

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693

ISSN Online: 2617-4707

NAAS Rating (2026): 5.29

IJABR 2026; 10(8): 616-623

www.biochemjournal.com

Received: 26-06-2026

Accepted: 28-07-2026

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Effect of different cooking methods on nutrient composition, lipid oxidation and muscle protein profile of carabeef from culled buffaloes

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i8h.9681>

Abstract

The present study evaluated the effects of pressure cooking, microwave cooking, sous-vide cooking and deep-fat frying on the nutrient composition, lipid oxidation and muscle protein profile of carabeef obtained from mature female buffaloes. Aged *Gluteus medius* muscle was subjected to the four cooking treatments while raw aged meat served as the control. Cooking significantly altered the proximate composition of carabeef. Moisture decreased from 77.33% in raw meat to 53.27-63.31% after cooking, with sous-vide retaining the highest moisture content among the cooked treatments. Crude protein increased from 18.71% in raw meat to 30.31-36.44% after cooking, largely reflecting the concentration effect associated with moisture loss. Deep-fat frying produced the highest ether extract value of 3.86%, compared with 1.36% in raw meat, indicating substantial uptake of cooking oil. Total ash and nitrogen-free extract also increased significantly following cooking. Thiobarbituric acid-reactive substances increased numerically from 0.34 mg malonaldehyde/kg in raw meat to 0.42-0.57 mg malonaldehyde/kg after cooking, although treatment differences were not significant. Electrophoretic analysis revealed marked cooking-induced alterations in both salt-soluble and water-soluble muscle protein fractions. Raw meat exhibited distinct myofibrillar and soluble protein bands whereas cooking markedly reduced the number and intensity of resolvable bands. Sous-vide retained comparatively more identifiable protein bands, particularly in the 30–60 kDa region, while microwave cooking and deep-fat frying caused extensive loss of resolvable bands. Overall, cooking method markedly influenced the nutritional composition and electrophoretic protein profile of carabeef, with sous-vide providing superior moisture retention and comparatively greater preservation of extractable muscle proteins.

Keywords: Buffalo meat, cooking methods, proximate composition, lipid oxidation, muscle proteins, sodium dodecyl sulphate-polyacrylamide gel electrophoresis

1. Introduction

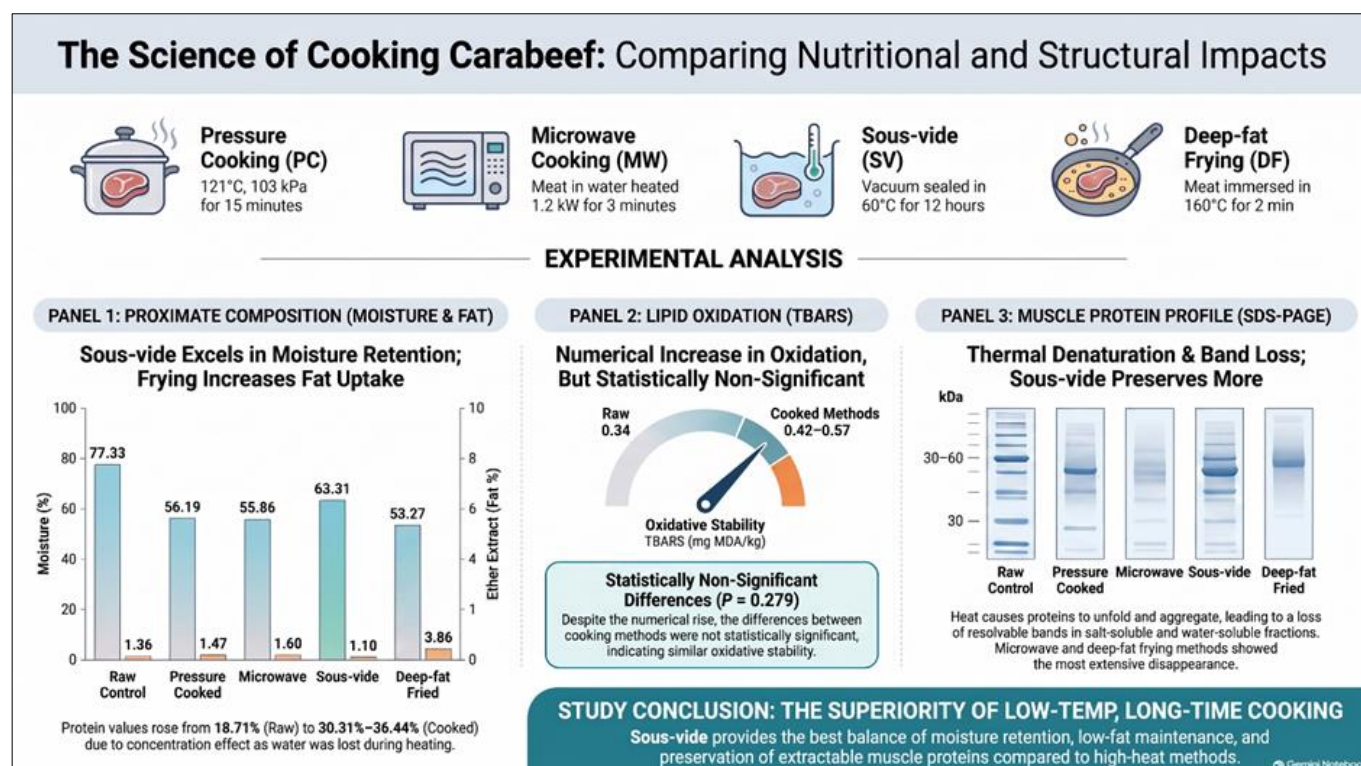
Buffalo meat or carabeef is an important source of high-quality animal protein and essential nutrients. Compared with conventional beef, carabeef is generally characterised by relatively low fat and cholesterol contents while providing high-quality protein, essential amino acids and micronutrients [1]. These nutritional characteristics, together with its favourable processing properties, make buffalo meat suitable for the development of a wide range of value-added meat products [2, 3]. Carabeef from mature or culled animals is particularly important to the meat-processing sector because it provides an economical source of animal protein from animals at the end of their productive life [4]. However, meat from older animals generally contains more mature and highly cross-linked connective tissue, which may contribute to increased toughness and reduced processing quality compared with meat from younger animals [5].

Cooking is essential for improving the palatability, digestibility and microbiological safety of meat [6] but thermal processing also produces substantial changes in its nutritional and structural characteristics. Heating causes denaturation and aggregation of sarcoplasmic and myofibrillar proteins, muscle-fibre shrinkage and redistribution or loss of water. The magnitude of these changes depends largely on the temperature, duration and mechanism of heat transfer [7]. Cooking-related moisture loss can consequently increase the apparent concentrations of protein, fat, ash and other solids in the cooked product. At the same time, thermal processing may promote oxidative reactions involving lipids and proteins, potentially affecting nutritional quality, protein solubility and the functional characteristics of muscle proteins [8].

Different cooking methods expose meat to markedly different thermal environments. Pressure cooking applies high-temperature moist heat, microwave cooking generates rapid volumetric heating within the muscle and deep-fat frying exposes the meat surface to high-temperature cooking oil. In contrast, sous-vide processing combines vacuum packaging with precisely controlled low-temperature long-time heating. Sous-vide cooking has been reported to improve moisture retention and heating uniformity while limiting some of the structural changes associated with more severe thermal processing [9]. Deep-fat frying, on the other hand, can cause substantial moisture loss and uptake of cooking oil and may favour oxidative deterioration because of the high processing temperature [10]. Microwave and conventional heating have also been shown to produce characteristic alterations in the electrophoretic patterns of muscle proteins, reflecting heat-induced denaturation, aggregation and changes in protein solubility [11]. Such changes in extractability and electrophoretic banding patterns provide useful indicators of the extent of thermal

modification of sarcoplasmic and myofibrillar proteins [12, 13].

Although the effects of individual cooking methods on meat quality have been widely investigated [6], direct comparative information on their influence on the nutritional composition, lipid oxidation and muscle protein profile of carabeef from culled buffaloes remains limited. Evaluation of these attributes is particularly relevant because changes in moisture and fat can substantially alter the apparent nutrient composition of cooked meat while heat-induced modification of sarcoplasmic and myofibrillar proteins provides information on the biochemical severity of processing [14]. The present study was therefore undertaken to compare the effects of pressure cooking, microwave cooking, sous-vide cooking and deep-fat frying on the proximate composition, lipid oxidation and electrophoretic profiles of salt-soluble and water-soluble proteins of carabeef from culled buffaloes. The study further aimed to identify a cooking method that provides a favourable nutritional composition while limiting undesirable alterations in the extractable muscle protein profile.



2. Materials and Methods

2.1 Experimental material and sample conditioning

The study was conducted at the Meat Technology Unit, Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India. Six female buffaloes (*Bubalus bubalis*) aged more than six years were slaughtered at the Meat Technology Unit. Following slaughter, the carcasses were subjected to low-voltage electrical stimulation at 100–110 V for 1.5–2 min and chilled at 2–4 °C for 24 h.

The *Gluteus medius* muscles were excised, trimmed of visible external fat and fascia and portioned into uniform cubes of approximately 5 × 5 cm with a thickness of 4–6 cm. The samples were aged for 72 h at 2–4 °C, individually packed in low-density polyethylene pouches and frozen at −18 ± 2 °C until further analysis. Before cooking, the samples were thawed at 4 ± 1 °C for 18–20 h and surface

moisture was removed by blotting. The experimental design comprised raw aged carabeef as the control and four cooking treatments.

2.2 Cooking treatments

Four cooking procedures representing different modes and intensities of heat transfer were evaluated. For pressure cooking, meat samples were cooked with an equal mass of potable water in a domestic pressure cooker at 121 °C and 103 kPa (15 lb/in²) for 15 min. For microwave cooking, samples submerged in an equal mass of potable water were heated in a domestic microwave oven (BPL-Sanyo Model 700 T, 1.2 kW, 2450 MHz) at full power for 3 min.

For deep-fat frying, samples were immersed in refined sunflower oil maintained at 160 °C for 2 min, removed from the oil and drained on absorbent paper. For sous-vide cooking, meat samples were vacuum-sealed at −0.08 MPa in polyester/low-density polyethylene pouches and heated in a thermostatically controlled water bath maintained at 60 ±

0.2 °C for 12 h. Raw aged carabeef served as the uncooked control.

2.3 Proximate composition

Moisture, crude protein, ether extract, total ash and nitrogen-free extract were determined according to standard procedures of the Association of Official Analytical Chemists [15].

2.3.1 Moisture

Approximately 20 g of finely chopped sample was accurately weighed in an evaporating dish and dried in a hot-air oven at 105 °C for 16-18 h. The dried sample was cooled in a desiccator and weighed. Moisture content was calculated from the loss in weight during drying and expressed as percentage of fresh sample weight.

2.3.2 Ether extract

Approximately 1 g of moisture-free sample was subjected to solvent extraction using petroleum benzene ether with a boiling range of 40-60 °C in a Socs-Plus Solvent Extraction System (Pelican Equipments, India). The extracted fat was dried to constant weight at 100 °C, cooled and weighed. Ether extract determined on a dry-matter basis was converted to a fresh-weight basis and expressed as percentage.

2.3.3 Crude protein

Crude protein was determined by the Micro-Kjeldahl method. Approximately 2 g of sample was digested with concentrated sulphuric acid in the presence of sodium sulphate and copper sulphate as catalysts using a Micro-Kjeldahl digestion unit (Kelplus-KES06LE, Pelican Equipment, Chennai, India). Following digestion, the sample was distilled with 40% sodium hydroxide and liberated ammonia was collected in 2% boric acid containing mixed indicator. The distillate was titrated against 0.1 N sulphuric acid. Protein content was calculated on a dry-matter basis, converted to a fresh-weight basis and expressed as percentage.

2.3.4 Total ash

Approximately 2 g of sample was transferred to a porcelain crucible and incinerated in a muffle furnace at 600 °C for 2 h. The crucible was subsequently cooled in a desiccator and weighed. The residual inorganic material was expressed as total ash percentage of the sample.

2.3.5 Nitrogen-free extract

Nitrogen-free extract was calculated by difference on a fresh-weight basis using the equation:

$$\text{Nitrogen-free extract (\%)} = 100 - [\text{Moisture (\%)} + \text{Crude protein (\%)} + \text{Ether extract (\%)} + \text{Total ash (\%)}]$$

The proximate values used for the calculation were obtained from the same experimental samples.

2.4 Lipid oxidation

Lipid oxidation was assessed by determining thiobarbituric acid-reactive substances using the extraction method of Witte *et al.* [16]. Twenty grams of meat sample was homogenised with 50 mL of chilled extracting solution containing 20% trichloroacetic acid in 2 M orthophosphoric acid for approximately 1.5–2 min. The homogenate was transferred to a 100 mL volumetric flask, made up to

volume with deionised distilled water and filtered through Whatman No. 1 filter paper.

Five millilitres of the filtrate were mixed with an equal volume of 0.005 M 2-thiobarbituric acid solution in a screw-capped tube. A reagent blank containing 5 mL distilled water and 5 mL thiobarbituric acid solution was prepared simultaneously. The tubes were heated in a boiling water bath at 100 °C for 30 min and subsequently cooled under running water for 10 min. Absorbance of the developed colour was measured at 530 nm using a UV-visible spectrophotometer (Systronics-119, Ahmedabad, India). Results were expressed as mg malonaldehyde/kg meat.

2.5 Extraction of muscle protein fractions

Water-soluble and salt-soluble protein fractions were prepared from raw and cooked carabeef for electrophoretic analysis.

For extraction of the fraction designated as water-soluble protein, approximately 4 g of finely minced meat was homogenised with 8 mL of 0.6 M sodium chloride solution. The homogenate was incubated at 37 °C and subsequently centrifuged at 3000 rpm for 10 min. The resulting supernatant was collected and protein concentration was estimated using a Nano Drop™ 2000C spectrophotometer.

For extraction of salt-soluble proteins, approximately 2 g of the residue remaining after the first extraction was homogenised with 4 mL of extraction buffer containing 0.5 M sodium chloride and 50 mM phosphate buffer at pH 6.8. The homogenate was centrifuged at 10 000 rpm for 10 min and the resulting supernatant was collected as the salt-soluble protein fraction. Protein concentration was determined using a Nano Drop™ 2000C spectrophotometer.

2.6 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

Electrophoretic profiles of the water-soluble and salt-soluble protein fractions were determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis using a mini-vertical electrophoresis unit (Bio-Rad, USA) according to the method of Laemmli [17]. Electrophoresis was performed in duplicate.

Protein extracts were adjusted to provide approximately 50 µg protein per 10 µL loading volume. Samples were mixed with 2× loading buffer containing 100 mM Tris-Cl, 4% sodium dodecyl sulphate, 0.2% bromophenol blue, 20% glycerol and freshly added dithiothreitol.

Separating and stacking polyacrylamide gels were prepared using Tris-Cl buffers at pH 8.8 and 6.8 respectively. Ammonium per sulphate and N, N, N', N'-tetramethyl ethylenediamine were used to initiate polymerisation. Ten microlitres of each prepared protein sample was loaded into the wells together with a molecular-weight marker.

Electrophoresis was initially performed at 80 V through the stacking gel and subsequently at 120 V through the separating gel until the tracking dye reached the bottom of the gel. The gels were stained with Coomassie Brilliant Blue and destained until distinct protein bands were obtained. The electrophoretic patterns were visualised using a gel documentation system with white-light illumination and photographed for comparative assessment of heat-induced alterations in the muscle protein profile.

2.7 Statistical analysis

The experiment comprised six biological replicates for each treatment. Numerical data on proximate composition and

thiobarbituric acid-reactive substances were analysed by one-way analysis of variance following the procedures described by Snedecor and Cochran [19]. Treatment means were compared using Duncan's multiple range test [20]. Results were expressed as mean $\pm$ standard error and differences were considered statistically significant at $p < 0.05$. The sodium dodecyl sulphate-polyacrylamide gel electrophoretic profiles were interpreted descriptively by comparing the presence, disappearance and relative intensity of protein bands among raw and cooked treatments. The thesis confirms the use of one-way analysis of variance and Duncan's post-hoc procedure for instrumental data.

3. Results

3.1 Proximate composition

Cooking method significantly affected all components of the proximate composition of carabeef ($p < 0.001$) (Table 1). Raw carabeef contained $77.33 \pm 0.44\%$ moisture. Moisture content decreased significantly following cooking, with deep-fat frying producing the lowest value of $53.27 \pm 0.56\%$. Pressure-cooked and microwave-cooked samples contained $56.19 \pm 0.71\%$ and $55.86 \pm 0.70\%$ moisture respectively, whereas sous-vide cooking retained significantly more moisture than the other cooked treatments, recording $63.31 \pm 0.32\%$.

Crude protein increased from $18.71 \pm 0.21\%$ in raw meat to 30.31 – 36.44% in the cooked samples. Deep-fat frying recorded the highest value of $36.44 \pm 0.61\%$, followed by pressure cooking at $36.31 \pm 0.92\%$ and microwave cooking at $35.08 \pm 0.69\%$. Sous-vide cooking recorded a significantly lower crude protein value of $30.31 \pm 0.22\%$ than the other cooked treatments.

Total ash increased significantly following cooking. Raw carabeef contained $0.93 \pm 0.02\%$ ash, while cooked samples ranged from $1.36 \pm 0.02\%$ in sous-vide meat to $1.74 \pm 0.13\%$ in fried meat. Pressure and microwave cooking recorded intermediate values of $1.62 \pm 0.13\%$ and $1.59 \pm 0.12\%$ respectively.

Ether extract was $1.36 \pm 0.11\%$ in raw carabeef. Pressure cooking, microwave cooking and sous-vide cooking recorded values of $1.47 \pm 0.23\%$, $1.60 \pm 0.16\%$ and $1.10 \pm 0.07\%$ respectively and did not differ significantly from the raw control. In contrast, deep-fat frying significantly increased the ether extract content to $3.86 \pm 0.51\%$.

Nitrogen-free extract also differed significantly among treatments. Raw carabeef contained $1.67 \pm 0.38\%$, while pressure cooking, microwave cooking, sous-vide cooking and deep-fat frying recorded values of $3.98 \pm 0.15\%$, $5.49 \pm 0.21\%$, $3.93 \pm 0.16\%$ and $4.69 \pm 0.44\%$ respectively. Microwave cooking produced the highest nitrogen-free extract value.

3.2 Lipid oxidation

Thiobarbituric acid-reactive substances were numerically higher in all cooked samples than in raw carabeef, although the differences among treatments were not statistically significant ($p = 0.279$).

Raw meat recorded a value of 0.34 ± 0.02 mg malonaldehyde/kg. Pressure-cooked and sous-vide samples recorded 0.42 ± 0.09 and 0.45 ± 0.09 mg malonaldehyde/kg respectively. Microwave cooking and deep-fat frying showed the highest numerical values at 0.57 ± 0.11 and 0.57 ± 0.09 mg malonaldehyde/kg respectively.

Thus, despite the numerical increase following cooking, lipid oxidation as measured by thiobarbituric acid-reactive substances did not differ significantly among treatments.

3.3 Electrophoretic profile of salt-soluble proteins

The sodium dodecyl sulphate-polyacrylamide gel electrophoretic profiles of the salt-soluble protein fractions showed marked differences between raw and cooked carabeef (Fig. 1). Raw meat exhibited several distinct protein bands, including a prominent high-molecular-weight myosin heavy-chain band at approximately 200–220 kDa, bands in the 95–135 kDa region, a distinct band at approximately 43 kDa and several lower-molecular-weight bands in the 30–40 kDa region. Cooking markedly reduced both the number and intensity of the salt-soluble protein bands. Pressure-cooked meat retained faint bands in the myosin heavy-chain and 95–135 kDa regions, while bands below approximately 75 kDa were markedly attenuated. Microwave-cooked and deep-fat fried samples were almost devoid of clearly resolvable bands, with only faint staining remaining in the high-molecular-weight region. Sous-vide cooked meat also showed a marked reduction in overall staining intensity compared with raw meat but retained comparatively more discernible protein material than the more intensely heated treatments.

3.4 Electrophoretic profile of water-soluble proteins

Raw carabeef exhibited numerous distinct water-soluble protein bands distributed over a molecular-weight range of approximately 15–100 kDa (Fig. 1). All cooking treatments markedly reduced the number and intensity of these bands. Pressure-cooked, microwave-cooked and deep-fat fried samples exhibited only faint residual bands at approximately 60 kDa and below. Sous-vide cooked meat retained comparatively greater band intensity in the 30–60 kDa region than the other cooked treatments. Overall, the electrophoretic profiles demonstrated substantial heat-induced alteration of both salt-soluble and water-soluble muscle protein fractions.

Loss of resolvable bands was most pronounced following microwave cooking and deep-fat frying, whereas sous-vide cooking showed comparatively greater retention of identifiable protein bands, particularly within the 30–60 kDa region.

Table 1: Effect of different cooking methods on proximate composition of carabeef

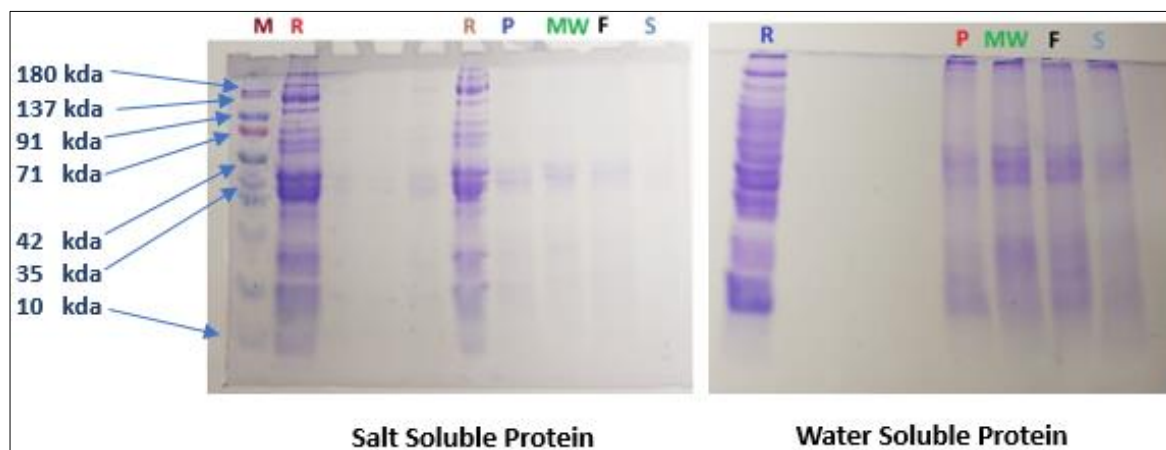
Parameter (%)	Raw meat	Pressure cooking	Microwave cooking	Sous-vide cooking	Deep-fat frying	F value	p value
Moisture	77.33 ± 0.44^d	56.19 ± 0.71^b	55.86 ± 0.70^b	63.31 ± 0.32^c	53.27 ± 0.56^a	297.68	<0.001
Crude protein	18.71 ± 0.21^a	36.31 ± 0.92^c	35.08 ± 0.69^c	30.31 ± 0.22^b	36.44 ± 0.61^c	156.96	<0.001
Total ash	0.93 ± 0.02^a	1.62 ± 0.13^{bc}	1.59 ± 0.12^{bc}	1.36 ± 0.02^b	1.74 ± 0.13^c	10.84	<0.001
Ether extract	1.36 ± 0.11^a	1.47 ± 0.23^a	1.60 ± 0.16^a	1.10 ± 0.07^a	3.86 ± 0.51^b	17.91	<0.001
Nitrogen-free extract	1.67 ± 0.38^a	3.98 ± 0.15^b	5.49 ± 0.21^c	3.93 ± 0.16^b	4.69 ± 0.44^{bc}	23.77	<0.001

values are expressed as mean $\pm$ se (n = 6). means in the same row bearing at least one common superscript do not differ significantly ($p < 0.05$).

Table 2: Effect of different cooking methods on lipid oxidation of carabeef

Treatment	TBARS (mg malonaldehyde/kg)
Raw meat	0.34 ± 0.02
Pressure cooking	0.42 ± 0.09
Microwave cooking	0.57 ± 0.11
Sous-vide cooking	0.45 ± 0.09
Deep-fat frying	0.57 ± 0.09
F value	1.35
p value	0.279

Values are expressed as mean ± SE (n = 6). Differences among treatments were not statistically significant ($p > 0.05$). TBARS – thiobarbituric acid-reactive substances.



M – molecular-weight marker; R – raw carabeef; P – pressure cooking; MW – microwave cooking; F – deep-fat frying; S – sous-vide cooking.

Fig 1: Sodium dodecyl sulphate-polyacrylamide gel electrophoretic profiles of salt-soluble and water-soluble proteins of raw and cooked carabeef.

4. Discussion

4.1 Effect of cooking on proximate composition

Cooking produced marked changes in the proximate composition of carabeef, with the magnitude of change depending on the heating method. The most prominent alteration was the reduction in moisture content, which was accompanied by corresponding increases in the concentrations of several solid components on a fresh-weight basis. Such changes are expected during thermal processing because denaturation and contraction of muscle proteins reduce water-holding capacity and promote the expulsion of intra- and extracellular water [7].

The greatest moisture retention among the cooked treatments was observed in sous-vide carabeef whereas deep-fat frying produced the lowest moisture content. The relatively high moisture content of sous-vide meat can be attributed to the lower cooking temperature and vacuum packaging, which restrict evaporative losses and limit heat-induced muscle-fibre contraction [9]. In contrast, the high surface temperature during deep-fat frying promotes rapid evaporation of water while facilitating the replacement of some lost moisture by cooking oil [21]. Larkeson *et al.* [10] also reported marked compositional alterations associated with frying. These findings demonstrate that the time-temperature combination and mode of heat transfer strongly influence water retention in cooked meat.

The apparent increase in crude protein following cooking should be interpreted primarily as a concentration effect rather than as an actual increase in protein quantity. Protein concentration on a fresh-weight basis increased as water was removed from the meat. Accordingly, the driest treatments, frying and pressure cooking, recorded the highest crude protein percentages whereas sous-vide, which retained more

moisture, showed the lowest protein percentage among the cooked treatments. Similar inverse relationships between moisture and apparent protein concentration have been reported in cooked meat [22, 23]. Thus, higher crude protein values following more intense heating should not be interpreted as superior protein retention without determining true nutrient retention based on both nutrient concentration and cooking yield. Structural contraction and expulsion of intracellular fluid are closely related to the extent of heat-induced protein denaturation [24, 25].

Total ash exhibited a comparable concentration effect. Cooking increased ash from 0.93% in raw meat to 1.36–1.74% in cooked meat, with the highest value occurring in fried samples and the lowest among the cooked treatments in sous-vide meat. This pattern broadly corresponded with the degree of moisture removal. Sen *et al.* [26] similarly demonstrated that thermal treatment can alter the apparent concentration of meat constituents even when the absolute nutritional contribution does not necessarily change to the same extent.

Ether extract clearly differentiated deep-fat frying from the other cooking methods. Pressure cooking, microwave cooking and sous-vide cooking did not significantly alter ether extract relative to raw meat whereas frying increased the value from 1.36 to 3.86%. This increase can be attributed to absorption of sunflower oil during immersion frying in addition to the concentrating effect of water loss. Similar increases in lipid content following frying have been reported previously [10]. This finding has practical nutritional significance because one of the advantages of carabeef is its naturally low-fat content and deep-fat frying partially diminishes this characteristic. The differences observed among treatments further demonstrate the importance of the

cooking medium in determining the final lipid content of cooked meat ^[27].

Nitrogen-free extract also increased after cooking, with the highest value recorded following microwave cooking. However, nitrogen-free extract was calculated by difference rather than measured directly. These values should therefore be regarded as the residual fraction remaining after subtraction of moisture, crude protein, ether extract and ash rather than as evidence of an actual increase in carbohydrate content. Changes in this fraction may reflect concentration following moisture loss, redistribution or loss of soluble constituents and cumulative analytical variation in the other components.

4.2 Effect of cooking on lipid oxidation

Cooking produced a numerical increase in thiobarbituric acid-reactive substances compared with raw carabeef although the treatment effect was not statistically significant. Raw meat recorded 0.34 mg malonaldehyde/kg whereas cooked samples ranged from 0.42 to 0.57 mg malonaldehyde/kg. Microwave cooking and deep-fat frying showed the highest numerical values while pressure cooking recorded the lowest value among the cooked treatments.

Thermal processing may accelerate lipid oxidation by disrupting cellular membranes, releasing pro-oxidant constituents and increasing interactions between unsaturated lipids and reactive oxygen species ^[28]. The comparatively higher numerical value after microwave cooking may be associated with rapid and spatially heterogeneous heating while the value observed following frying may additionally reflect exposure to heated unsaturated cooking oil. Broncano *et al.* ^[29] similarly demonstrated cooking-method-dependent lipid oxidation, with frying and microwave heating producing appreciable oxidative changes. Klinhom *et al.* ^[30] also reported that the extent of lipid oxidation in buffalo meat was influenced by cooking method.

Despite these numerical trends, the absence of a significant treatment effect indicates that none of the cooking procedures produced a sufficiently consistent increase in secondary lipid oxidation products to distinguish the treatments statistically. The findings should therefore be interpreted as a numerical tendency towards increased lipid oxidation following cooking rather than as evidence of a significant cooking-method effect. The relatively low value following pressure cooking may have resulted from the comparatively short moist-heat treatment and limited exposure to atmospheric oxygen during processing ^[31], although this interpretation should be considered cautiously because oxygen availability was not measured directly.

4.3 Effect of cooking on salt-soluble muscle proteins

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis demonstrated substantial modification of the salt-soluble protein profile following cooking. Raw carabeef exhibited a complex electrophoretic pattern dominated by high-molecular-weight myofibrillar proteins, including a prominent myosin heavy-chain region together with bands corresponding approximately to actin and other structural proteins. Cooking reduced both the number and staining intensity of these bands.

These alterations are consistent with heat-induced unfolding, aggregation and loss of solubility of myofibrillar proteins. Myosin is particularly susceptible to thermal denaturation within the temperature range encountered during meat cooking while increasing temperatures subsequently affect other myofibrillar proteins ^[7].

Comparable heat-induced changes in the electrophoretic pattern of muscle proteins have been reported previously ^[18]. Importantly, disappearance of a band from the soluble electrophoretic fraction does not necessarily indicate destruction of the protein molecule. Aggregation and cross-linking may render proteins insoluble under the extraction and electrophoretic conditions, thereby reducing their representation in the gel.

Pressure cooking retained faint high-molecular-weight bands but caused substantial attenuation of lower-molecular-weight proteins. Microwave cooking and deep-fat frying produced very few clearly resolvable salt-soluble bands, indicating greater loss of extractable myofibrillar proteins. Taskiran *et al.* ^[11] similarly reported marked changes in myofibrillar and sarcoplasmic protein band patterns following thermal treatment. Increasing meat temperature has also been shown to alter muscle-protein solubility and the structural characteristics of cooked muscle ^[33].

Sous-vide cooking produced considerably lower staining intensity than raw meat but retained more discernible protein material than the higher-temperature treatments, particularly within the intermediate molecular-weight region. This comparatively greater retention is compatible with the lower cooking temperature used during sous-vide processing because temperature is an important determinant of muscle-protein denaturation, aggregation and extractability ^[7, 33]. Nevertheless, prolonged heating at 60 °C was sufficient to cause substantial alteration of the myofibrillar protein profile. The findings therefore indicate relative preservation of extractable proteins rather than preservation of an unchanged native protein structure.

4.4 Effect of cooking on water-soluble muscle proteins

The fraction designated as water-soluble protein exhibited numerous bands between approximately 15 and 100 kDa in raw carabeef. Cooking markedly reduced band intensity in all treatments, demonstrating the thermal sensitivity of proteins present in this soluble fraction ^[32]. Pressure-cooked, microwave-cooked and fried samples showed only weak residual bands whereas sous-vide treatment retained comparatively greater staining in the 30–60 kDa region.

Sarcoplasmic proteins include numerous metabolic enzymes that are susceptible to thermal denaturation and coagulation. Their declining extractability following cooking is therefore consistent with progressive thermal modification of the soluble muscle-protein fraction ^[34]. The extensive disappearance of bands following microwave cooking and frying suggests rapid unfolding, aggregation and in solubilisation of proteins under these conditions. In contrast, the comparatively greater retention of bands following sous-vide treatment indicates less extensive loss of extractable proteins at the lower processing temperature despite the prolonged cooking period.

The electrophoretic findings are consistent with previous reports demonstrating reductions in sarcoplasmic and myofibrillar protein band intensity following thermal processing. Taskiran *et al.* ^[11] observed clear modifications in electrophoretic profiles of chicken muscle following microwave and conventional cooking. In the present study, the differences among treatments further demonstrate that the mode of heat transfer, temperature and duration of heating collectively influence the extent of muscle-protein modification.

Taken together, the electrophoretic results demonstrate that all four cooking methods substantially altered the

extractable muscle protein profile but the magnitude of alteration differed among treatments. Microwave cooking and deep-fat frying resulted in extensive disappearance of resolvable protein bands whereas sous-vide cooking retained comparatively more identifiable protein material, consistent with previous comparisons of microwave and sous-vide processing on muscle protein structural changes^[36]. These findings indicate that low-temperature long-time processing provides a comparatively milder thermal environment for extractable muscle proteins than the high-temperature cooking methods evaluated. However, the present SDS-PAGE results provide evidence of differences in protein extractability, solubility and electrophoretic profile and should not be interpreted as direct evidence of protein digestibility or biological protein quality^[37].

5. Conclusion

Cooking method markedly influenced the nutritional composition, lipid oxidation and electrophoretic protein profile of carabeef from culled buffaloes. All cooking treatments reduced moisture content, resulting in an apparent increase in the concentration of several solid components on a fresh-weight basis. Sous-vide cooking retained the highest moisture content among the cooked treatments whereas deep-fat frying produced the lowest moisture content and significantly increased ether extract through uptake of cooking oil. The higher crude protein percentages observed following pressure cooking, microwave cooking and deep-fat frying were primarily associated with moisture loss and should therefore be interpreted as concentration effects rather than increased protein retention.

Cooking resulted in only a numerical increase in thiobarbituric acid-reactive substances, with no significant differences among treatments, indicating limited variation in secondary lipid oxidation under the conditions employed. Electrophoretic analysis demonstrated substantial heat-induced modification of both salt-soluble and water-soluble muscle protein fractions. Microwave cooking and deep-fat frying caused the greatest loss of clearly resolvable protein bands whereas sous-vide cooking retained comparatively more identifiable bands, particularly in the 30–60 kDa region.

Overall, low-temperature long-time sous-vide cooking provided a comparatively favourable combination of moisture retention, maintenance of the naturally low-fat character of carabeef and greater retention of extractable muscle proteins. Pressure cooking and microwave cooking resulted in greater compositional concentration associated with moisture loss whereas deep-fat frying substantially increased the lipid content of the cooked meat. These findings demonstrate that the choice of cooking method can significantly influence the nutritional composition and biochemical characteristics of carabeef from mature buffaloes.

6. Acknowledgments

The authors gratefully acknowledge the Dean, College of Veterinary and Animal Sciences, Mannuthy and the Departments of Veterinary Biochemistry and Livestock Products Technology, Kerala Veterinary and Animal Sciences University for providing the facilities required for this study. The authors also acknowledge the teaching and technical staff of the Department of Veterinary

Biochemistry and the staff of the Meat Technology Unit, Mannuthy for their assistance during the experimental work. This article forms part of the M.Sc. dissertation work of the first author submitted to Kerala Veterinary and Animal Sciences University.

7. Conflict of interest

The authors declare that they have no conflict of interest.

8. Funding

The authors received no specific funding for this research

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