.54±0.02 ^{dC}	3.25±0.03 ^{eC}

15	6.67±0.05 ^{aB}	4.83±0.05 ^{bB}	4.41±0.05 ^{cB}	3.99±0.03 ^{dB}	3.63±0.03 ^{eB}
20	7.58±0.03 ^{aA}	5.31±0.02 ^{bA}	4.85±0.04 ^{cA}	4.36±0.05 ^{dA}	3.91±0.02 ^{eA}
Lactic acid bacteria (log₁₀ CFU/g)					
Day	AP0	VP0	VP5	VP10	VP15
0	2.16±0.04 ^{aE}	2.16±0.04 ^{aE}	2.07±0.03 ^{abE}	1.93±0.03 ^{bcE}	1.79±0.03 ^{cE}
5	3.43±0.04 ^{bD}	3.70±0.05 ^{aD}	3.23±0.04 ^{cD}	2.97±0.04 ^{dD}	2.59±0.03 ^{eD}
10	4.53±0.04 ^{bC}	5.01±0.04 ^{aC}	4.37±0.03 ^{cC}	3.88±0.04 ^{dC}	3.34±0.04 ^{eC}
15	5.47±0.03 ^{bB}	6.13±0.03 ^{aB}	5.43±0.05 ^{bB}	4.70±0.03 ^{cB}	4.09±0.04 ^{dB}
20	6.27±0.03 ^{bA}	7.08±0.04 ^{aA}	6.27±0.04 ^{bA}	5.38±0.03 ^{cA}	4.62±0.03 ^{dA}
Coliform count					
Day	AP0	VP0	VP5	VP10	VP15
0	Not detected	Not detected	Not detected	Not detected	Not detected
5	Not detected	Not detected	Not detected	Not detected	Not detected
10	Not detected	Not detected	Not detected	Not detected	Not detected
15	Not detected	Not detected	Not detected	Not detected	Not detected
20	Not detected	Not detected	Not detected	Not detected	Not detected
Yeast and mould count (log₁₀ CFU/g)					
Day	AP0	VP0	VP5	VP10	VP15
0	1.27±0.03 ^{aE}	1.27±0.03 ^{aE}	1.16±0.01 ^{bE}	0.98±0.02 ^{cE}	0.82±0.02 ^{dE}
5	1.82±0.02 ^{aD}	1.38±0.03 ^{bD}	1.24±0.00 ^{cD}	1.05±0.02 ^{dD}	0.87±0.02 ^{eD}
10	2.43±0.03 ^{aC}	1.50±0.03 ^{bc}	1.34±0.01 ^{cC}	1.11±0.02 ^{dC}	0.92±0.02 ^{eC}
15	3.12±0.02 ^{aB}	1.64±0.02 ^{bB}	1.45±0.01 ^{cB}	1.18±0.02 ^{dB}	0.96±0.02 ^{eB}
20	3.83±0.03 ^{aA}	1.79±0.03 ^{bA}	1.53±0.01 ^{cA}	1.26±0.02 ^{dA}	1.02±0.02 ^{eA}

Values are mean ± SE (n = 6). Within a storage day, means with different lowercase superscripts differ significantly. Within a treatment, means with different uppercase superscripts differ significantly ($P < 0.05$). AP0 = aerobic packaging without pepper paste; VP0 = vacuum packaging without pepper paste; VP5, VP10 and VP15 = vacuum packaging with 5%, 10% and 15% green bell pepper paste respectively. Treatment condition, storage day and their interaction were significant for all variables ($P < 0.01$).

Table 7: Texture-profile stability during refrigerated storage at 4±1°C

Hardness (N)					
Day	AP0	VP0	VP5	VP10	VP15
0	67.33±0.45 ^{aA}	67.33±0.45 ^{aA}	60.88±0.39 ^{bA}	54.98±0.39 ^{cA}	48.07±0.39 ^{dA}
5	62.62±0.42 ^{bB}	64.52±0.53 ^{aB}	59.09±0.43 ^{cB}	53.66±0.40 ^{dB}	47.13±0.39 ^{eB}
10	55.89±0.38 ^{cC}	60.44±0.48 ^{aC}	56.26±0.39 ^{bc}	51.62±0.42 ^{dC}	46.19±0.42 ^{eC}
15	47.81±0.32 ^{dD}	55.59±0.42 ^{aD}	52.59±0.39 ^{bD}	49.04±0.41 ^{cD}	44.28±0.31 ^{eD}
20	39.05±0.26 ^{eE}	50.38±0.30 ^{aE}	48.54±0.33 ^{bE}	46.02±0.39 ^{cE}	42.27±0.32 ^{dE}
Cohesiveness					
Day	AP0	VP0	VP5	VP10	VP15
0	0.617±0.004 ^{aA}	0.617±0.004 ^{aA}	0.590±0.005 ^{bA}	0.560±0.005 ^{cA}	0.500±0.005 ^{dA}
5	0.580±0.004 ^{bB}	0.594±0.003 ^{aB}	0.577±0.004 ^{bB}	0.549±0.005 ^{cB}	0.494±0.005 ^{dB}
10	0.524±0.004 ^{dC}	0.561±0.004 ^{aC}	0.549±0.005 ^{bc}	0.533±0.005 ^{cC}	0.484±0.004 ^{eC}
15	0.456±0.003 ^{dD}	0.520±0.004 ^{aD}	0.520±0.004 ^{aD}	0.507±0.004 ^{bD}	0.469±0.004 ^{cD}
20	0.382±0.003 ^{dE}	0.477±0.004 ^{bE}	0.483±0.004 ^{aE}	0.480±0.004 ^{abE}	0.453±0.005 ^{cE}
Springiness					
Day	AP0	VP0	VP5	VP10	VP15
0	0.795±0.005 ^{aA}	0.795±0.005 ^{aA}	0.760±0.005 ^{bA}	0.720±0.005 ^{cA}	0.670±0.005 ^{dA}
5	0.755±0.005 ^{bB}	0.775±0.005 ^{aB}	0.744±0.005 ^{cB}	0.705±0.005 ^{dB}	0.662±0.004 ^{eB}
10	0.700±0.004 ^{cC}	0.738±0.004 ^{aC}	0.717±0.004 ^{bc}	0.689±0.004 ^{dC}	0.653±0.004 ^{eC}
15	0.628±0.004 ^{eD}	0.693±0.004 ^{aD}	0.689±0.004 ^{bD}	0.671±0.004 ^{cD}	0.636±0.004 ^{dD}
20	0.540±0.003 ^{eE}	0.643±0.005 ^{aE}	0.646±0.004 ^{aE}	0.639±0.004 ^{aE}	0.617±0.005 ^{bE}
Chewiness (N)					
Day	AP0	VP0	VP5	VP10	VP15
0	33.03±0.64 ^{aA}	33.03±0.64 ^{aA}	27.32±0.57 ^{bA}	22.19±0.50 ^{cA}	16.12±0.40 ^{dA}
5	27.42±0.53 ^{bB}	29.74±0.57 ^{aB}	25.37±0.53 ^{cB}	20.80±0.48 ^{dB}	15.44±0.38 ^{eB}
10	20.50±0.40 ^{cC}	25.03±0.46 ^{aC}	22.17±0.49 ^{bc}	18.96±0.44 ^{dC}	14.61±0.34 ^{eC}
15	13.71±0.27 ^{dD}	20.07±0.42 ^{aD}	18.85±0.40 ^{bD}	16.68±0.37 ^{cD}	13.24±0.29 ^{eD}
20	8.08±0.16 ^{dE}	15.45±0.31 ^{aE}	15.14±0.33 ^{aE}	14.13±0.32 ^{bE}	11.82±0.30 ^{cE}
Resilience					
Day	AP0	VP0	VP5	VP10	VP15
0	0.380±0.005 ^{aA}	0.380±0.005 ^{aA}	0.350±0.005 ^{bA}	0.320±0.005 ^{cA}	0.280±0.005 ^{dA}
5	0.353±0.005 ^{bB}	0.364±0.006 ^{aB}	0.340±0.005 ^{cB}	0.312±0.005 ^{dB}	0.275±0.005 ^{eB}
10	0.311±0.004 ^{cC}	0.340±0.004 ^{aC}	0.322±0.004 ^{bc}	0.302±0.004 ^{dC}	0.270±0.005 ^{eC}
15	0.262±0.003 ^{dD}	0.308±0.004 ^{aD}	0.302±0.004 ^{bD}	0.285±0.005 ^{cD}	0.261±0.004 ^{dD}
20	0.209±0.003 ^{dE}	0.277±0.003 ^{aE}	0.276±0.004 ^{aE}	0.267±0.004 ^{bE}	0.249±0.004 ^{cE}

Values are mean ± SE (n = 6). Within a storage day, means with different lowercase superscripts differ significantly. Within a treatment, means with different uppercase superscripts differ significantly ($P < 0.05$). AP0 = aerobic packaging without pepper paste; VP0 = vacuum packaging without pepper paste; VP5, VP10 and VP15 = vacuum packaging with 5%, 10% and 15% green bell pepper paste respectively. Treatment condition, storage day and their interaction were significant for all variables ($P < 0.01$).

Table 8: Sensory quality during refrigerated storage at 4±1°C

General appearance					
Day	AP0	VP0	VP5	VP10	VP15
0	7.26±0.04 ^{aA}	7.26±0.04 ^{aA}	7.32±0.03 ^{aA}	7.40±0.06 ^{aA}	6.92±0.06 ^{bA}
5	6.68±0.05 ^{cB}	6.94±0.04 ^{bB}	7.13±0.05 ^{aB}	7.24±0.05 ^{aB}	6.81±0.04 ^{bcA}
10	5.92±0.04 ^{dC}	6.54±0.03 ^{cC}	6.76±0.02 ^{bC}	7.02±0.05 ^{aC}	6.64±0.02 ^{bcB}
15	5.00±0.02 ^{dD}	6.02±0.02 ^{cD}	6.44±0.03 ^{bD}	6.68±0.02 ^{aD}	6.43±0.04 ^{bC}
20	4.00±0.04 ^{dE}	5.45±0.03 ^{cE}	5.90±0.03 ^{bE}	6.24±0.03 ^{aE}	6.14±0.03 ^{aD}
Flavour					
Day	AP0	VP0	VP5	VP10	VP15
0	7.14±0.03 ^{bA}	7.14±0.03 ^{bA}	7.38±0.05 ^{aA}	7.46±0.04 ^{aA}	7.15±0.04 ^{bA}
5	6.58±0.03 ^{dB}	6.83±0.02 ^{cB}	7.15±0.03 ^{aB}	7.26±0.03 ^{aB}	7.04±0.02 ^{bB}
10	5.72±0.03 ^{dC}	6.35±0.03 ^{cC}	6.76±0.03 ^{bC}	6.99±0.02 ^{aC}	6.84±0.02 ^{bC}
15	4.63±0.06 ^{dD}	5.76±0.04 ^{cD}	6.27±0.04 ^{bD}	6.56±0.01 ^{aD}	6.61±0.03 ^{aD}
20	3.49±0.04 ^{eE}	5.13±0.02 ^{dE}	5.66±0.04 ^{cE}	6.14±0.05 ^{bE}	6.31±0.02 ^{aE}
Juiciness					
Day	AP0	VP0	VP5	VP10	VP15
0	6.93±0.05 ^{bA}	6.93±0.05 ^{bA}	7.46±0.03 ^{aA}	7.42±0.04 ^{aA}	6.89±0.05 ^{bA}
5	6.60±0.03 ^{cB}	6.78±0.03 ^{bB}	7.26±0.01 ^{aB}	7.29±0.05 ^{aB}	6.76±0.02 ^{bB}
10	5.87±0.05 ^{dC}	6.46±0.01 ^{cC}	7.02±0.04 ^{aC}	7.09±0.04 ^{aC}	6.62±0.03 ^{bC}
15	5.00±0.07 ^{dD}	6.06±0.01 ^{cD}	6.71±0.03 ^{aD}	6.79±0.02 ^{aD}	6.37±0.04 ^{bD}
20	4.20±0.06 ^{dE}	5.72±0.04 ^{cE}	6.25±0.02 ^{bE}	6.47±0.03 ^{aE}	6.10±0.03 ^{bE}
Sensory texture					
Day	AP0	VP0	VP5	VP10	VP15
0	7.17±0.03 ^{bA}	7.17±0.03 ^{bA}	7.41±0.02 ^{aA}	6.81±0.04 ^{cA}	6.47±0.03 ^{dA}
5	6.75±0.06 ^{cB}	6.94±0.04 ^{bB}	7.19±0.03 ^{aB}	6.67±0.03 ^{cB}	6.35±0.04 ^{dA}
10	6.15±0.06 ^{dC}	6.62±0.03 ^{bC}	6.89±0.03 ^{aC}	6.44±0.04 ^{cC}	6.20±0.04 ^{dB}
15	5.32±0.04 ^{dD}	6.17±0.03 ^{bD}	6.55±0.02 ^{aD}	6.21±0.02 ^{bD}	6.02±0.02 ^{cC}
20	4.42±0.06 ^{dE}	5.65±0.03 ^{cE}	6.12±0.02 ^{aE}	5.84±0.03 ^{bE}	5.79±0.02 ^{bcD}
Binding					
Day	AP0	VP0	VP5	VP10	VP15
0	7.26±0.03 ^{aA}	7.26±0.03 ^{aA}	7.34±0.05 ^{aA}	7.41±0.03 ^{aA}	6.78±0.02 ^{bA}
5	6.67±0.03 ^{dB}	6.94±0.03 ^{cB}	7.12±0.03 ^{bB}	7.31±0.03 ^{aA}	6.61±0.04 ^{dB}
10	6.06±0.05 ^{dC}	6.60±0.03 ^{cC}	6.77±0.03 ^{bC}	7.01±0.03 ^{aB}	6.45±0.04 ^{cC}
15	5.25±0.04 ^{dD}	6.15±0.03 ^{cD}	6.44±0.03 ^{bD}	6.74±0.03 ^{aC}	6.33±0.02 ^{bC}
20	4.16±0.04 ^{dE}	5.57±0.02 ^{cE}	5.94±0.04 ^{bE}	6.29±0.04 ^{aD}	6.05±0.01 ^{bD}
Saltiness					
Day	AP0	VP0	VP5	VP10	VP15
0	6.44±0.04 ^{cA}	6.44±0.04 ^{cA}	7.24±0.04 ^{bA}	7.38±0.02 ^{abA}	7.51±0.05 ^{aA}
5	6.31±0.04 ^{cA}	6.34±0.03 ^{cA}	7.13±0.05 ^{bA}	7.41±0.05 ^{aA}	7.48±0.03 ^{aA}
10	6.07±0.03 ^{cB}	6.20±0.02 ^{cB}	6.96±0.05 ^{bB}	7.30±0.04 ^{aAB}	7.37±0.05 ^{aAB}
15	5.78±0.04 ^{dC}	5.98±0.03 ^{cC}	6.90±0.03 ^{bB}	7.18±0.04 ^{aB}	7.27±0.04 ^{aB}
20	5.41±0.04 ^{dD}	5.71±0.03 ^{cD}	6.53±0.03 ^{bC}	6.96±0.02 ^{aC}	7.03±0.06 ^{aC}
Overall Acceptability					
Day	AP0	VP0	VP5	VP10	VP15
0	6.92±0.05 ^{cA}	6.92±0.05 ^{cA}	7.22±0.06 ^{bA}	7.48±0.05 ^{aA}	6.96±0.06 ^{cA}
5	6.27±0.05 ^{dB}	6.61±0.04 ^{cB}	6.98±0.05 ^{bB}	7.29±0.04 ^{aB}	6.82±0.02 ^{bB}
10	5.33±0.03 ^{dC}	6.12±0.02 ^{cC}	6.66±0.04 ^{bC}	6.97±0.04 ^{aC}	6.66±0.03 ^{bC}
15	4.29±0.03 ^{eD}	5.62±0.02 ^{dD}	6.19±0.03 ^{cD}	6.60±0.02 ^{aD}	6.36±0.05 ^{bD}
20	3.04±0.05 ^{dE}	5.00±0.03 ^{cE}	5.68±0.04 ^{bE}	6.12±0.03 ^{aE}	5.99±0.03 ^{aE}

Values are mean ± SE (n = 6). Within a storage day, means with different lowercase superscripts differ significantly. Within a treatment, means with different uppercase superscripts differ significantly ($P < 0.05$). AP0 = aerobic packaging without pepper paste; VP0 = vacuum packaging without pepper paste; VP5, VP10 and VP15 = vacuum packaging with 5%, 10% and 15% green bell pepper paste respectively. Treatment condition, storage day and their interaction were significant for all variables ($P < 0.01$).

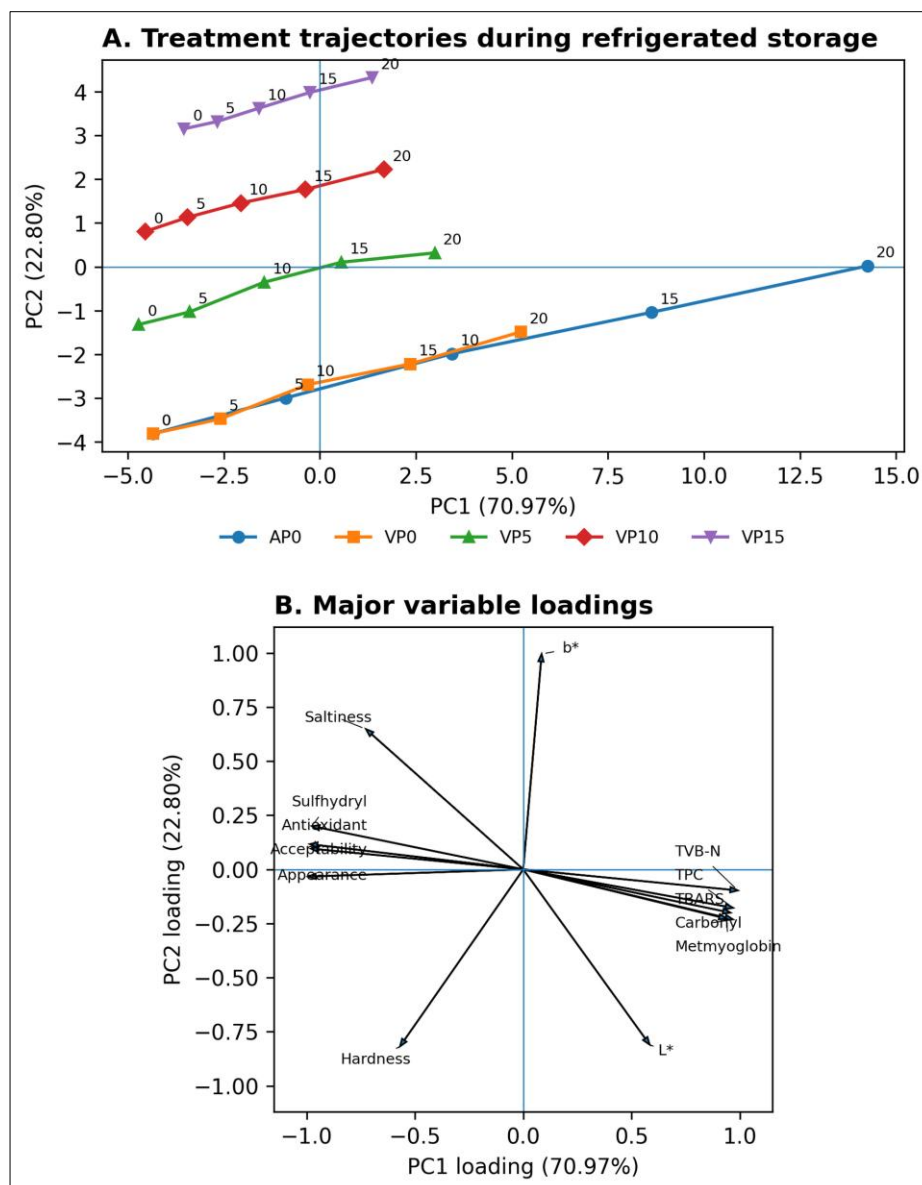


Fig 1: Principal component analysis of 28 standardised physicochemical, oxidative, colour, microbiological, textural and sensory variables in restructured buffalo meat steaks during refrigerated storage at $4\pm 1^\circ\text{C}$. (A) Score trajectories for AP0, VP0, VP5, VP10 and VP15 on days 0, 5, 10, 15 and 20. Numbers adjacent to symbols indicate storage day. (B) Loading plot showing selected major variables contributing to PC1 and PC2. PC1 and PC2 explained 70.97% and 22.80% of the total variance respectively. AP0 = aerobic packaging without pepper paste; VP0 = vacuum packaging without pepper paste; VP5, VP10 and VP15 = vacuum packaging with 5%, 10% and 15% green bell pepper paste respectively.

5. Conclusion

Vacuum packaging substantially delayed the physicochemical, oxidative, microbial, textural and sensory deterioration of restructured buffalo meat steaks stored at $4\pm 1^\circ\text{C}$. Compared with aerobic packaging, it reduced lipid and protein oxidation, metmyoglobin formation, TVB-N accumulation and aerobic microbial growth and improved texture and sensory retention.

Green bell pepper paste provided additional concentration-dependent protection under vacuum packaging. Incorporation at 15% produced the lowest oxidative and microbial deterioration, greatest sulfhydryl and antioxidant retention and shortest multivariate deterioration trajectory. However, this level reduced initial redness, firmness and binding because of greater meat-protein dilution.

The vacuum-packaged formulation containing 10% green bell pepper paste achieved the highest overall acceptability and provided the best balance between oxidative and microbial stability, structural quality and sensory

performance. It is therefore recommended as the optimum formulation, whereas 15% may be selected where maximum preservation is prioritised over initial texture and appearance.

The findings support the combined use of vacuum packaging and green bell pepper paste as a natural preservation strategy for refrigerated restructured buffalo meat products. Commercial validation should include defined packaging specifications, consumer testing and predetermined microbial, chemical and sensory rejection limits.

Author statement

All authors have read and approved the final version of the manuscript. The authors agree to its submission to the *International Journal of Agriculture and Food Science* and confirm that the manuscript has not been published previously and is not under consideration for publication elsewhere.

Author contributions

Irshad A.: Conceptualisation, methodology, investigation, data curation, formal analysis and writing - original draft.

M. Muthulakshmi: Methodology, supervision, validation and writing - review and editing.

R. Rajkumar: Methodology, supervision, validation and writing - review and editing.

B. D. Sharma: Conceptualisation, supervision, project administration and writing - review and editing.

All authors reviewed and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Acknowledgements

The authors gratefully acknowledge the Director, ICAR-Indian Veterinary Research Institute, Izatnagar and the Head, Division of Livestock Products Technology, ICAR-Indian Veterinary Research Institute, Izatnagar for providing the facilities required to conduct this research.

The authors also acknowledge the technical staff of the Division of Livestock Products Technology for their assistance with product preparation, laboratory analyses and refrigerated storage studies. The contribution of the sensory panel members is sincerely acknowledged.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethics statement

The study did not involve experimentation on live animals. Buffalo meat was obtained from commercially slaughtered animals through a licensed meat outlet.

Sensory evaluation was conducted using adult semi-trained panellists after obtaining their voluntary informed consent. The panellists were informed about the nature of the products and their right to withdraw from the evaluation.

References

- Dushyanthan K, Narendra Babu R, Vasanthi C, Venkataramanujam V. Processing of buffalo meat nuggets utilizing different binders. *Tamilnadu J Vet Anim Sci*. 2008;4(2):77-83.
- Anandh MA. Shelf life of boiled restructured buffalo meat rolls in refrigerated storage under vacuum packaging condition. *J Appl Anim Res*. 2015;43(3):318-323. doi:10.1080/09712119.2014.978769.
- Brito TRR, Valeriano HHC, Ítavo LCV, Duarte MT, Pereira MWF, Corrêa SM, *et al.* Effect of including oilseed grains in bovine diets on fatty acid profile, lipid stability and sensory aspects of burgers. *Front Vet Sci*. 2022;9:923937. doi:10.3389/fvets.2022.923937.
- Limbo S, Torri L, Sinelli N, Franzetti L, Casiraghi E. Evaluation and predictive modelling of shelf life of minced beef stored in high-oxygen modified-atmosphere packaging at different temperatures. *Meat Sci*. 2010;84(1):129-136. doi:10.1016/j.meatsci.2009.08.035.
- Anandh MA, Venkatachalapathy RT, Radha K, Lakshmanan V. Quality and shelf life of cooked buffalo tripe rolls at refrigerated storage under vacuum packaging condition. *J Food Sci Technol*. 2014;51(7):1370-1376. doi:10.1007/s13197-012-0646-7.
- Sahoo J, Anjaneyulu ASR. Effect of natural antioxidants and vacuum packaging on the quality of buffalo meat nuggets during refrigerated storage. *Meat Sci*. 1997;47(3-4):223-230. doi:10.1016/S0309-1740(97)00053-3.
- Guo R, Yang F, Liu C, Qiu J, Liang W. Effect of different packaging methods on the quality characteristics of buffalo meat during refrigerated storage. *Sci Technol Food Ind*. 2023;44(4):369-377. doi:10.13386/j.issn1002-0306.2022030387.
- Jaspal MH, Badar IH, Ghani MU, Ijaz M, Yar MK, Manzoor A, *et al.* Effect of packaging type and ageing on the meat quality characteristics of water buffalo bulls. *Animals (Basel)*. 2022;12(2):130. doi:10.3390/ani12020130.
- Sun T, Xu Z, Wu CT, Janes M, Prinyawiwatkul W, No HK. Antioxidant activities of different coloured sweet bell peppers (*Capsicum annuum* L.). *J Food Sci*. 2007;72(2)-S102. doi:10.1111/j.1750-3841.2006.00245.
- Marín A, Ferreres F, Tomás-Barberán FA, Gil MI. Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuum* L.). *J Agric Food Chem*. 2004;52(12):3861-3869. doi:10.1021/jf0497915.
- Howard LR, Talcott ST, Brenes CH, Villalon B. Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. *J Agric Food Chem*. 2000;48(5):1713-1720. doi:10.1021/jf990916t.
- Zhang D, Hamauzu Y. Phenolic compounds, ascorbic acid, carotenoids and antioxidant properties of green, red and yellow bell peppers. *J Food Agric Environ*. 2003;1(2):22-27. doi:10.1234/4.2003.334.
- Shin JY, Kim HS. Quality characteristics of chicken nuggets containing *Protaetia brevitaris seulensis* larvae during refrigerated storage. *Food Sci Anim Resour*. 2026;46(1):41. doi:10.1007/s44463-025-00020-1.
- Rao DN, Sachindra NM. Modified atmosphere and vacuum packaging of meat and poultry products. *Food Rev Int*. 2002;18(4):263-293. doi:10.1081/FRI-120016206.
- Pateiro M, Gómez-Salazar JA, Jaime-Patlán M, Sosa-Morales ME, Lorenzo JM. Plant extracts obtained with green solvents as natural antioxidants in fresh meat products. *Antioxidants (Basel)*. 2021;10(2):181. doi:10.3390/antiox10020181.
- Aminzare M, Hashemi M, Ansarian E, Bimkar M, Azar HH, Mehrasbi MR, *et al.* Using natural antioxidants in meat and meat products as preservatives: a review. *Adv Anim Vet Sci*. 2019;7(5):417-426. doi:10.17582/journal.aavs/2019/7.5.417.426.
- Horbańczuk OK, Kurek MA, Atanasov AG, Brnčić M, Brnčić SR. The effect of natural antioxidants on quality and shelf life of beef and beef products. *Food Technol*

- Biotechnol. 2019;57(4):439-447. doi:10.17113/ftb.57.04.19.6267.
18. Cocan I, Cadariu AI, Negrea M, Alexa E, Obiștioiu D, Radulov I, *et al.* Investigating the antioxidant potential of bell pepper processing by-products for the development of value-added sausage formulations. *Appl Sci.* 2022;12(23):12421. doi:10.3390/app122312421.
 19. Manessis G, Kalogianni AI, Lazou T, Moschovas M, Bossis I, Gelasakis AI. Plant-derived natural antioxidants in meat and meat products. *Antioxidants (Basel).* 2020;9(12):1215. doi:10.3390/antiox9121215.
 20. Ibrahim N, Fayez S, Katary SH, Ibrahim H, Ahmed LI, Ezzat GA. Green valorization of melon and prickly pear byproducts for functional use in minced beef. *NPJ Sci Food.* 2026;10(1):184. doi:10.1038/s41538-026-00922-4.
 21. Conway EJ. Microdiffusion analysis and volumetric error. 3rd rev ed. London: Crosby Lockwood & Son; 1950.
 22. Shantha NC, Decker EA. Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. *J AOAC Int.* 1994;77(2):421-424. doi:10.1093/jaoac/77.2.421.
 23. Tarladgis BG, Watts BM, Younathan MT, Dugan L Jr. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J Am Oil Chem Soc.* 1960;37(1):44-48. doi:10.1007/BF02630824.
 24. Brand-Williams W, Cuvelier ME, Berset C. Use of a free-radical method to evaluate antioxidant activity. *LWT Food Sci Technol.* 1995;28(1):25-30. doi:10.1016/S0023-6438(95)80008-5.
 25. Oliver CN, Ahn BW, Moerman EJ, Goldstein S, Stadtman ER. Age-related changes in oxidized proteins. *J Biol Chem.* 1987;262(12):5488-5491. doi:10.1016/S0021-9258(18)45598-6.
 26. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, *et al.* Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol.* 1990;186:464-478. doi:10.1016/0076-6879(90)86141-H.
 27. Mercier Y, Gatellier P, Viau M, Rémignon H, Renner M. Effect of dietary fat and vitamin E on colour stability and on lipid and protein oxidation in turkey meat during storage. *Meat Sci.* 1998;48(3-4):301-318. doi:10.1016/S0309-1740(97)00113-7.
 28. Liu G, Xiong YL, Butterfield DA. Chemical, physical and gel-forming properties of oxidized myofibrils and whey- and soy-protein isolates. *J Food Sci.* 2000;65(5):811-818. doi:10.1111/j.1365-2621.2000.tb13592.
 29. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys.* 1959;82(1):70-77. doi:10.1016/0003-9861(59)90090-6.
 30. Krzywicki K. Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of beef. *Meat Sci.* 1979;3(1):1-10. doi:10.1016/0309-1740(79)90019-6.
 31. Tang J, Faustman C, Hoagland TA. Krzywicki revisited: equations for spectrophotometric determination of myoglobin redox forms in aqueous meat extracts. *J Food Sci.* 2004;69(9):C720. doi:10.1111/j.1365-2621.2004.tb09922.
 32. Morton RD. Aerobic plate count. In: Downes FP, Ito K, editors. *Compendium of methods for the microbiological examination of foods.* 4th ed. Washington, DC: American Public Health Association; 2001. p. 63-67. doi:10.2105/9780875531755ch07.
 33. de Man JC, Rogosa M, Sharpe ME. A medium for the cultivation of lactobacilli. *J Appl Bacteriol.* 1960;23(1):130-135. doi:10.1111/j.1365-2672.1960.tb00188.
 34. Beuchat LR, Cousin MA. Yeasts and molds. In: Downes FP, Ito K, editors. *Compendium of methods for the microbiological examination of foods.* 4th ed. Washington, DC: American Public Health Association; 2001. p. 209-215. doi:10.2105/9780875531755ch20.
 35. Bourne MC. Texture profile analysis. *Food Technol.* 1978;32(7):62-66, 72.
 36. Gómez-Estaca J, Pintado T, Jiménez-Colmenero F, Cofrades S. The effect of household storage and cooking practices on quality attributes of pork burgers formulated with PUFA- and curcumin-loaded oleogels as healthy fat substitutes. *LWT.* 2020;119:108909. doi:10.1016/j.lwt.2019.108909.
 37. Jaber R, Kaban G, Kaya M. Effects of vacuum and high-oxygen modified-atmosphere packaging on physicochemical and microbiological properties of minced water buffalo meat. *Asian-Australas J Anim Sci.* 2019;32(3):421-429. doi:10.5713/ajas.18.0391.
 38. Ghidelli C, Pérez-Gago MB. Recent advances in modified atmosphere packaging and edible coatings to maintain quality of fresh-cut fruits and vegetables. *Crit Rev Food Sci Nutr.* 2018;58(4):662-679. doi:10.1080/10408398.2016.1211087.
 39. Bernardez-Morales GM, Douglas SL, Nichols BW, Barrazaeta-Cordero RJ, Belk AD, Brandebourg TD, *et al.* Vacuum packaging can protect ground beef colour and oxidation during cold storage. *Foods.* 2024;13(17):2841. doi:10.3390/foods13172841.
 40. Domínguez R, Pateiro M, Munekata PES, Zhang W, Garcia-Oliveira P, Carpena M, *et al.* Protein oxidation in muscle foods: a comprehensive review. *Antioxidants (Basel).* 2022;11(1):60. doi:10.3390/antiox11010060.
 41. Domínguez R, Pateiro M, Gagaoua M, Barba FJ, Zhang W, Lorenzo JM. A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants (Basel).* 2019;8(10):429. doi:10.3390/antiox8100429.
 42. Sembring HS, Chin KB. Antioxidant activities of eggplant (*Solanum melongena*) powder with different drying methods and addition levels to pork sausages. *Food Sci Anim Resour.* 2021;41(4):715-730. doi:10.5851/kosfa.2021.e31.
 43. Sohaib M, Anjum FM, Arshad MS, Imran M, Imran A, Hussain S. Oxidative stability and lipid oxidation flavouring volatiles in antioxidant-treated chicken meat patties during storage. *Lipids Health Dis.* 2017;16:27. doi:10.1186/s12944-017-0426-5.
 44. Bariya AR, Patel A, Gamit VV, Bhedi KR, Parmar R. Assessment of antioxidant and sensory properties of amla (*Embllica officinalis*) fruit and seed-coat powder incorporated cooked goat meat patties. *Int J Curr Microbiol Appl Sci.* 2018;7(7):3306-3318. doi:10.20546/ijemas.2018.707.385.
 45. Wu H, Bak KH, Goran GV, Tatiyaborworntham N. Inhibitory mechanisms of polyphenols on heme protein-mediated lipid oxidation in muscle food: new insights

- and advances. *Crit Rev Food Sci Nutr.* 2024;64(15):4921-4939. doi:10.1080/10408398.2022.2146654.
46. Papuc C, Goran GV, Predescu CN, Nicorescu V, Ștefan G. Plant polyphenols as antioxidant and antibacterial agents for shelf-life extension of meat and meat products: classification, structures, sources and action mechanisms. *Compr Rev Food Sci Food Saf.* 2017;16(6):1243-1268. doi:10.1111/1541-4337.12298.
 47. Zhu W, Han M, Bu Y, Li X, Yi S, Xu Y, *et al.* Plant polyphenols regulating myoglobin oxidation and colour stability in red meat and certain fish: a review. *Crit Rev Food Sci Nutr.* 2024;64(8):2276-2288. doi:10.1080/10408398.2022.2122922.
 48. Manzoor A, Haque A, Ahmad S, Hopkins DL. Incorporation of betel leaf extract provides oxidative stability and improves phytochemical, textural, sensory and antimicrobial activities of buffalo meat sausages. *Meat Sci.* 2023;200:109157. doi:10.1016/j.meatsci.2023.109157.
 49. Torres-Martínez BDM, Sánchez-Escalante A, Torrescano-Urrutia GR, Vargas-Sánchez RD. Natural ingredients to enhance the antioxidant capacity in different meat products: a review. *Foods.* 2026;15(5):852. doi:10.3390/foods15050852.
 50. Cava R, Ladero L. Using polyphenol-rich extracts from tropical fruit by-products to control lipid and protein oxidation in cooked chicken models. *Eur Food Res Technol.* 2024;250:2809-2820. doi:10.1007/s00217-024-04577-x.
 51. Woo SH, Sung JM, Park H, Kim J, Kim YJ, Kim TK, *et al.* Inhibitory effect of natural extract mixtures on microbial growth and lipid oxidation of sausages during storage. *J Anim Sci Technol.* 2023;65(1):225-243. doi:10.5187/jast.2022.e92.
 52. Lušnic Polak M, Kuhar M, Zahija I, Demšar L, Polak T. Oxidative stability and quality parameters of veal during ageing. *Pol J Food Nutr Sci.* 2023;73(1):24-31. doi:10.31883/pjfn/157248.
 53. Baugreet S, Kerry JP, Allen P, Gallagher E, Hamill RM. Physicochemical characteristics of protein-enriched restructured beef steaks with phosphates, transglutaminase and elasticised package forming. *J Food Qual.* 2018;2018:4737602. doi:10.1155/2018/4737602.
 54. Sánchez-Escalante A, Torrescano G, Djenane D, Beltrán JA, Roncalés P. Stabilisation of colour and odour of beef patties by using lycopene-rich tomato and peppers as a source of antioxidants. *J Sci Food Agric.* 2003;83(3):187-194. doi:10.1002/jsfa.1298.
 55. Alakomi HL, Puupponen-Pimiä R, Aura AM, Helander IM, Nohynek L, Oksman-Caldentey KM, *et al.* Weakening of Salmonella with selected microbial metabolites of berry-derived phenolic compounds and organic acids. *J Agric Food Chem.* 2007;55(10):3905-3912. doi:10.1021/jf070190y.
 56. Beya MM, Netzel ME, Sultanbawa Y, Smyth HE, Hoffman LC. Plant-based phenolic molecules as natural preservatives in comminuted meats: a review. *Antioxidants (Basel).* 2021;10(2):263. doi:10.3390/antiox10020263.
 57. Maharjan S, Rayamajhee B, Chhetri VS, Sherchan SP, Panta OP, Karki TB. Microbial quality of poultry meat in an ISO 22000:2005-certified poultry processing plant of Kathmandu Valley. *Int J Food Contam.* 2019;6(1):8. doi:10.1186/s40550-019-0078-5.
 58. Indriani S, Srisakultiew N, Benjakul S, Andriani C, Thumanu K, Kingwascharapong P, *et al.* Molecular structural changes of Korat chicken meat as affected by packaging conditions during cold storage: correlation to textural traits and sensory acceptance. *Poult Sci.* 2026;105(2):106315. doi:10.1016/j.psj.2025.106315.
 59. Draszanowska A, Karpińska-Tymoszczyk M, Olszewska MA. Effect of the addition of chilli pepper fruits and refrigerated storage time on the quality of pasteurised canned meat. *Czech J Food Sci.* 2020;38(5):301-307. doi:10.17221/52/2020-CJFS.
 60. Mir SA, Masoodi FA. Effect of packaging on lipid oxidation, sensory and colour attributes of value-added mutton meatballs during refrigeration. *J Nutr Health Food Eng.* 2017;7(3):301-309. doi:10.15406/jnhfe.2017.07.00240.
 61. Singh P, Wani AA, Saengerlaub S, Langowski HC. Understanding critical factors for the quality and shelf-life of modified-atmosphere-packaged fresh meat: a review. *Crit Rev Food Sci Nutr.* 2011;51(2):146-177. doi:10.1080/10408390903531384.
 62. Zhao Y, Wells JH, McMillin KW. Applications of dynamic modified-atmosphere packaging systems for fresh red meats: review. *J Muscle Foods.* 1994;5(3):299-328. doi:10.1111/j.1745-4573.1994.tb00538.