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Moringa pod derived antioxidant dietary fibre as a quality enhancer in goat meat nuggets

Annada Das,[a] S. Biswas,[a] P. K. Nanda,[b] Niloy Chatterjee,[c] Srija Pal,[c] Pubali Dhar,[c] Arun K. Verma,[d] Dipanwita Bhattacharya,[e] Rojison Koshy[f] and Arun K. Das *[b]

This study evaluated the quality traits and oxidative storage stability of meat nuggets enriched with immature moringa pod powder (MPP) at 1.5% and 3.0% levels in comparison to control samples over a 12 day storage period under refrigerated conditions. MPP is a rich source of protein (18.96%), ash (7.42%), dietary fiber (DF, 43.64%), and a notable concentration of total phenolics (TP, 9.20 mg GAE g^{-1}). The MPP analyzed by GC-MS showed the presence of different phenolic acids, such as cinnamic, benzoic, phthalic, vanillic, *p*-coumaric, ferulic, and caffeic acids and catechin, with concentrations ranging from 1.031 ppm to 2.949 ppm. Incorporating MPP as a source of DF had a negligible impact ($p > 0.05$) on the pH levels of both the emulsion and meat nuggets. However, it notably improved the emulsion stability, cooking yield, ash content, DF content, and TP content of the nuggets. Immature MPP at the 3% level significantly ($p < 0.05$) influenced the lightness and redness of the nuggets. Moreover, the MPP in meat formulations demonstrated a significant ($p < 0.05$) ability to inhibit lipid oxidation and had no adverse effect on the sensory attributes of meat nuggets. This finding highlights the potential of MPP to enhance oxidative stability during refrigerated storage for up to 12 days. This study suggests that immature moringa pods can serve as a natural functional ingredient by improving the nutritional quality and functionality of meat products while extending their shelf life through their antioxidative properties.

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Sustainability spotlight

This research highlights the potential of immature moringa (*Moringa oleifera*) pods as a natural ingredient that can improve the nutritional quality and functionality of meat products while extending their shelf life through its antioxidative properties. This not only helps contribute to sustainable, eco-conscious food production, aligning with the growing demand for healthy and environment-friendly food options but also offers health advantages to consumers.

Introduction

Meat is a rich source of highly digestible protein, minerals, vitamins (especially B_{12}), and trace elements, such as iodine, iron, and zinc, and hence is preferred while formulating a healthy diet.[1,2] However, meat lacks dietary fibre (DF) and is susceptible to oxidative deterioration owing to its lipid components. In order to meet the expectations of health-conscious consumers, novel functional meat products with a variety of non-meat functional bioactive compounds, such as dietary fibre possessing antioxidant capacity, are being examined to improve the nutritional and health values of meat.[3–5] Dietary fibre is the indigestible cell wall, which is primarily obtained from different edible components of plants and is low in calories.[6] In addition to providing protective benefits against significant diseases, dietary fibre is also associated with a healthy lifestyle.[2,4] It is widely recognized that including foods rich in fiber or utilizing fiber supplements can lower the likelihood of developing coronary heart disease (CHD), hypertension, diabetes, obesity, and specific gastrointestinal conditions.[6,7] When dietary fiber is used in food formulation, it influences both quality characteristics and sensory properties, such as cooking yield, texture, gel-forming ability, and water-holding capacity.[8,9] Incorporating dietary fiber into a meat matrix preserves its juiciness through water retention, reduces cooking losses, and contributes to the gradual release of the volatile compounds that are responsible for enhancing the flavor profile of meat products.[2,10] Therefore, the utilization of dietary fibre in processed meat is of considerable interest as it

[a]*Department of Livestock Products Technology, West Bengal University of Animal and Fishery Sciences, Kolkata-700 037, India*

[b]*Eastern Regional Station, ICAR-Indian Veterinary Research Institute, Kolkata-700 037, India. E-mail: arun.das@icar.gov.in*

[c]*Laboratory of Food Science and Technology, University of Calcutta, 20B, Judges Court Road, Alipore, Kolkata 700 027, India*

[d]*Goat Products Technology Laboratory, ICAR-CIRG, Makhdoom, Mathura-281 122, India*

[e]*Department of Livestock Products Technology, Faculty of Veterinary and Animal Sciences, Banaras Hindu University, Varanasi-221 005, India*

[f]*R&D Centre, Natural Remedies Pvt. Ltd., Bangalore 560100, India*

not only improves the functional and eating properties but also adds value to the products, benefitting both the consumer and industry.[11] Hence, an increasing trend is being noticed regarding the use of such fibre in meat product formulations.

Dietary fibre possessing antioxidant activity, known as antioxidant dietary fibre (ADF), from many plant sources like amaranth and quinoa,[12] bael,[13] aloe vera,[14] litchi,[15] dragon fruit peel,[9] soy,[16] curry leaf,[17] moringa flower,[18,19] and moringa leaves,[20,21] has been incorporated in a variety of meat products, but no information has been available to date regarding the use of the drumstick or moringa pod as a source of fibre in meat food formulation.

Moringa oleifera, also called the 'miracle tree', 'horseradish tree', and 'drumstick tree',[22] is one of the most widely grown and cultivated species, mainly native to the sub-Himalayan region of India.[19] Nearly all parts of this miracle tree, such as the root, flowers, bark, gum, leaf, fruit and drumsticks (green pods), possess a plethora of therapeutic compounds and are used for the treatment of various ailments, including cardiovascular, gastrointestinal, haematological and hepato-renal disorders.[23–25] Of all the tree's parts, the immature green pods are a substantial source of good quality protein with all the major essential amino acids and are rich in dietary fiber, lipids, ash, and non-structural carbohydrates, along with many vitamins and other nutrients.[26,27] In addition to playing a significant role in combating malnutrition and diarrhoea,[26] the pods have excellent antioxidant capacity,[28] hypotensive potential,[29] and immune-modulatory and antiviral activities (useful during COVID-19)[30] and are reported to have an ameliorative effect on the pathogenesis of inflammatory-associated chronic diseases.[31–33] Furthermore, the fibrous pods are useful in treating colon cancer[34] and also have industrial applications due to their pollutant- and pesticide-absorbing properties.[35]

In spite of their enormous benefits, research on moringa pods is mostly focused on their use as a feed supplement or additive in poultry diets to study the productive performance and health status of chickens.[36,37] A few studies have evaluated the effects of moringa pods in functional semolina pasta[38] and pork meat balls,[39] but the information available regarding its use in functional muscle food formulation is scarce. Hence, this work evaluates the detailed *in vitro* antioxidant capacities of MPP in terms of total phenolic content, DPPH radical scavenging activity, ferrous ion chelating activity, *etc.* At the same time, meat nuggets were formulated by incorporating immature moringa pod powder at different levels (1.5% and 3%) as an ADF source and the proximate composition, physico-chemical quality, sensory properties and oxidative storage stability in comparison to control samples were evaluated.

Materials and methods

Materials and reagents

Tender and green moringa pods (drumsticks) less than two weeks of age were harvested from trees located in the campus. The pods were sorted (damaged, diseased and broken pieces were left out), chopped into small pieces, washed thoroughly in clean water and dried in a hot air oven (50 °C) for 8 h. The fine powder obtained after grinding and sieving (using 60 mesh) the dried moringa pods was used for meat product formulation. Goat meat samples (leg and loin cuts) were purchased from a supermarket in Kolkata and were kept at −18 °C until further processing. The chemicals (analytical grade) and other reagents used were procured from standard firms like Hi-Media (Mumbai, India) and Sigma-Aldrich (USA).

Extract from moringa pod powder

Extract was prepared from moringa pod powder (MPP) using sterile distilled water as the solvent (40 °C) and the total phenolics and antioxidant capacities were determined as per the method described by Madane *et al.*[19] with slight modification. Briefly, to 5 g MPP, 200 mL water was added in a conical flask and continuous stirring at normal room temperature was performed for 10 h. The contents were centrifuged (REMI NEYA 8, Kolkata, India) at 5000 g for 10 min and filtered using filter paper. The extract obtained was stored at 2 °C until further analysis.

In vitro antioxidant capacities of moringa pod powder extract

2,2-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity. This activity of MPP extract was determined by slightly modifying the method of Rahman *et al.*[40] In a test tube, 1 mL of MPP extract was combined with 2 mL of a 0.1 mM methanolic DPPH solution. After incubating in darkness for 30 minutes, the color change from deep violet to a light-yellow hue, signifying radical scavenging activity, was quantified spectrophotometrically at 517 nm. Methanol was used to establish the baseline absorbance, while Trolox, BHT, and BHA served as positive controls. The percentage of inhibition or DPPH activity was determined using the following formula.

$$\text{Percentage of inhibition } (I\%) = [(A_0 - A)/A_0] \times 100$$

Here, A_0 is the absorbance of the blank, while A is the absorbance of the test extract. The IC_{50} values were determined based on the inhibition of free radical formation (I%) by the MPP extract.

Hydroxyl radical scavenging (HRS) assay. The HRS of the MPP extract was evaluated using the Fenton reaction.[41] Various concentrations of the MPP extract (1 mL each) were combined with 2 mL of 1.5 nM $FeSO_4$, 1 mL of 6 mM H_2O_2, and 0.3 mL of 20 mM sodium salicylate. This reaction mixture was then heated to 37 °C for one hour and cooled to room temperature, and its absorbance at 510 nm was measured. In this assay, positive controls such as Trolox, BHA, and BHT were employed, and the IC_{50} values were calculated using the inhibition percentage (I%).

Ferrous ion chelating activity (FICA). The FICA of MPP was evaluated by the reduction in absorbance at 562 nm resulting from the formation of the iron(II) and ferrozine complex.[3] Here, 1 mL of the test sample (with a concentration of 5 mg) was blended with 1 mL of methanol and 0.1 mL of 2 mM $FeCl_2$. The reaction was commenced by adding 0.2 mL of 5 mM ferrozine, and the mixture was allowed to incubate for 10 minutes at room

temperature before measuring the absorbance at 562 nm. For positive controls, Trolox and BHT were used, while sterile distilled water served as the blank. The FICA was calculated with the following equation.

$$\text{Metal ion chelating rate } (M\%) = [1 - (A/A_0)] \times 100$$

Here, A is the absorbance of the MPP extract and A_0 is the absorbance of the blank/control at 562 nm. The IC_{50} values were derived from M%.

Superoxide anion radical scavenging activity. The test involved a reaction mixture comprised of 0.2 mL of MPP at various concentrations, 0.2 mL of 60 mM phenazine methosulphate (PMS), 0.2 mL of 677 mM NADH, and 0.2 mL of 144 mM nitroblue tetrazolium (NBT), all of which were dissolved in a phosphate buffer with a concentration of 0.1 mol l^{-1} and a pH of 7.4. The absorbance of the reaction mixture was subsequently measured at 560 nm using spectrophotometry.[42]

$$\text{Superoxide radical scavenging activity } (\%) = [(A_0 - A_1)/A_0 \times 100]$$

Here, A_0 is the absorbance of the control (Trolox and BHT) and A_1 is the absorbance of the sample.

Total antioxidant activity (β-carotene-linoleic acid method). In this specific assay for measuring antioxidant activity, we evaluated the ability to hinder the formation of the volatile organic compounds and conjugated diene hydroperoxides generated during the oxidation of linoleic acid.[43] Initially, a stock solution was prepared by dissolving 0.5 mg of β-carotene in 1 mL of chloroform, supplemented with 25 μl of linoleic acid and 200 mg of Tween 40. The chloroform was subsequently removed *via* vacuum evaporation. Following this step, 100 mL of distilled water was added to the mixture, and it was vigorously agitated. Then, 2.5 mL of this reaction mixture was dispensed into test tubes, and different concentrations of MPP extract were introduced, alongside the positive controls BHA, BHT, and Trolox, with sterile distilled water serving as a blank. The emulsion system was incubated at 50 °C for up to 2 hours. During this incubation period, we monitored the absorbance of the mixture at 490 nm at 15 minute intervals. To determine the rates of β-carotene degradation for both the MPP extract and the positive controls, we applied first-order kinetics, following the protocol outlined by Al-Saikhan *et al.*[44]

Reducing power (RP) assay. To perform the RP assay, we initiated the process by adding 2.5 mL of 0.2 M phosphate buffer (with a pH of 6.6) and 2.5 mL of $K_3Fe(CN)_6$ (at a concentration of 1% w/v) to 1 mL of the MPP extract.[45] This mixture was then incubated at 50 °C for 20 minutes. After this incubation period, we introduced 2.5 mL of trichloroacetic acid (at a concentration of 10% w/v) and subsequently centrifuged the mixture at 3000 rpm for 10 minutes to collect the supernatant, which amounted to 2.5 mL. This supernatant was combined with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (at a concentration of 0.1% w/v). The absorbance of the resulting solution was measured at 700 nm, using sterile distilled water as a reference blank. Furthermore, we also conducted a ferric reducing antioxidant power (FRAP) assay, following the procedure outlined by Madane *et al.*[19]

Total phenolic content and phenolic composition by GC-MS. For measurement of the total phenolic content (TPC), the classical Folin–Ciocalteu (FC) method[46] was used. The process was initiated by adding 0.75 μl of the FC reagent to each 100 μl of extracts derived from both MPP and nuggets. Subsequently, we increased the volume tenfold by diluting it with distilled water. Following this step, 750 μl of a 7.5% sodium carbonate solution was introduced to the mixture. We mixed the resulting combination for 5 minutes to ensure a thorough reaction and then placed it in darkness at room temperature for a duration of 1 hour and 30 minutes to facilitate the development of color. After this incubation period, the absorbance at 725 nm against a blank sample was recorded. To determine the total phenolic content (TPC), we created a standard curve using various concentrations of gallic acid. The TPC within the MPP sample was subsequently quantified in terms of milligrams of gallic acid equivalents (GAE) per gram of MPP on a dry weight basis.

For phenolic composition estimation, GC-MS analysis of extracts of moringa pod was performed using a GC-MS triple quadrupole (GC-MS TQ8030, Shimadzu, Japan) as per the standard procedure of Al-Owaisi *et al.*[47] The process of identifying phenolic acids within the extract entailed a systematic comparison of the retention times and mass spectral data of the unidentified compounds with well-established calibration standards. To quantify these compounds, we relied on the ratio of the peak area of the compound of interest to that of an internal standard. Each of the calibration standards and the moringa pod samples underwent triplicate runs for analysis.

Goat meat nugget formulation

Nuggets were prepared in three separate batches using a standardized procedure.[13] The initial batch served as the control group, consisting of meat without MPP. In the other two batches, labeled T_1 and T_2, MPP was incorporated at concentrations of 1.5% and 3%, respectively, replacing an equivalent percentage of meat. The goat meat was first thawed and then minced using an electric meat mincer (Tallers Ramon, Model P-22, Barcelona, Italy). Subsequently, it was chopped in conjunction with all other non-meat ingredients needed to prepare the emulsion (as outlined in Table 1). Approximately 400–500 grams of the resulting emulsion were steam-cooked in a mold for a duration of 40 minutes, and the cooking yield was recorded. Afterward, the cooked goat meat was sliced into small

Table 1 Meat and non-meat ingredients used for goat meat nugget formulation

Ingredients (%)	Control	(T_1)	(T_2)
Minced goat meat	71.00	69.50	68.00
Salt	1.50	1.50	1.50
Ice flakes	10.00	10.00	10.00
Refined oil	8.00	8.00	8.00
Garlic and onion paste	4.00	4.00	4.00
Dry spice mix	2.00	2.00	2.00
Refined wheat flour	3.50	3.50	3.50
Moringa pod powder	0.00	1.50	3.00

nuggets, which were then hermetically sealed in low-density polyethylene (LDPE) pouches and stored at a consistent refrigerated temperature of 4 ± 1 °C. Analysis of storage stability in terms of TBARS value was conducted at 4 day intervals over a span of 12 days.

pH, emulsion stability and cooking yield

For pH estimation, a 10 gram portion of the sample, whether emulsion or final product, was combined with 50 mL of distilled water (DW) within a plastic centrifuge tube. Subsequently, the sample with water was homogenized for the duration of one minute, utilizing a tissue homogenizer (Model PT-MR-2100, Kinematica AG, Switzerland). For the evaluation of emulsion stability, the meat emulsion (25 g) was placed into a polypropylene pouch. This pouch containing the meat was heated at a controlled temperature of 80 °C for 20 minutes within a temperature-controlled water bath. The percentage of emulsion stability was determined subsequent to the removal of accumulated exudate from the pouches.[19] To express the product or cooking yield (as a percentage), the weight of the emulsion both before and after the cooking process was recorded. The product yield was measured based on the following equation.

$$\text{Cooking yield (\%)} = \text{cooked meat weight/raw meat weight} \times 100$$

Proximate analysis and dietary fibre content

The proximate compositions (moisture, total protein, fat and total ash) of the MPP and meat nuggets were analysed as per the method described by AOAC,[48] wherein the enzymatic-gravimetric method was used for determination of dietary fibre content.[49] Briefly, the dietary fibre estimation procedure involved the placement of both MPP and fat-free nuggets into a phosphate buffer. Subsequently, they underwent sequential enzymatic digestion, employing heat-stable amylase, protease, and aminoglycosidase enzymes.[9] The analysis yielded measurements for both soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). The total dietary fiber (TDF) was then calculated as the combined sum of IDF and SDF.

Expressible water

The expressible water content of the nuggets was assessed using a modified centrifuge method as previously described.[50] Approximately 5 grams of finely minced cooked nuggets were sandwiched between two layers of filter paper and then placed inside a 50 mL centrifuge tube. These tubes were subjected to centrifugation at 1500 g using a centrifuge (Remi India) for a duration of 15 minutes, after which their weight was recorded. The expressible water content was calculated as a percentage using the following formula.

$$\text{Expressible water (\%)} = (\text{initial weight} - \text{final weight})/\text{initial weight} \times 100$$

Texture profile analysis (TPA)

The TPA of the cooked goat meat nuggets was carried out utilizing a texturometer (Stable Micro System Model TA.XT 2i/25, UK). For each treatment group, central cores measuring 1.5 cm^3 were prepared. These cores underwent a double compression to 80% of their initial height, facilitating the assessment of several crucial parameters such as hardness, springiness, cohesiveness, gumminess, and chewiness. The compression procedure was conducted at a consistent speed of 2 mm per second.

Instrumental colour attributes

To record the color parameters of the samples, a Hunter color lab system (Mini XE, Portable HunterLab, Reston, USA) enabled the measurement of Hunter L^* (representing brightness on a scale from 0 to 100), a^* (indicating + redness or -greenness), and b^* (denoting + yellowness or -blueness). Prior to use, the instrument underwent proper calibration, involving the use of a light trap, black glass, and a white tile provided with the system. Measurements were taken at four distinct points on the samples.

Thiobarbituric acid reacting substance (TBARS) value

The TBARS values were analysed to evaluate the lipid oxidation in the goat meat nugget samples.[51] For this, a nugget (10 g) from each treatment was homogenized with 25 mL of 20% trichloroacetic acid (TCA, pre-cooled) for 2 min. The TCA extract was prepared after filtering the homogenized content. In a test tube, 3 mL of thiobarbituric acid (TBA) reagent and an equal quantity of TCA extract were added and boiled in a water bath at 70 °C for 35 minutes. Likewise, the control or blank was taken by mixing 10% TCA solution and TBA reagent. The absorbance of the mixture was quantified with the spectrophotometer (Eppendorf BioSpectrometer, USA) at a wavelength of 532 nanometers. The TBARS values were represented as milligrams of malonaldehyde per kilogram of the meat sample.

Sensory evaluation

The sensory qualities, encompassing attributes such as appearance, flavor, texture, and overall acceptability, of nuggets containing MPP as a dietary fiber source were assessed using an 8-point descriptive scale. This scale ranged from 1, indicating extremely poor, to 8, representing excellent quality.[15] To ensure unbiased evaluation, the panelists were given a briefing about the experiment's nature, though the specific sample coding remained undisclosed. They were then instructed to assess both the treated and control nugget samples. Before conducting the evaluation, the samples were gently heated in a microwave oven to ensure uniform temperature. Additionally, each panelist was provided with filtered water to rinse their mouths between sample tastings.

Statistical analysis

Data analysis was carried out using IBM-SPSS software, version 20.0, and all parameters were meticulously recorded in duplicate. To assess lipid oxidation, a two-way analysis of

variance (ANOVA) was employed, considering treatments (control, T_1, and T_2) and storage periods (0 days, 4th day, 8th day, and 12th day) as the primary factors within a 3 × 4 factorial design. Furthermore, for the comparison of parameter means, Duncan's multiple range test was utilized.[52] The resulting mean values are presented alongside their corresponding standard errors (SE), and statistical significance was determined at a 95% confidence level.

Results and discussion

Chemical composition and dietary fibre contents of MPP

The proximate composition of MPP and its dietary fibre content are presented in Table 2. The results indicate that MPP had a good amount of protein (18.96%) and ash (7.42%). The findings of this study are in line with those of Sánchez-Machado *et al.* (2010), who indicated that the protein and ash contents in immature moringa pods were 19.34% and 7.62%, respectively, but differed from Manzoor *et al.*,[53] where protein content was higher, ranging from 20.66–30.07% in mature seeds of *M. oleifera*. Analyzing the proximate composition of *M. oleifera* pods, Razzak *et al.*[54] found the moisture (83.12% and 86.03%) and total ash (2.01% and 1.80%) contents in raw and thermally treated samples, respectively. Furthermore, the thermally processed moringa pods had crude protein (3.00%), fat (0.10%) and carbohydrate (3.20%) contents. Such variations in proximate or chemical composition could be attributed to many factors, including the nature of the soil, agro-climatic conditions of the area, stage of maturity of plant part, cultivars, *etc.*, as reported by many researchers.[3,19]

Further, the MPP had a total dietary fibre of 43.64% with a higher fraction of insoluble (32.30%) than soluble fibre (11.34%). In a previous study, Sánchez-Machado *et al.*[26] reported a similar percentage of dietary fibre in moringa pods. Likewise, Mallillin *et al.*[55] also stated that moringa pods are excellent source of total (34.0 ± 0.2 g/100 g), insoluble (22.7 ± 0.2 g/100 g) and soluble (11.3 ± 0.2 g/100 g) dietary fiber, corroborating our findings that moringa pods have a good amount of dietary fibre.

As shown in Table 2, the aqueous extract of MPP had a total phenolics content of 9.20 mg GAE g^{-1}. Few reports regarding the TPC of immature pods are available in the literature to substantiate our findings. In one study, Ravani *et al.*[56] reported that extracts of *M. oleifera* (var. PKM-1) pod pulp had a TPC of 11.02 mg GAE g^{-1}. Razzak *et al.*[54] and Prasajak *et al.*[39] found higher values for the TPC of *M. oleifera* pods, *i.e.*, 28.13 mg GAE g^{-1} and 55.17 mg GAE g^{-1}, respectively. Hadi *et al.*[57] quantified a much higher value (500.05 mg GAE g^{-1}) for the TPC of crude alcoholic extract of *M. oleifera* pods. On the other hand, Golla *et al.*[58] reported that the TPC values of fresh and dried *M. oleifera* pods were in the range of 5.40–21.74 mg GAE g^{-1}, which is similar to our findings.

Phenolic composition of moringa pod extract

Understanding the phytochemical profile of immature moringa pods is vital for assessing their beneficial bio-activities and optimizing their utilization in nutritional, pharmaceutical and industrial applications. In our study, cinnamic acid, 3-hydroxybenzoic acid, 4-hydroxy-benzoic acid, phthalic acid, *p*-coumaric acid, vanillic acid, ferulic acid, caffeic acid and catechin were the major phytochemical compounds identified in immature moringa pods (Fig. 1). Catechin, the naturally occurring flavonoid, was identified and quantified at a concentration of 4.329 ppm; it is popular for its antioxidant and anti-inflammatory effects. The concentrations of different phenolic acids contributing to the antioxidant and antimicrobial activities ranged from 1.031–2.949 ppm (Fig. 1). Our results corroborate the findings of Salem *et al.*,[59] who identified various polyphenols, like vanillic acid (5053.49 mg/100 g), benzoic acid (262.98 mg/100 g), *etc.*, as the main components of mature moringa pods from an HPLC chromatogram. The above identified compounds are well known for contributing to the antioxidant activities of moringa pods. In another similar study, Hadi *et al.*[57] identified phenolic acids like 4-hydroxy benzoic

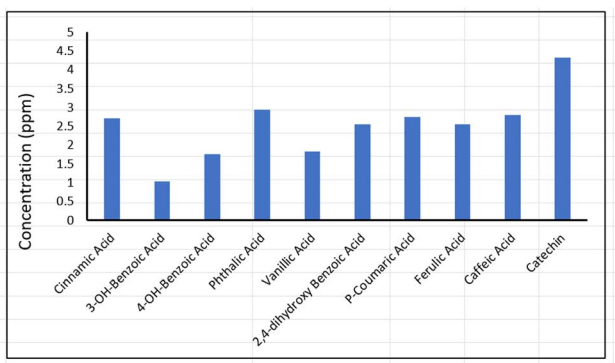

Fig. 1 Phenolic composition of immature moringa pod extract.

Table 2 Chemical composition and dietary fibre contents of moringa pod powder

Content	(g/100 g dry weight)
Protein	18.96 ± 0.38
Ash	7.42 ± 0.38
Lipid	1.35 ± 0.17
Total dietary fibre	43.64 ± 1.82
Soluble dietary fibre	11.34 ± 1.04
Insoluble dietary fibre	32.30 ± 0.98
Non-structural carbohydrates	28.63 ± 1.15
Total phenolics (mg gallic acid equivalent/g) content	9.20 ± 2.14

acid (37.57%) as the principal polyphenolic compounds in the ethanolic and methanolic extracts of moringa pods through GC-MS analysis.

In vitro antioxidant activity of moringa pod powder

DPPH radical scavenging activity. The *in vitro* antioxidant activity of the MPP extract was evaluated using the DPPH radical scavenging method. The results were checked with those of established antioxidants, including BHA, BHT and Trolox. The findings are summarized in Table 3. As indicated, the DPPH radical was efficiently scavenged by the MPP extract and, at a very low concentration of 751.27 μg mL^{-1}, it showed 50% inhibition. Standard antioxidants like BHA, BHT and Trolox were much more competent in destroying reactive radicals and were about 99% more effective than the test MPP extract. In a recently conducted study, the DPPH radical scavenging activity of *M. oleifera* leaves was found to be within 46.32–58.09% and 56.76–69.72% for methanol and ethanol extracts, respectively.[60] In another study, a methanolic extract of moringa plant bark showed an IC_{50} of 40 μg mL^{-1}, whereas its leaf and stem extracts achieved the same at 320 μg mL^{-1} and 720 μg mL^{-1}, respectively.[61] The studies by Madane *et al.*[19] and[20] showed that the IC_{50} values for the DPPH radical scavenging activity of aqueous extracts of *M. oleifera* flowers and leaves were 126.20 μg mL^{-1} and 18.54 μg mL^{-1}, respectively. Chumark *et al.*[62] also found that the IC_{50} of aqueous extract of *M. oleifera* leaves and Trolox were 75.15 ± 0.92 and 2.14 ± 0.12 μg mL^{-1}, respectively. On the other hand, Siddhuraju *et al.*[23] observed a dose-dependent increase in DPPH activity (2.3–65.03%) in the pericarp of the immature drumstick and flower of *M. oleifera* and the flower and leaf of *Sesbania grandiflora.* Likewise, Ramamurthy *et al.*[41] indicated that the aqueous extract of *M. oleifera* leaves showed a DDPH free radical scavenging activity ranging from 67.52–88.52% in a concentration dependant manner (10–50 μl).

Hydroxyl radical scavenging assay. The MPP extract scavenged 50% radicals at 810.13 μg mL^{-1}, compared to BHA, BHT and Trolox at 51.27, 81.91 and 68.14 μg mL^{-1}, which respectively showed 93.67%, 89.89% and 91.59% more potency than the MPP extract. As per Ramamurthy *et al.*,[41] an aqueous extract of *M. oleifera* leaves exhibited hydroxyl radical scavenging activity ranging from 72.65% to 90.21% at a concentration range of 10–50 μl. In an another study, Siddhuraju *et al.*[23] observed high hydroxyl radical scavenging activity (21.6–82.4%) in acetone rather than ethanol extracts of *M. oleifera* (pericarp of immature drumstick and flower) and *S. grandiflora* (flower and leaf).

Ferrous ion chelating activity. The antioxidant properties of plant extracts can be ascertained from their ability to chelate transition metal ions like Fe^{2+} and Cu^{2+}. The MPP extract was very potent in degrading such species, showing 50% inhibition at 757 μg mL^{-1}. Standard antioxidants like BHA, BHT and Trolox were equally efficacious at much lower concentrations of 38.99, 53.08 and 44.92 μg mL^{-1}, respectively, and were 94.85, 92.99 and 94.07% more effective than the examined MPP extract. In contrast to this, Das *et al.*[3] observed 50% chelation activity by bamboo essential oil at a much lower concentration of 0.53 μl mL^{-1}. However, these findings show similarity with the results of Arawande,[63] who observed that the iron chelating activity (%) of moringa pods ranged from 28.14–36.73%. Likewise, Verma *et al.*[64] reported that the IC_{50} values for the ferrous ion chelating activity of various extracts of *M. oleifera* leaves varied from 0.28–2.17 mg mL^{-1}.

Superoxide anion radical scavenging. In this study, the efficacy of MPP extract destroying free radicals was evident, but was relatively lower than those of other standards (BHA, BHT and Trolox). The IC_{50} of the MPP extract was observed at 950 μg mL^{-1} in comparison to much lower concentrations of BHA, BHT and Trolox; thus, MPP showed 95.81, 93.43 and 95.31% less activity, respectively. Likewise, Verma *et al.*[64] found potent IC_{50} values of the crude extract, aqueous extract and ethanolic fraction of *M. oleifera* leaves for scavenging superoxide anion radicals at concentrations of 0.25 mg mL^{-1}, 1.86 mg mL^{-1} and 0.17 mg mL^{-1}, respectively.

Total antioxidant activity by β-carotene–linoleic acid method. The IC_{50} of the MPP extract in this study was found to be 94.76%, 92.09% and 93.93% less than the standard antioxidants BHA, BHT and Trolox, respectively. In a study, Verma *et al.*[64] noticed that the total antioxidant activities (%) in terms of the bleaching of β-carotene were 80.36%, 22.36% and 89.35% for the crude extract, aqueous extract and ethanolic extract of *M. oleifera* leaves, respectively. Similarly, Elmastaş *et al.*[65] observed that the water and ethanol extracts of bay leaf concentrations at 20, 40 and 60 μg mL^{-1} showed 84.9%, 95.7% and 96.8% and 94.2%, 97.7% and 98.6% inhibition of lipid peroxidation linoleic acid emulsion, respectively.

Reducing power assay. The reducing power of MPP extract was very effective, as evidenced by the increase per 100 μg of sample. The MPP extract showed 12.36% reducing power in comparison to standard antioxidants like BHA, BHT and Trolox, which have 66.95%, 44.93% and 54.57% reducing power, respectively (Table 3). Verma *et al.*[64] also estimated the reducing

Table 3 *In vitro* antioxidant potentials of moringa pod powder extract as compared to standard antioxidants

In vitro antioxidant potential	BHA	BHT	Trolox	MPP extract
DPPH radical scavenging activity (IC_{50}) (μg mL^{-1})	7.01 ± 1.55	13.01 ± 0.10	7.13 ± 0.09	751.27 ± 15.11
Ferrous ion chelating activity (IC_{50}) (μg mL^{-1})	38.99 ± 0.02	53.09 ± 0.05	44.92 ± 1.32	757.30 ± 6.13
Hydroxyl radical scavenging activity (IC_{50}) (μg mL^{-1})	51.27 ± 0.31	81.91 ± 0.26	68.15 ± 1.22	810.13 ± 0.95
β-Carotene–linoleic acid method (IC_{50}) (μg mL^{-1})	46.57 ± 0.39	70.32 ± 0.14	53.98 ± 0.49	888.81 ± 11.19
Superoxide radical scavenging activity (IC_{50}) (μg mL^{-1})	40.15 ± 1.01	62.97 ± 1.32	44.99 ± 0.44	958.63 ± 3.89
Reducing power assay (% increase per 100 μg)	66.95 ± 1.37	44.93 ± 0.81	54.57 ± 0.01	12.36 ± 0.76
Ferric reducing antioxidant power ($FeSO_4$ mole equivalent (M mg^{-1}))	0.28 ± 0.03	0.13 ± 0.01	0.11 ± 0.02	0.035 ± 0.01

power of *M. oleifera* leaves using different solvents as an ascorbic acid equivalent and the values ranged from 0.25–2.18 ASE per mL.

Ferric reducing antioxidant power (FRAP) assay. In this assay, an $FeSO_4$ mole equivalent is reduced to ferrous ions (Fe^{2+}) from ferric (Fe^{3+}). In this phenomenon, an electron is donated by various antioxidants and the FRAP is measured. The MPP extract showed a FRAP efficacy of 0.0353 M mg^{-1}, which is about 87.57%, 74.044% and 69.57% lower than those of BHA, BHT and Trolox, respectively. The study conducted by Iqbal *et al.*[66] reported a nearly similar value of FRAP for *M. oleifera* leaf extracts with a value ranging from 20–48 μM at a concentration range of 5–20%. Similarly, Asekunowo *et al.*[67] observed that the methanolic extracts of moringa pods showed stronger FRAP activity at a concentration of 49 923 μg mL^{-1}. Ramamurthy *et al.*[41] also found that the aqueous extract of *M. oleifera* leaves showed FRAP activities of 71.52%, 73.52%, 79.58%, 83.25% and 89.25% at concentrations of 10 μl, 20 μl, 30 μl, 40 μl and 50 μl, respectively. In studies conducted by Tekle *et al.*[68] and Madane *et al.*,[19] the alcoholic extracts of *M. oleifera* leaf (7.5–10 mg mL^{-1}) and flower, respectively, also exhibited good FRAP activities.

Physico-chemical properties of goat meat nuggets

Effects of MPP incorporation on pH and emulsion stability of nuggets. The effects of MPP on the pH and emulsion stability of goat meat nuggets were also examined (Table 4). The results indicated that pod powder used at both levels (1.5 and 3.0%) decreased the pH of the emulsion, although non-significantly ($p > 0.05$). The lower pH value in the MPP treated emulsions could be due to the acidic pH of the MPP. In a similar study, incorporation of moringa flower powder decreased the pH of chicken meat nuggets.[19] The findings of this study showing a non-significant ($p < 0.05$) decrease in emulsion pH are in partial conformity with the results of Habib *et al.*,[69] where a significant decrease in the pH of carabeef after incorporation of different levels of pomegranate rind powder was reported. The addition of MPP as a fibre source significantly ($p < 0.05$) improved the emulsion stability. Meat nuggets with 3.0% MPP had the highest emulsion stability (88.27%), whereas it was the least in the control group (84.97%). Improvement in the emulsion stability of various meat products has also been reported by different researchers with the incorporation of guava antioxidant dietary fibre,[70] dragon fruit peel fibre,[9] and wheat fibre mix.[71]

Effects of MPP on cooking yield and composition of nuggets. The effects of MPP as a source of fiber on the chemical composition and cooking yield of goat meat nuggets were studied and are summarized in Table 4. The inclusion of MPP at 3% led to a statistically significant improvement ($p < 0.05$) in the cooking yield of the nuggets. Interestingly, there was no discernible distinction ($p > 0.05$) in cooking yield observed between the meat nuggets prepared with 1.5% and 3.0% MPP. In a previous study, Fang *et al.*[72] indicated that the use of sugarcane fibre in chicken sausage formulation resulted in increased cooking yield and reduced water and fat loss. It is well established that use of dietary fibres in emulsion-based meat, as well as non-meat components, increases the cooking yield and decreases the water loss during cooking.[13] This could be due to the higher surface area and porosity of pod fibres that favour the binding of more water and fat molecules, resulting in less loss during the cooking process and higher yield, as found in the MPP treated goat meat nuggets. A similar trend was also reported by Anderson and Berry,[73] where pea fibre incorporated into beef patties with 10% fat had increased cooking yield.

Incorporating MPP at both concentrations, 1.5% and 3.0%, resulted in a statistically significant ($p < 0.05$) increase in the moisture content of the nuggets compared to the control sample. This substantial increase in moisture content within the treated nuggets can be attributed to the enhanced water-binding capacity of the fiber present in the pod powder. Swelling of the moringa pod fibre, gelatinization of the starch in the flour during cooking and overall increased water binding by the moringa pod fibre might have played vital roles in improving the water retention of treated nuggets. Further various factors should be taken into account when considering the water holding capacity in meat products, including the conformational characteristics of amino acids within protein molecules and the solubility of dietary fibre.[74] Interestingly, while the protein and fat contents of the nuggets, both in the control and treatment groups, showed no statistically

Table 4 Effects of moringa pod powder on quality characteristics of meat emulsion and nuggets[a]

Parameters	Control	Moringa pod powder (1.5%-T_1)	Moringa pod powder (3.0%-T_2)
Emulsion pH	6.16 ± 0.02	6.14 ± 0.02	6.13 ± 0.03
Emulsion stability (%)	84.97 ± 0.25^{b}	87.29 ± 0.22^{a}	88.27 ± 0.26^{a}
Product pH	6.28 ± 0.01	6.29 ± 0.01	6.30 ± 0.01
Cooking yield (%)	92.02 ± 0.41^{b}	94.82 ± 0.36^{a,b}	95.28 ± 0.44^{a}
Moisture (%)	64.48 ± 2.38^{b}	66.31 ± 0.86^{a}	67.19 ± 0.32^{a}
Protein (%)	16.10 ± 0.80	16.09 ± 0.48	15.82 ± 0.45
Fat (%)	10.24 ± 0.47	9.28 ± 0.94	9.31 ± 0.27
Ash (%)	2.22 ± 0.03^{b}	2.50 ± 0.02^{a}	2.57 ± 0.04^{a}
Expressible water (%)	23.08 ± 2.42	21.85 ± 2.36	19.25 ± 2.34
Total dietary fibre (%)	0.68 ± 0.06^{a}	1.21 ± 0.08^{b}	1.78 ± 0.10^{c}
Total phenolic content (mg GAE g^{-1})	0.062 ± 0.42^{c}	0.342 ± 0.39^{b}	0.582 ± 0.42^{a}

[a] a–c Data (mean ± SE) with different superscript(s) row-wise differ significantly ($p < 0.05$).

significant differences ($p > 0.05$), there was a noticeable increase in ash content in the treated groups. This rise in ash content can be attributed to the inherently higher ash content (4.72%) present in MPP. Similar to the present study, Al-Juhaimi *et al.*[75] recorded that moringa seed flour increased the ash content of patties. Again, the use of the flower from moringa as an antioxidant dietary fibre has been reported to significantly improve ash content in treated nuggets.[19]

Effects of MPP on expressible water, total dietary fibre and total phenolic contents of nuggets. The expressible water content plays an important role in determining the water holding capacity (WHC) of emulsion-based meat products. The relation between these two is simple: the lower the percentage of extracted water, the greater the WHC.[50] In this study, no significant difference ($p > 0.05$) in expressible water percent between the control and treated nuggets (T_1-1.5% and T_2-3.0%) was found. This observation suggests that the nuggets with the higher MPP concentration (T_2-3.0%) had a non-significantly ($p > 0.05$) lower percentage of expressible water. Many reports are available on the effects of fibre on the expressible water content of various meat products. For instance, incorporation of sugar beet fibre was reported to improve the WHC of frankfurters without any significant influence on sensory attributes.[76] Furthermore, inclusion of *M. oleifera* flower at different levels lowered the expressible water, indicating the retention of more water in chicken nuggets.[19] In fact, plant fibres obtained from sugarcane, pea, millet, oat *etc.*, are hydrophilic polyhydroxy compounds which might contribute to the high water-binding capacity.[77] The insoluble fraction of polysaccharides might help to bind water, thereby reducing the cooking loss and allowing more water retention.[2,4] Our findings clearly indicate that replacing an equal part of lean meat with MPP was beneficial in increasing the cooking yield and lowering the expressible water.

Dietary fiber is widely recognized for its important role in enhancing both the palatability and health-related merits of meat products. In our study, the incorporation of MPP as a substitute for lean meat resulted in a significant increase ($p < 0.05$) in the total dietary fiber (TDF) and total phenolic contents within the goat meat nuggets. Remarkably, the nuggets treated with 3.0% MPP exhibited a notably higher TDF content (1.78%) compared to the nuggets with 1.5% MPP (1.21%) and the control group (0.68%), and this difference was statistically significant ($p < 0.05$).

Similarly, higher total phenolics contents (TPC) (0.342 and 0.582 mg GAE g^{-1}) were recorded in the nuggets incorporated with 1.5% MPP and 3.0% MPP, respectively, than in the control (0.062 mg GAE g^{-1}). This increased TDF content and TPC in the treated nuggets can be attributed to the substantial TDF content (43.64%) and total phenolic content (9.20 mg GAE g^{-1}) present in the MPP itself. Similar to these findings, incorporation of amaranth and quinoa seed flour[12] or dragon fruit peel powder in meat product formulation has also been reported to significantly increase the TDF content.[9] Improvement in the dietary fibre and TPC of emulsion-based products was also reported when moringa flower powder[19] and bael pulp residues were incorporated as ADFs in meat formulations.[13]

Effects of MPP on textural properties and instrumental colour values of nuggets. The incorporation of MPP did not affect textural properties such as adhesiveness, springiness, gumminess, and chewiness of meat nuggets, but hardness was non-significantly ($p > 0.05$) lower in treated samples, indicating slightly softer nuggets than the control (Table 5). The textural properties of meat products may be influenced by the type of meat and plant fibres[11] used in the formulation. In a study, Verma *et al.*[70] recorded that use of guava powder did not significantly affect different textural properties of meat products. Adhesiveness is often comparable to the sensory attribute "stickiness to mouth".[78] In this study, nuggets with MPP had non-significantly lower ($p > 0.05$) adhesive value than the control nuggets. This could be due to the presence of fibre in the MPP. Likewise, incorporation of MPP non-significantly ($p > 0.05$) decreased the springiness of nuggets, which was probably due to their higher moisture content than the control, as also reported by Das *et al.*[78] Other textural properties of nuggets treated with MPP were non-significantly ($p > 0.05$) different from the control nuggets, although they decreased slightly with increasing levels of MPP in the nuggets.

The analysis of colour values is very important, as colour influences the acceptance of meat products by consumers. The effects of MPP on the colour values of cooked goat meat nuggets are presented in Table 5. The incorporation of MPP at 3% concentration influenced the colour values, with significant ($p < 0.05$) decreases in the lightness (L^*) and redness (a^*) values of the T_2 nuggets. However, the nuggets with 1.5% MPP had similar colour values as the control samples, and a non-significant difference ($p > 0.05$) was observed for the lightness (L^*) and redness (a^*) values of T_1 and control. The yellowness

Table 5 Effects of MPP on textural attributes, colour values and sensory attributes of goat meat nuggets[a]

Parameters	Control	T_1 1.5% MPP	T_2 3.0% MPP
Textural properties			
Hardness	92.76 ± 4.31	90.17 ± 4.75	89.59 ± 6.12
Adhesiveness	−0.03 ± 0.02	−0.02 ± 0.01	−0.02 ± 0.02
Springiness	0.80 ± 0.01	0.77 ± 0.01	0.75 ± 0.01
Cohesiveness	0.38 ± 0.01	0.36 ± 0.01	0.37 ± 0.01
Gumminess	35.20 ± 1.92	34.09 ± 2.21	34.14 ± 2.86
Chewiness	27.25 ± 1.67	26.16 ± 1.90	25.89 ± 1.83
Colour values			
L^* value	45.74 ± 0.22^{a}	44.45 ± 0.18^{a,b}	44.17 ± 0.20^{b}
a^* value	7.12 ± 0.18^{a,b}	6.68 ± 0.19^{a,b}	6.47 ± 0.18^{b}
b^* value	12.36 ± 0.20	12.43 ± 0.28	12.56 ± 0.32
Sensory attributes			
Appearance	7.08 ± 0.13	6.94 ± 0.12	6.86 ± 0.18
Flavour	7.03 ± 0.25	6.96 ± 0.23	6.87 ± 0.22
Juiciness	7.01 ± 0.10	7.13 ± 0.12	7.18 ± 0.08
Texture	6.80 ± 0.20	6.62 ± 0.23	6.50 ± 0.22
Overall acceptability	6.92 ± 0.13	6.88 ± 0.12	6.84 ± 0.17

[a] MPP: moringa pod powder; L^* value: lightness; a^* value: redness; b^* value: yellowness. a–c Data (mean ± SE) with different superscripts row-wise differ significantly ($p < 0.05$).

(b^*) values of nuggets in all groups (control, 1.5% MPP and 3.0% MPP) were similar ($p > 0.05$). Various researchers have reported that the use of plant fibre influences the colour properties of meat nuggets. Madane *et al.*[19] observed a significant increment in L^* values and a decrease in a^* values in nuggets incorporated with *M. oleifera* flower as antioxidant dietary fibre. Again, both fresh and cooked sausages prepared with sugarcane fibre had increased L^* values and b^* values, but decreased a^* values.[11] Likewise, sheep meat nuggets with guava fibre have also been reported to have higher L^* values and lower a^* values.[70]

Thiobarbituric acid reacting substances (TBARS) value of meat nuggets. The measurement of malonaldehyde is one of the most commonly used indicators to know the degree of secondary lipid oxidative changes in meat products during storage, where a lower value indicates lower lipid oxidation. The TBARS values of cooked goat meat nuggets (control and treated) during storage for 12 days at 4 ± 1 °C under aerobic packaging conditions are presented in Fig. 2. The TBARS values in both control and treated nuggets increased, with a maximum of 1.25 mg malonaldehyde/kg for the control and 0.81 malonaldehyde per kg for treated samples after 12 days (Fig. 2). However, nuggets treated with MPP showed a comparatively ($p < 0.05$) slower rate of lipid oxidation compared to the control samples, indicating less oxidative deterioration (more storage stability), which may be due to the higher TPC and antioxidant potentials of the MPP. In a study, Jayawardana *et al.*[79] observed a significant reduction of TBARS values during storage in chicken sausages enriched with *M. oleifera* leaves. Moringa flower powder also retarded lipid oxidation in chicken nuggets, keeping the oxidation well below the unacceptable limit during the entire storage period.[19] Likewise, Das *et al.*[20] recorded 47% lower TBARS values in meat patties treated with *M. oleifera* leaf extract than in the control sample. Interestingly, in an *ex-vivo* assay conducted by Chumark *et al.*,[62] an aqueous extract of *M. oleifera* leaf retarded the LDL oxidation in human plasma by reducing the formation of TBARS and completely blocking it at higher concentration (50 μg mL^{-1}). Overall, the results from this study indicate that the inclusion of moringa pod demonstrated a notable antioxidant capacity, effectively inhibiting lipid oxidation in the meat nuggets. This antioxidant action extended the shelf life of the nuggets when stored under refrigeration conditions (4 ± 1 °C) in aerobic packaging.

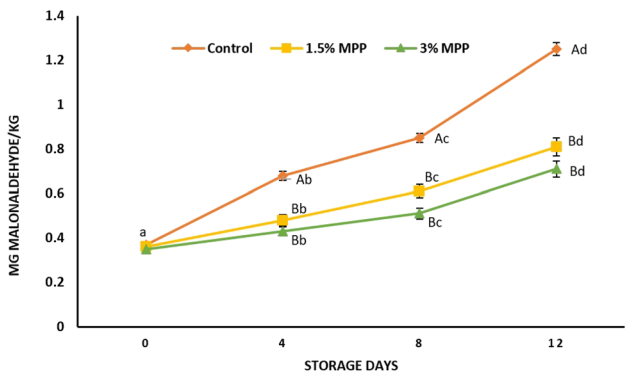

Fig. 2 The effect of moringa pod powder (MPP) on oxidative changes in nuggets during storage. $^{A-B}$ Indicate significant differences ($p < 0.05$) between treatments. $^{a-d}$ Indicate significant differences ($p < 0.05$) between storage periods.

Sensory attributes of meat nuggets. Sensory attributes play a vital role in the consumer acceptance of a new food product. The level of MPP incorporation had no significant influence on sensory attributes, although numerically lower ($p > 0.05$) appearance scores (pale colour) of treated nugget samples than control were noticed (Table 4). This could be due to the difference in colour of goat meat and pod fibre, with the replacement of goat meat with whitish MPP making the nuggets relatively paler. Also, it can be correlated with the fact that the yellowness (b^*) values of the T_1 and T_2 nuggets were non-significantly ($p > 0.05$) higher than that of the control nuggets. Similarly, no significant differences ($p > 0.05$) in flavor scores between control (C) and treated samples (T_1 and T_2) were observed, which indicates that nuggets with MPP had no adverse effect on flavor scores. In another study, Al-Juhaimi *et al.*[75] reported that increasing levels of moringa seed flour decreased the sensory attributes like appearance, flavour and acceptability of meat products (except tenderness), but the rates of decline were non-significant ($p > 0.05$).

As far as texture scores are concerned, no significant differences ($p > 0.05$) between the treated (T_1 and T_2) and control (C) samples were observed. The addition of MPP resulted in non-significantly ($p > 0.05$) lower texture scores, indicating a slight improvement in tenderness (softer texture) which could be due to the lower chewiness in the treated samples (Table 4). The nuggets with MPP had non-significantly ($p > 0.05$) better juiciness scores than the control samples. In a study, chicken nuggets incorporated with *M. oleifera* flower powder were reported to be juicer than control nuggets.[19] This could be due to the increased water holding capacity and moisture retention by the pod fibre of the meat nuggets during cooking process. The overall acceptability score of goat meat nuggets also exhibited similar variations relative to the appearance, texture and flavour scores. In a similar work, chicken sausages prepared with 0.25% and 0.5% *M. oleifera* leaves[79] and meat patties with 0.1% *M. oleifera* leaves[20] had no difference in sensory attributes compared to the controls. From this study, it can be concluded that the goat meat nuggets formulated with 1.5 and 3.0% MPP had acceptable sensory scores.

Conclusion

The findings of this study indicate that immature moringa pod powder is a good source of dietary fibre, protein, ash and total phenolics, including many phenolic acids, and possesses intense antioxidant properties. Incorporating immature moringa pods at both concentrations (1.5% and 3.0%) in meat formulations significantly ($p < 0.05$) enhanced the emulsion stability and increased the phenolic and dietary fiber contents of the meat nuggets. Although the moringa pod incorporation at a 3% level significantly ($p < 0.05$) increased the cooking yield and decreased ($p < 0.05$) the redness values, it did not influence the textural properties and overall sensory acceptability of the

end products. Furthermore, the inclusion of immature moringa pods enhanced the storage stability of the meat nuggets, and MPP therefore could be used as a natural functional ingredient for the development of healthier meat products providing potential health advantages to consumers.

Data availability

All data and materials are available.

Conflicts of interest

The authors report no conflicts of interest.

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