Antioxidant, chemical, microbial and sensory quality characteristics of Moringa (*Moringa oleifera*) leaf powder added chicken patties

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Abstract

Chicken, accounting for 37% of the world's total meat production, is increasingly popular due to its affordability and health benefits. However, processed meat foods are more susceptible to lipid peroxidation due to various processing conditions. Synthetic antioxidants have been used to prevent oxidation-induced damage to meat, but their genotoxic effects have led to concerns. The industrial sector is now shifting towards natural antioxidants from plant materials high in polyphenols. The purpose of this study was to investigate the potential of *Moringa oleifera* leaves powder (MOLP) as a phytopreservative for enhancing the storage stability of chicken patties. MOLP was added added in the formulation at the rate of 1% (T1), 1.5% (T2), and 2% (T3) compared to control and positive control (BHT added). The product with best sensory score T2 was further analyzed to check pH, oxidative changes, antioxidant potential, microbial quality, and sensory characteristics during storage. The study found that MOLP significantly improved patties' quality, with lower pH, TBARS values, and higher DPPH inhibition, maintaining good microbial and sensory quality up to 12 days of storage. It can be concluded that MOLP, up to 1.5% level, can effectively control deteriorative changes without affecting sensory characteristics.

Keywords: Natural antioxidant; *Moringa oleifera* leaves powder; MOLP; Chicken meat patties; Sensory evaluation; Physico-chemical quality; Antimicrobial activity; DPPH activity

Introduction

Approximately 37% of the world's total meat production consists of chicken. Chicken accounts for more than half of the annual per capita meat intake and has one of the highest per capita consumption rates in the world (OECD/FAO, 2021). People are increasingly opting to consume chicken. This is because poultry is less expensive than other types of meat in low- and middle-income countries, whereas in high-income nations, people are gravitating towards white meats since they are easier to prepare and are believed to be healthier (Uzundumlu & Dilli, 2022). Ready-to-eat processed foods, particularly meat and meat by-products, are in high demand in more and more urbanising developing countries as a result of shifting eating habits and customer preferences.

The processed meat foods are more susceptible to lipid peroxidation due to application of various processing conditions. Although synthetic antioxidants like butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ), propyl gallate (PG), and butylated hydroxytoluene (BHT), have been used for a long time to prevent oxidation-induced damage to meat, their possible genotoxic effects have brought them under closer scrutiny. The current industrial trend, then, is towards natural antioxidants generated from a wide range of plant materials that are high in polyphenols that can neutralise free radicals. The perishable nature of meat foods make it more prone to microbial spoilage (Saucier, 2016). Extending the shelf life of meat products is a complicated phenomenon that relies heavily on the sequential regulation of oxidation and microbial spoiling processes during both ambient and chilled storage. Consumers are increasingly mindful of health benefits, convenience, shelf life, and safety when choosing processed foods due to awareness programme by social media platforms and food research organization (Hygreeva & Pandey, 2016). For this reason, there has been a recent shift in the industrial sector towards the use of natural antioxidants produced through a wide range of plant materials that are high in radical-scavenging polyphenols.

Moringa oleifera, also referred to as drumstick tree or the horse-radish tree, is a highly productive crop in many parts of Southeast Asia. Since the plant's constituents have medicinal value, it has been in use for quite some time. Several phytonutrients, including zeaxanthin, lutein, alpha- and beta-carotene, and chlorophyll, can be found in Moringa. Phytonutrients have been shown to improve liver function, boost immunity, aid in the production of new blood cells, and revitalise the body at the cellular level (Sarkar et al., 2022). The leaves and fruits of various plants are considered vegetables in their native nations. Dried leaves can be used in infusions or processed into powder for more convenient storage and use. Moringa leaves include phytochemicals like alkaloids, protein, quinine, saponins, flavonoids, tannin, steroids, glycosides making them a potential source of antioxidants and antimicrobials (Bagheri et al., 2020).

Some information regarding adding the powdered leaves of the *M. oleifera* plant to meat and meat products has been published (Das et al., 2012; Khomola et al., 2021; Madane et al., 2019). The study was carried out to check effect of *M. oleifera* leaves powdered in chicken patties during at refrigerated storage.

Materials and Methods

In order to conduct the experiment, fresh, boneless chicken was purchased from a meat shop in Junagadh, Gujarat, India. Polyethylene bags were used to retain the chicken meat in the fridge at a temperature of (4±1°C) for an entire night before it was used. Before grinding, cold meat was sliced into small cubes. Fat and connective tissue that could be separated did so. Onions and garlic were purchased from a Junagadh city market. Garlic and onion were peeled, chopped, and crushed into a fine paste individually before being combined for use in the condiment mix. The condiment blend was made using a 4:1 ratio of onion to garlic. Chicken masala powder (Everest) readily available in market was used as a spice mix. Wheat flour, refined vegetable oil, and salt were all bought at nearby shops. Analytical grade chemicals and media were used from standard firms like Sigma, Merck, SRL and Hi-media etc. for the analysis of product.

Preparation of MOLP

Moringa oleifera leaves powder (MOLP) was prepared as per method described by Mashau *et al.*, (2021) with suitable modifications. Leaves of the *Moringa oleifera* plant were picked from the campus of Junagadh Agricultural University, Junagadh and rinsed under running water to eliminate any debris. After washing, the leaves were spread out on a tray and given around 24 hours to dry in the air. The leaves were then dried in a hot air oven at 50 degrees Celsius for three hours. The powder was made by grinding the dried leaves in a mixer grinder. The powder was sieved through a 36-mesh screen to ensure a high degree of fineness. Powder of an extremely fine consistency was sealed in a plastic jar of amber colour and kept in a cool, dry place.

Estimatipon of total phenolic content of MOLP

Total phenolic content (TPC) of MOLP was measured spectrophotometrically using a suitable modifications in the method provided by Singleton & Rossi (1965). For the preparation of alcoholic extract of MOLP, about 5 g of *Moringa oleifera* leaves powder was mixed with 20 ml methanol and ethanol (1:1) and left for 24 hrs in a shaker. Sample was filtrated by whatman filter paper no.1. Filtrate was used as crude extract in further procedure. Briefly, 0.2 mL of the crude extract was added into tubes containing 1.0 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in distilled water. The sample was incubated for 10 minutes before 0.8 ml of a sodium carbonate solution (7.5% w/v) was added. After letting the tubes stand out for 30 minutes at room temperature,

the absorbance at 743 nm was taken. Blank preparation consisted of taking the same volume of distilled water as the sample. The TPC was reported as milligrammes of gallic acid equivalents per kilogram of sample (mg GAE/kg). Polyphenol concentration of sample was calculated using a standard curve of gallic acid with concentrations ranging from 5 to 70 mg GAE/kg.

Estimation of DPPH radical scavenging activity of MOLP

Moringa oleifera leaves powder was tested for its potential to scavenge the 2,2-diphenyl -1-picrylhydrazyl (DPPH) radical using a technique provided by Brand-Williams et al., (1995) with slight modifications. Briefly, 6.92 mg of DPPH (Sigma-Aldrich) was taken in amber coloured reagent bottle and dissolved in 6.25 ml methanol by stirring over magnetic stirrer overnight. The final volume was adjusted with methanol to 12.5 ml using a volumetric flask and the stock solution was stored at -20°C. 2 ml of stock solution was diluted with 18 ml methanol in a volumetric flask to prepare working solution. The working solution was prepared freshly prior to analysis and kept in an amber glass bottle.

About 0.2 ml of MOLP extract and 1.8 ml of freshly made DPPH working solution were added together in a 10 ml test tube, stirred with a vortex stirrer, and incubated in the dark at 37 degrees Celsius for 120 minutes after covering the test tube with aluminium foil. Absorbance at 515 nm was taken using a visible spectrophotometer (WENSAR, WSP-V500). For the blank determination, 0.2 ml of methanol was used in place of the sample, and the absorbance was immediately measured against methanol. Radical scavenging activity was calculated using the following formula.

$$\% \text{ inhibition} = \left(\frac{\text{A blank} - \text{A sample}}{\text{A blank}}\right) x\ 100$$

Preparation of Chicken Meat Patties

Chicken meat patties were prepared following method as described by Talukder et al., (2020). Briefly, Chicken meat was minced (8-mm plate) using a meat mincer (MADO, MEW 710, Germany). Minced meat and all other ingredients (Table-1) were thoroughly mixed in a bowl chopper (SCHARFEN, TC11, Germany) to prepare the emulsion for each treatment. Emulsion was moulded in a petri-plates (90 mm x 15 mm) to form patties and were cooked in a pre-heated oven at 180±5°C for 15 min, after which they were turned and allowed to get cooked for 10 more minutes till internal temperature reaches about 75±20C. After cooling to room temperature, the patties were aerobically packaged in a low-density polyethylene (LDPE) pouches and were stored at refrigerated temperature until its acceptability tested by quality parameters.

Sensory evaluation of chicken meat patties

A nine-point descriptive hedonic scale was used to rate the product on a variety of sensory aspects, including its appearance, flavour, texture, juiciness and overall acceptability. A score of nine indicated the highest quality, while a score of one indicated the lowest. The panel consisted of staff members with moderate experience who were familiar with meat products and their qualities. The coded samples were served at room temperature in separate booths. Prior to the sensory test, the patties were heated for 20 seconds in the microwave. Between tastings, participants were offered water to rinse their mouths. The most acceptable product based on sensory score was used for further analysis and storage study.

Determination of cooking yield in chicken meat patties

Raw chicken meat patties (10 g) were weighed using a weighing balance, then cooked according to the prescribed protocol, and chilled to room temperature for both the treatment and control groups. The cooked chicken patties were weighed again after being surface-dried on filter paper. The yield from cooking was calculated by comparing the pre- and post-cooking weights of chicken patties.

$$Cooking\ yield = \frac{Weight\ of\ cooked\ chicken\ meat\ patties}{Weight\ of\ raw\ chicken\ meat\ patties}\ x\ 100$$

Table 1. Formulation of chicken meat patties with different concentrations of *Moringa oleifera* leaves powder

Ingredients	Control (%)	Positive control (%) (BHT)	**Treatments**		
			T1 1% MOLP)	T2 1.5% MOLP)	T3 2% MOLP)
Chicken meat	62	62	61	60.5	60
Ice flakes	15	15	15	15	15
Refined vegetable oil	8	8	8	8	8
Salt	1.5	1.5	1.5	1.5	1.5
Condiments*	4.5	4.5	4.5	4.5	4.5
Refined wheat flour	3	3	3	3	3
Dry spice mix**	2.7	2.7	2.7	2.7	2.7
Sodium tripolyphosphate	0.30	0.30	0.30	0.30	0.30
Sodium nitrite (ppm)	150	150	150	150	150
MOLP	0	0.01(BHT)	1	1.5	2

* Condiments: Onion and Garlic (4:1). ** Dry spice mix: commercially available chicken masala powder (Everest Chicken Masala)

Determination of pH in chicken meat patties

The pH of the cooked patties were measured using a technique provided by Muthukumar et al., (2014). Briefly, 10 g of sample was homogenized with 50 milliliters of distilled water for 60 seconds. Standardized electrode of a pH meter (Thermo scientific, ORION STAR A111, Indonesia) was dipped directly into the suspension to determine its pH value.

Determination of Thio-barbituric acid reactive substances (TBARS) value of patties

Using a suitable modification of the extraction procedure published by Witte et al., (1970), the TBARS value (mg malonaldehyde/kg) of patties was calculated. In a nutshell, a 10 g. sample was homogenized with 25 mL of a 20% trichloroacetic acid solution in 2M H_3PO_4, and the resulting slurry was centrifuged at 3,000 g for 10 minutes. Supernatant (5 mL) was combined with 0.005M thiobarbituric acid (also 5 mL) in glass test tubes and left at room temperature overnight. The TBARS values were computed using a factor of 5.2 and represented in mg malonaldehyde/kg based on the mixture's absorbance at 532 nm.

Estimation of DPPH radical scavenging activity of patties

A sample of meatballs was prepared specifically for DPPH analysis, following the methodology described by Verma et al., (2023). In summary, a sample weighing five grams was subjected to trituration within a conical flask that contained a mixture of ethanol and methanol in a 1:1 proportion, with a total volume of 20 mL. Subsequently, the resulting mixture filtrated using Whatman filter paper (No. 42). The analysis was conducted on the extract of the samples. The above mentioned method for DPPH analysis of MOLP extract was further used.

Microbiological quality of chicken meat patties

Standard American Public Health Association procedures (Salfinger & Tortorello, 2015) were used to analyze all of the microbiological characteristics of the patties i.e. Standard plate count (SPC), Coliform count (CC) and Yeast and mold count (Y&M).

Storage Stability and Shelf-life of chicken meat patties

The product with best sensory attributes along with control and positive control chicken patties were packed in LDPE pouches and stored in refrigerated temperature (4±1°C). The quality changes in the patties were evaluated based on various (pH, TBARS, DPPH radical scavenging activity, Microbial, and Sensory) parameters after drawing sample periodically at three days interval up to its acceptability tested by quality parameters.

Statistical Analysis

Mean ± SE values for a number of parameters were computed from the collected data, and the results were analyzed with an ANOVA, after which the significance levels between any two means were determined with the help of Duncan's Multiple Range Test (DMRT). It was determined that there was a statistically significant difference between the groups when the probability (p value) was less than 0.05.

Results and Discussion

TPC and DPPH radical scavenging activity of alcoholic crude extract of MOLP

Using the linear regression equation derived from the gallic acid standard plot, TPCs were determined. The crude alcoholic extract of MOLP was found to contain 462.74 ± 21.18 mg GAE/kg of dried leaves powder. However, the value (105 mg gallic acid equivalents/100 g) reported by Adedapo et al., (2009) was higher than the present study. Sulastri et al., (2018) reported that the total phenolic content of ethanolic extract of MOLP from different regions were ranges between 2.5 to 3.0 mg GAE/100mg of dried extract. Many factors, including as genetics, environment, harvest timing, and storage, could account for the variations (Stohs & Hartman, 2015). The quality and amount of bioactive components in the crude extracts are also affected by parameters such as solvents, extraction methods, and extraction time (Vongsak et al., 2013). The percentage DPPH free radical scavenging activity of MOLP was 64.28 ± 2.53 %. This result is in close agreement with the value reported by Shahriar et al., (2012) who reported that the methanolic and ethanolic extract of MOLP have a 67.59 % and 69.35 % DPPH radical scavenging activity. Meat and meat products benefit from MOLP's natural antioxidant properties, as evidenced by its total phenolic content (TPC) and radical scavenging activity (Mashau et al., 2021).

Sensory evaluation of chicken meat patties

The sensory score for different sensory attributes of MOLP incorporated chicken meat patties is presented in Figure 1. Color and appearance score of the patties made with MOLP is not differ significantly compared to the control and positive control. The results showed that increasing the inclusion of *Moringa oleifera* leaves powder significantly affected color and appearance scores. However, T3 (2%) patties showed lower ratings (6.88 ± 0.15) in color and appearance, indicating a significant drop ($p < 0.05$). The results of flavor score showed that there is a statistically significant difference ($p < 0.05$) between T1 (1%), T2 (1.5%), and T3 (2%). T2 chicken meat patties scored highest for flavor score and it was significantly higher ($p < 0.05$) than the control and positive control. Samples of treatment T3 had significantly reduced taste and flavor acceptability due to the bitter flavor of MOLP. T1 and T2 experimental samples were rated as having a more desirable texture than the control and positive control treatment groups, respectively. A statistically significant difference ($p < 0.05$) was also observed between the T2 treatment group and the other groups. In T3 treatment group texture score was found to be lower

than those of the other treatment groups which means that addition of MOLP up to 1.5% gives desired texture to the product. The sensory panelists'' ratings of juiciness followed a similar pattern, with the T3 treatment patties receiving a lower juiciness score compared to the other treatment groups. There was no statistically significant difference in the scores given by panelists between the control, positive control, and T1 patties. The juiciness score for the T2 was higher as compared to other treatments. The overall acceptability score showed that the T2 patties received highest score among all the treatments. However, the score for the T2 is not significantly differ ($p > 0.05$) with the control group patties. The higher values for the 1.5 % MOLP added patties were due to the effect of the MOLP on the sensory characteristics of the product. However, the increasing concentration of MOLP at 2% level had given negative effect due to changes in the taste and intensity of colour as well as flavor. Similar trend was also noticed by Mashau et al., (2021). They stated that there may be a correlation between the green hue of MOLP and the low color scores observed in cooked ground beef incorporated with MOLP. The bitterness in MOLP originates from the activity and specificity of myrosinase, which has been shown to break down the main glucosinolates (Chodur et al., 2018). Madane et al., (2019) reported that addition of moringa flower powder at 1% level (T1) increased the desirability of all sensory properties. However, addition of moringa flower at 2% level decreased sensory score compare to T1 and control. Our findings are also in agreements with the other studies carried out using various leaf powder (Al-Juhaimi et al., 2018). The treatment product T2 scored highest in sensory characteristics among all, so it is used for further investigation.

Determination of cooking yield in chicken meat patties

The cooking yield of the control, positive control and Treatment T2 was examined. It was observed that the inclusion of MOLP has an impact on the cooking characteristics of chicken meat patties. The cooking yield of the control, positive control, and T2 patties were found to be 75%, 80%, and 88.3% respectively. The observed enhancement in cooking yield of the group treated with moringa powder could potentially be ascribed to the capacity of MOLP to absorb both fat and water, hence aiding in the retention of moisture inside the chicken meat patties (Mashau et al., 2021; Serdaroğlu et al., 2021). Similar trend was also noticed by Madane et al., (2019) during use of drumstick flower powder in chicken meat nuggets. In a study conducted by Ham et al. (2017), cooked pork sausage was prepared with the incorporation of Lotus Rhizome powder at varying concentrations (0%, 1%, 2%, and 3% of the total weight). The findings of the study demonstrated that the addition of Lotus Rhizome powder had a beneficial impact on the cooking yield, resulting in an increase from 90% to 94%.

Storage Stability and Shelf-life of chicken meat patties

The assessment of storage quality was conducted on T2 meat patties, which had been selected from an above mentioned sensory score, in comparision with control and positive control. The evaluation focused on physico-chemical, microbiological, and sensory criteria. The products were packed aerobically in low density polyethylene pouches. These packaged products were thereafter stored at a temperature of 4±1ºC. The products underwent analysis at consistent intervals of three days until deteriorative changes observed by various parameters.

Determination of pH in chicken meat patties

The effect on the pH of chicken meat patties samples was presented in figure 2. It was revealed that the pH value of aerobically packaged control, positive control and T2 treatment chicken meat patties were 5.84 ± 0.05, 5.79 ± 0.030 and 5.73 ± 0.01 respectively at day 0. The pH values of all patty samples exhibited a notable increase within the initial six days of storage. Subsequently, the pH values remained rather consistent or experienced a minor reduction across all treatment groups as the duration of storage progressed. The patties (T2) made with MOLP exhibited the lowest pH value among the three groups tested throughout the whole storage period. The release of alkaline metabolites by bacteria might be the reason for the ensuing increase in pH during the storage period (Al-Juhaimi et al., 2018). When the glucose in the product runs out, the bacteria start using the amino acids that were liberated during the protein breakdown process, which causes the pH to rise because of the accumulation of ammonia (Gill, 1983). Similar results were observed by Das et al. (2011), who studied the effects of curry leaf (*Murraya koenigii*) on the pH of cooked chevon meat over the course of 20 days of refrigeration. The results were also in agreement with the studies reported by various researchers (Khomola et al., 2021; Madane et al., 2020; Mashau et al., 2021; Serdaroğlu et al., 2021).

Determination of Thio-barbituric acid reactive substances (TBARS) value of patties

Thiobarbituric acid value of aerobically packaged control, positive control and T2 meat patties are presented in figure 3. It was clearly observed that there was a significantly ($p \leq 0.05$) increase in TBARS value in all three group with advancement of storage period. The TBARS levels increased linearly with time in storage. Up to 12th day of storage, none of the treatment had mean TBARS readings higher than the recommended threshold of 2 mg malonaldehyde/kg of meat (Greene & Cumuze, 1982). When compared to both the "positive control" patties (which contain BHT) and the "T2" patties (which contain 1.5% MOLP), the rate at which the TBARS value increased was much higher in the "control" patties. Both BHT and MOLP have antioxidant

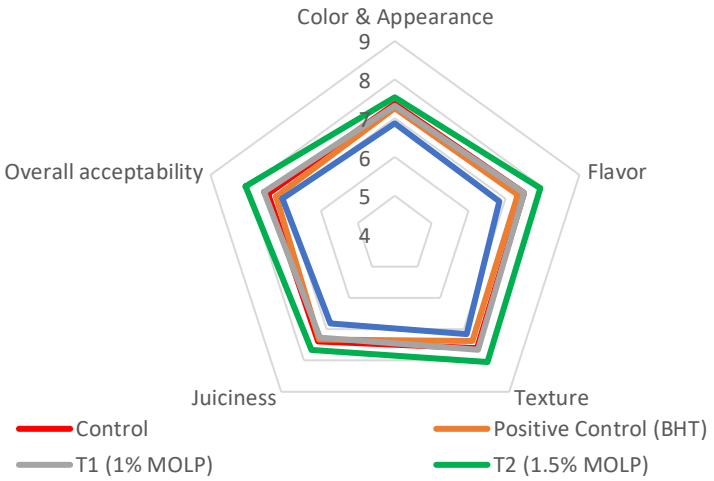

Figure 1. Sensory score of *Moringa oleifera* leaves powder incorporated chicken meat patties.

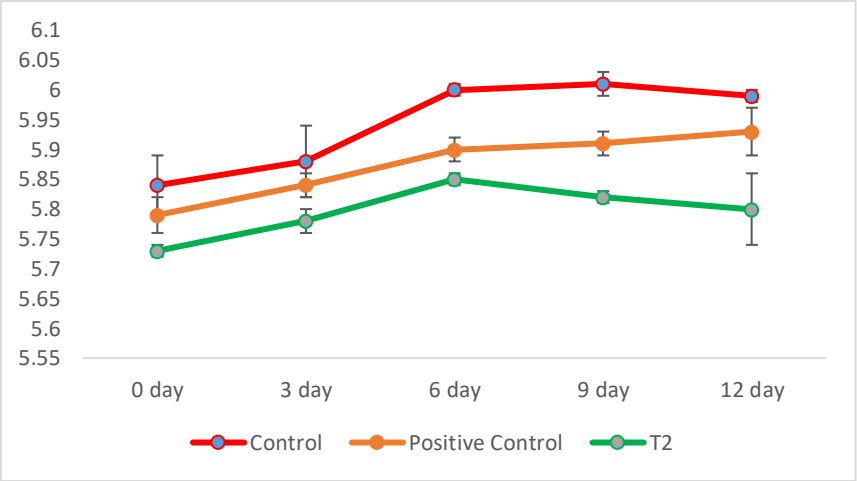

Figure 2. pH value for different treatment groups of chicken meat patties during refrigerated (4±1°C) storage.

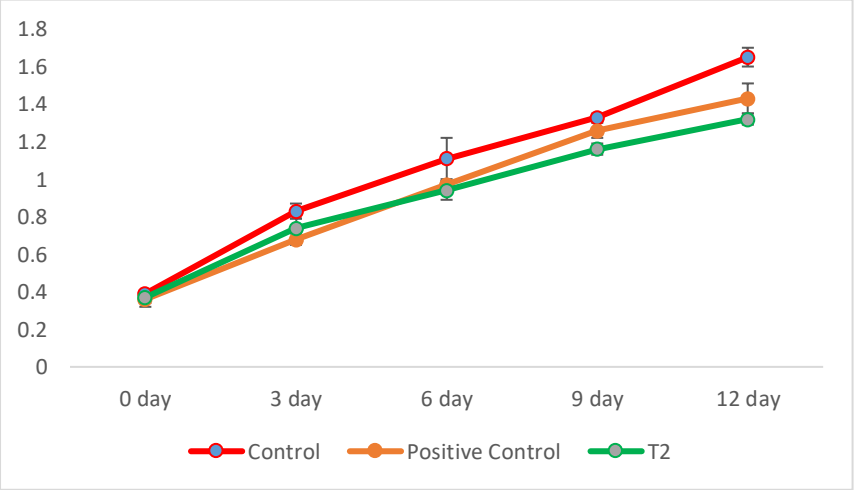

Figure 3. TBARS value (mg Malonaldehyde/Kg of sample) for different treatment groups of chicken meat patties during refrigerated (4±1°C) storage.

properties, although MOLP is natural whereas BHT is synthetic. This may explain why the rate of growth of positive control and T2 patties is relatively slow. The oxygen permeability of the packaging material may also be contributing factors for the lipid oxidation that resulted in the rise in TBARS levels during storage (Xiao et al., 2011). Redox characteristics in phenolic compounds of MOLP are responsible for much of their antioxidant activity, including their ability to absorb and neutralize free radicals, decompose peroxides, and quench singlet oxygen (Sreelatha & Padma, 2009). Similarly, other studies found that adding plant-based powders or extracts to meat substantially lowered lipid oxidation (Kaur et al., 2015; Khomola et al., 2021; Mashau et al., 2021). In addition, Das et al., (2012) discovered that adding 0.1% MOL extract to cooked goat meat patties dramatically reduced TBARS readings compared to the control.

Estimation of DPPH radical scavenging activity of patties

DPPH radical scavenging activity value of aerobically packaged control, positive control and T2 meat patties are presented in figure 4. The DPPH radical scavenging activity is quantified and given as a percentage (%). The DPPH activity value was found to be highest on day 0 in all respective groups. The DPPH readings exhibited a considerable decrease as the storage period progressed. The DPPH activity in the treatment group with MOLP remained higher compared to both the control group and the positive control group. As the duration of storage increased, the ability of antioxidants to scavenge free radicals was diminished. The values are in agreement with the results of TBARS value which indicates that as the radical scavenging property of the products decreases the lipid oxidation of the products increases. The positive control and the MOLP containing patties having a addition of synthetic and natural antioxidant respectively. So the rate of oxidation is lower in that products as compared to control patties. These results are consistent with the findings published by Verma et al., (2019) in use of porcine liver hydrolysate in meat emulsion and by Verma et al., (2023) in effect of mushroom extract on storage stability of Chevon Nuggets. A study has also reported comparable findings about the decline in DPPH scavenging activity in meat products containing oregano and bay during the storage period (Umaraw et al., 2020).

Microbiological quality of chicken meat patties

The effect of MOLP on microbial quality during refrigerated storage was observed. The result (log cfu/g) for standard plate count, coliform count and yeast & mold count of control, positive control and T2 meat patties were presented in table 2. At the 0-day mark, there was not a significant difference between the control and positive control groups. However, beginning at day 6, there were statistically significant differences ($p \leq 0.05$) in SPC value across all groups. The Standard Plate Count of control as well as positive control was above the standard permissible limit for cooked meat products (FSSAI, 2016) on 9^{th} day of storage. However, the SPC of T2 patties were observed over the permissible limit on 12^{th} day of storage. Patties with MOLP had a slower rate of development for microorganism compared to the control and positive control, reflecting a higher overall microbiological quality as determined by SPC evaluation. This might be due to the antimicrobial property of MOLP. MOLP contains high levels of phytochemicals such phenols, flavonoids, alkaloids, and saponin which is responsible for its potent antibacterial activity, giving the plant great medical value (Asghar et al., 2022). The results of this study are in agreement with the study of Muthukumar et al., (2014) who reported that addition of MOLP extract in meat affect the microbial quality of ground pork patties. Kaur et al., (2015) reported that total plate count of chicken nuggets revealed a statistically significant ($p \leq 0.05$) increasing tendency from Day 0 to Day 21 in all treatments; however, counts of products prepared with inclusion of pomegranate seed powder, grape seed extract, and tomato powder were statistically significantly ($p \leq 0.05$) lower than control throughout the storage at 4±1ºC.

The coliform count was not detected in any of the treatment on the day 0 and day 3 of storage. At the 6^{th} day of storage the control product was observed to have coliform count 1.30±0.05 log cfu/g. After day 6^{th} the increasing trend was found within the treatment. The coliform count was significantly ($p \leq 0.05$) lower in T2 patties as compared to other treatment groups. However, Kaur et al., (2015) revealed that both the control products and the products made with the addition of antioxidant sources remained free of coliforms during the storage period. This may be because these bacteria are killed off when subjected to temperatures well over their thermal death point of 57 °C, as is the case during cooking. In addition, hygienic methods during product preparation and packing may contribute to the absence of coliforms. Microbiological limits of Coliform Count for cooked or semi-cooked meat are set at 10^1-10^2/g by FSSAI (2016). The results are also in agreement with the work reported by researchers (Muthukumar et al., 2014; Mashau et al., 2021; Ali et al., 2022; Abdel-Naeem et al., 2022).

The yeast and mold count of all group are shown in table 2. Throughout the entire storage period, the yeast and mold count in the control patties increased significantly ($p \leq 0.05$). Moringa powder treated chicken patties were yeast and mold free on day 0 and day 3 of storage. Compared to the control group, the Moringa treatment group's patties had a less increase in yeast and mold count as the storage period advanced. It is possible that the antifungal capabilities of MOLP's bioactive components (Asghar et al., 2022) are responsible for the reduced numbers in the treated products. Microbiological limits for yeast and mold count in cooked or

Figure 4. DPPH inhibition (%) value for different treatment groups of chicken meat patties during refrigerated (4±1°C) storage.

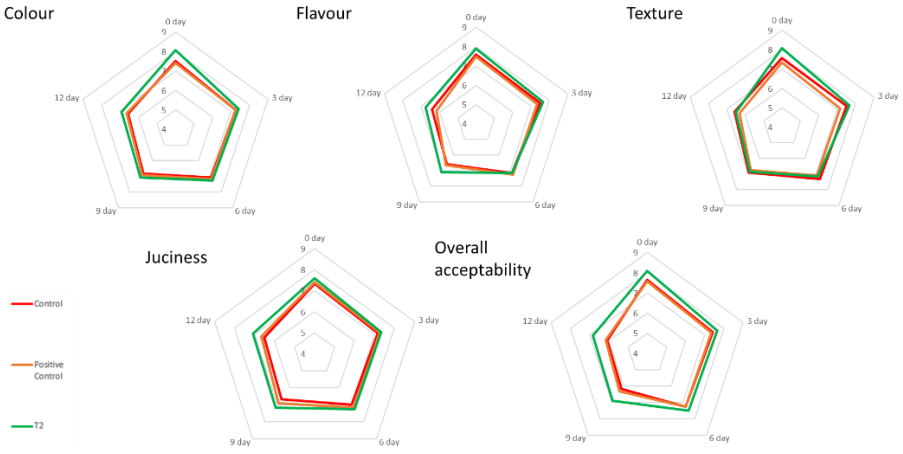

Figure 5. Sensory scores for different treatment groups of chicken meat patties during refrigerated (4±1°C) storage.

Table 2. Microbial quality evaluation (log CFU/g) for different treatment groups of chicken meat patties during refrigerated (4±1°C) storage.

Microbial Evaluation (log CFU/g)	Time interval in days	Treatments		
		Control	Positive Control	T2
Standard Plate Count	0	2.50 ± 0.07^{aB}	2.47 ± 0.06^{aB}	1.77 ± 0.04^{aA}
	3	2.76 ± 0.07^{aB}	2.50 ± 0.05^{aB}	1.86 ± 0.06^{aA}
	6	4.24 ± 0.06^{bB}	4.32 ± 0.04^{bB}	2.47 ± 0.05^{bA}
	9	5.49 ± 0.05^{dC}	5.27 ± 0.05^{cB}	3.15 ± 0.06^{cA}
	12	--	--	5.11 ± 0.06^{eA}
Coliform count	0	ND	ND	ND
	3	ND	ND	ND
	6	1.30 ± 0.05^{aA}	1.1 ± 0.06^{aA}	ND
	9	1.95 ± 0.06^{bC}	1.84 ± 0.06^{bB}	1.60 ± 0.03^{bA}
	12	--	--	1.89 ± 0.07^{cA}
Yeast and mold Count	0	1.67 ± 0.04^{aA}	ND	ND
	3	1.80 ± 0.06^{bB}	1.73 ± 0.04^{bA}	ND
	6	1.82 ± 0.01^{cC}	1.85 ± 0.05^{cB}	1.21 ± 0.06^{cA}
	9	2.02 ± 0.06^{dC}	1.88 ± 0.03^{dB}	1.76 ± 0.06^{dA}
	12	--	--	1.89 ± 0.04^{eA}

Values were given as mean ± SE (n = 3); ND = Not Detected; -- : Not Performed
(Values in the same column bearing different superscript small letters and same row bearing different superscript capital letters are significantly different ($p\leq0.05$)).

semi-cooked meat are set at 10^1-10^2/g by FSSAI (2016). Many researchers have also documented comparable outcomes (Das et al., 2012; Kaur et al., 2015; Khomola et al., 2021) in the preservation of mutton patties or chicken nuggets using various natural components, corroborating the aforementioned findings.

Sensory quality of chicken meat patties during refrigerated storage

The result of sensory analysis during storage of control, positive control and T2 meat patties are presented in figure 5. There was a downward tendency in color and appearance rating with each passing storage day. Overall, all treatments showed a statistically significant ($p \leq 0.05$) decrease in color and appearance score throughout all storage days. During the storage period, a significant difference was seen in the T2 group between day 0 and days 3 and 6. Throughout all of the storage periods, T2 patties received higher marks for color and appearance from the panelist than did the other treatment groups. At 0 days of storage, a significant difference was seen between the T2 group and the other treatment groups. Aerobically packaged patties may have a lower color and appearance score because of the oxidation of lipid and pigment, which causes non-enzymatic browning and surface dryness (Kumar et al., 2021). Giriprasad et al., (2015) noticed a loss of color and appearance quality in restructured chicken slices and buffalo meat streaks during storage.

At 0 days, the T2 had a higher flavor score than the other treatments. Flavor scores did not alter noticeably for the first three days of storage, but then steadily dropped as time passed on. The score for T2 treatment group was substantially different from the control and positive control patties. Microbial growth and oxidative deterioration may be responsible for the patties' declining flavor score. The bitterness observed in MOLP can be attributed to the existence of the glucosinolate and myrosinase, which is accountable for the bitter flavors (Chodur et al., 2018). Kaur et al., (2015) showed a comparable decrease in the flavor score of meat nuggets over a period of storage. In a study conducted by Muthukumar et al., (2014) it was demonstrated that the flavor of ground meat was significantly enhanced ($p \leq 0.05$) with the application of a crude extract of MOLP.

A notable variation in texture score was detected among all treatment groups on the initial day of the storage period. The texture score of the T2 treatment group consistently stayed at a higher level during the whole duration of the storage periods. Considerable differences were noted within the same treatment group as the study progressed. Abdel-Naeem et al., (2022) observed that the incorporation of lemon, orange, and grape fruit peel powders in the chicken patties formulations resulted in statistically significant ($p \leq 0.05$) improvements in tenderness scores when compared to the control samples. Furthermore, Ali et al., (2022) noted a significant difference in the texture score among the control group and the samples that had 6%, 9%, and 12% of cantaloupe peel (CP) and seeds (CS) powder.

The analysis of variance conducted on the mean score of juiciness revealed no statistically significant differences among the various products. The product made using T2 had a slightly higher juiciness score in comparison to both the control and positive control. The study also indicated that the chicken patty products from all groups exhibited a decreasing trend in juiciness score over the storage period, with the highest score seen on day 0 and the lowest score observed on day 12. The increased juiciness score seen in the T2 group patties may be attributed to their enhanced water retention ability. The observed phenomenon can perhaps be attributed to the evaporation of moisture from the product and the passage of oxygen into the packaging material (Madane et al., 2020). Similar trends were also reported by various researchers (Das et al., 2012; Khomola et al., 2021; Abdel-Naeem et al., 2022).

For a duration of up to six days, there was no notable alteration in the overall acceptability score. However, subsequent to this period, a decline in the overall acceptability of the patties was noted as the storage period progressed. The analysis of variance conducted on the mean scores of overall acceptance revealed no statistically significant differences among the different products. The T2 had a significantly higher overall acceptance score in comparison to both the control and positive control samples. The study also shown that the chicken patties from all groups exhibited a progressive decrease in overall acceptability score throughout the course of the storage period, with the highest score seen on day 0 and the lowest score observed on day 12. This phenomenon could perhaps be attributed to a decline in the perceived value of other sensory qualities, an increase in lipid oxidation, protein breakdown, and a reduction in flavor quality resulting from fat degradation (Khomola et al., 2021). Similar trend was also observed by Abdel-Naeem et al., (2022) in various fruit peel powder added chicken patties. Some previous studies have documented the adverse impact of the use of plant extracts on the sensory attributes and overall acceptance of meat-based products, such as patties (Al-Juhaimi et al., 2018; Mashau et al., 2021).

Conclusion

Chicken patties with added MOLP had increased functionality, qualitative attributes, and antioxidant activity. When compared to the control sample and the other treatments, the chicken patties supplemented with MOLP at a ratio of 1.5% demonstrated the highest overall acceptance. Incorporating MOLP successfully reduced lipid oxidation, making meat patties more durable on the shelf. It is also improving the meat product's cooking yield and pH value. Samples of chicken patties made with MOLP demonstrated superior microbiological quality than the control sample. Incorporating moringa leaves powder into the formulation of chicken meat patties could make it possible for the development of a product with beneficial oxidative and storage stability and good

to very good acceptance. However, additional study is required to investigate the widespread application of the MOLP in the production of various meat-based products, with the aim of assessing storage quality and shelf stability.

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