Research article
J. Adv. Nutri. Sci. Technol. 4(1-2): 1-8, 2024 **DOI:10.15228/ANST. 2024.v04.i01-2.P01**

In vitro study of antioxidant and antimicrobial potential of *Moringa oleifera* leaves as a green food preservative in chicken burger

Ali Raza Kashif[1], Saima Naz[1*], Adil Usman[1], Nighat Javed[2], Muhammad Usama Younas[1], Ashaan Zahoor[1]
[1] Department of Chemistry, Division of Science and Technology, University of Education, Lahore, Faisalabad campus 37300 Pakistan.
[2] Department of Chemistry, Government College University, Faisalabad
Corresponding author's e-mail: saima.naz@ue.edu.pk

ABSTRACT
Moringa oleifera L. (from the Moringaceae family) is highly valued for its rich nutritional profile, phytochemical composition, and abundance of crucial vitamins and minerals. This study aimed to investigate the impact of bioactive compounds found in *Moringa oleifera* leaves on the antimicrobial properties of chicken burgers, thus extending their shelf life. Five variants of chicken burgers were prepared, comprising a control group (having no antioxidant contents) and groups with 1% or 2% concentrations of *Moringa oleifera* polyphenol extract (MOPE) and whole *Moringa oleifera* powder (WMOP). Results indicated that after a 7-day storage period, these burgers treated with 1% and 2% concentrations of WMOP and MOPE exhibited substantially less total plate counts (TPCs) than the control group. Additionally, burgers supplemented with WMOP and MOPE demonstrated notably higher levels of total flavonoid, phenolic content, and antioxidant content compared to the control. Notably, the addition of WMOP or MOPE did not affect the acceptability of the chicken burgers. These findings suggest that incorporating WMOP and MOPE at 1% and 2% concentrations could serve as effective natural preservatives in chicken burgers, potentially extending their shelf life while maintaining consumer satisfaction.

Keywords: *Moringa oleifera*, Antioxidants, Antimicrobial agent, preservation

Highlight:

- *Moringa oleifera* L has a vibrant nutritional and phytochemical composition
- Antioxidant potential and antimicrobial activities of ethanolic leaf extract
- WMOP and MOPE act as an effective natural preservative in chicken burgers

1.0. INTRODUCTION

Moringa oleifera L (MO) belongs to the *Moringaceae family* and has scientific significance due to its abundant phytochemical composition, considerable vitamins, mineral content, and significant nutritional value (Islam et al., 2021; Meireles et al., 2020). The MO tree is commonly known as the "wonder tree" or "miracle tree" primarily due to its significant socioeconomic importance resulting from its diverse applications in pharmacology and industry (Abdel-Latif et al., 2022). All segments of the tree, including the bark, roots, sap, flowers, leaves, and seeds, are extensively utilized for both food items and medicinal purposes (Padayachee & Baijnath, 2020). The MO tree possesses characteristics such as lack of resistance, making it a valuable crop with substantial nutrient and medicinal attributes, particularly in dry and semiarid regions (Arora & Arora, 2021). MO has been extensively studied and documented for its remarkable abundance of bioactive compounds, including carotenoids, alkaloids, glycosides, glucosinolates, terpenoids, and flavonoids that are important in preventing various chronic disorders (Chhikara et al., 2021; Liu et al., 2022; Milla et al., 2021; Mutar et al., 2021). Additionally, previous studies have demonstrated that leaves of MO are a valuable source of protein with therapeutic properties (Abdel-Latif et al., 2022; Alqurashi & Aldossary, 2021; Ahmad et al., 2022).

In the food industry, there is a growing interest in consumers for natural products due to their superficial health benefits (Ahmad et al., 2022). Polyphenols derived from plants have gained attention as potential natural food preservatives to extend the shelf life of meat products (Alqurashi & Aldossary, 2021; Manessis et al., 2020). Research indicates that ingredients rich in antioxidants can mitigate microbial spoilage during meat storage. Chicken burgers, being a widely popular processed meat product worldwide, are favoured for their convenience and affordability, appealing to a large segment of the population (Gómez et al., 2020; Martillanes et al., 2020; Rudy et al., 2020). Numerous reports have highlighted the ability of ingredients rich in antioxidants to effectively minimize microbial decomposition in the preservation of meat products (Tayengwa et al., 2020). Chicken burgers are among the most widely consumed meat products all over the world (Assanti et al., 2021). These products have gained immense popularity and are widely encompassed by an extensive portion of the population, primarily because of their accessibility and affordability (Lorenzo et al., 2021). Over the last five years, Pakistan has shown a rapid growth in the number of local restaurants; therefore, chicken & other meats have less stability, probably as a result of

microbial activity and lipid content peroxidation which can have adverse impacts on safety and human (Ahmad et al., 2023; Alqurashi & Aldossary, 2021).

The bioactive ingredients of MO leaves developed at Al-Rahman farm, Pakistan, have not been thoroughly examined. Hence, this analysis aimed to investigate the influence of *Moringa oleifera* leaves and their bioactive components, antioxidant properties, and antimicrobial characteristics on chicken burgers. These components were explored as natural food preservatives with the potential to extend the shelf-life of the product.

2.0. MATERIALS AND METHODS

2.1. Chemicals used

Each chemical used in this study such as aluminum chloride, methanol, ethanol, sodium carbonate (Anhydrous), Folin-Ciocalteu reagent, ammonium thiocyanate, gallic acid, ascorbic acid, catechin, ferric chloride, ferrous chloride, BHT (butylated hydroxyl toluene), sodium nitrite and DPPH (2,2-diphenyl-1-picrylhydrazyl) were bought from Nadeem Scientific store Faisalabad, Pakistan.

2.2. Plant collection and pretreatment

Fresh *Moringa oleifera* (MO) leaves were manually harvested from fully grown trees selected in 2023 from the Al-Rahman farm located in Kabirwala, Punjab, Pakistan (Fig. 1). After thoroughly cleaning these MO leaves to remove any unwanted debris, washed with hot water and then with distilled water. They were then chopped into a fine powder (whole *Moringa oleifera* powder (WMOP) and set aside for air drying at room temperature (25°C) for 96 hours to prepare them for use in chicken burgers. After that, the solution was centrifuged at 4000 rpm for 10 minutes, and the supernatants were collected after each centrifugation. Using a rotary evaporator (Ecohim Ltd., Ekros Group of 20 enterprises, Saint-Petersburg) at 40°C and vacuum conditions provided by a vacuum pump, the mixed supernatants were concentrated. After that, the extracted materials were freeze-dried.

Fig. 1: MO leaves growth in Al-Rahman farm, kabirwala, Pakistan

2.3. Preparation of chicken burger

Fresh chicken from a local poultry market in Al-Syed, Pakistan, was used for the preparation of chicken burgers (C.B). The ingredients used for making the C.B are listed in Table (1) consists of 86% minced chicken breast, 6% whole egg, 1% salt, and 7% breadcrumbs. The C.B were treated with *Moringa oleifera* polyphenol extract (MOPE) and whole *Moringa oleifera* powder (WMOP) at varying concentrations (1% and 2%). Antioxidants were not used in the control group. The samples were individually made into C.B using a bowl chopper. The burgers were then aerobically packed in plastic bags and kept at 4°C. Analysis of raw C.B samples was performed at intervals of 0, 3, 5, and 7 days of storage under refrigerated conditions (4°C) to determine the Total Phenolic Content(TPC), Total Flavanoids Content (TFC) and antioxidant potential of these chicken burger. Furthermore, the control, WMOP and MOPE samples of cooked C.B were evaluated for their sensory qualities. Each experiment was carried out three times to ensure the reliability and strength of the results.

Table 1: **Ingredients used for the preparation of chicken burgers**

Ingredients	Control	1 % WMOP	2% WMOP	1% MOPE	2% MOPE
Whole MO powder	0.0	1.0	2.0	0.0	0.0
MO polyphenols extract	0.0	0.0	0.0	1.0	2.0
Brest chicken	86	86	86	86	86
Salt	1.0	1.0	1.0	1.0	1.0
Whole egg	6.0	6.0	6.0	6.0	6.0
Breadcrumbs	7.0	6.0	5.0	6.0	5.0

Control (without antioxidant), 1%, 2% WMOP (burgers with 1% & 2% whole MO powder), 1%, 2% MOPE (burgers with 1% & 2% MO polyphenol extract)

2.4. Estimation of TPC

The Folin-Ciocalteu method, followed by a slightly modification reported by Ahmad et al. (2022) was used to determine the TPC. To put it briefly, 15 g of chicken burger samples containing WMOP and MOPE at two concentrations of 1% and

2%, 75 ml of aqueous ethanol (80% v/v) was used to homogenize the control group which did not use antioxidants. After being mixed again and centrifuged for ten minutes at 4000 rpm, the mixture was let to incubate for 5 minutes. After taking 1.5 ml aliquot of the supernatant, it was transferred to tubes holding 0.5 ml of Folin-Ciocalteu reagent. The tubes were incubated at 25°C for 5 minutes before being filled with 4 ml of sodium carbonate solution and shaken for about half an hour. The absorbance of the resultant solution was measured at 755nm with a UV/Vis Spectrophotometer (Model: STA-8200, STALWART). Using a calibration curve built with gallic acid as a reference, the TPC was determined. The results were represented as gallic acid equivalents (GAE) mg/g of dried *Moringa oleifera* leaves.

2.5. Estimation of TFC

Total flavonoid contents (TFCs) were determined using the method with slight modification reported by Boeira et al. (2021). To put it briefly, 15 g of chicken burger samples containing WMOP and MOPE at two concentrations of 1% and 2% 75 ml of aqueous ethanol (80% v/v) was used to homogenize the control group, which did not use antioxidants. After being mixed again and centrifuged for 10 minutes at 4000 rpm, the mixture was let to stand for 5 minutes. The supernatants were then mixed with 0.2 ml of a 5% $NaNO_2$ solution. After incubating for 10 minutes at 25°C, 0.2 ml of 10% $AlCl_3$ solution was added. After a further incubation of 10 minutes, 2 ml of (1.0 M) NaOH solution was mixed, and the volume was finally adjusted to 10 mL using distilled water. Using a UV/Vis Spectrophotometer (Model: STA-8200, STALWART), the absorbance of the reaction mixture was determined at 510 nm. The catechin equivalents (mg CE)/g were used to determine the overall TFC.

2.6. DPPH radical scavenging assay

The antioxidant activity of C.B. was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay by following the methodology given by Ramli et al. (2020). The basis of this assay is the DPPH free radical's capacity to take up an electron and change into a stable diamagnetic molecule. In this experiment, 4 ml of 0.1 mM DPPH in methanol was combined with 4 ml of chicken burger samples that contained WMOP and MOPE at concentrations of 1% and 2%, as well as the control group (except antioxidants). After that, the mixture was incubated for 20 minutes at 25°C in a dark environment. After incubation, a UV-1800 spectrophotometer (Shimadzu, China) was used to measure the absorbance at 517 nm. The following equation was used to express the antioxidant activity as a percentage:

$$I\ (\%) = 100 \times (A_{blank} - A_{sample}/A_{blank})$$

Where I is inhibition, A_s and A_b represent sample and blank absorbance values, respectively.

2.7. Antimicrobial analysis

The samples of chicken burgers were divided into portions of 10 g each. The control group (without antioxidants) was compared to each treatment that included *Moringa oleifera* polyphenol extract (MOPE) and whole *Moringa oleifera* powder (WMOP) at different concentrations (1% and 2%). After standardizing each sample in 90 ml of sterile peptone water (0.1%), the samples were diluted to different quantities. The total plate count (TPC) was then calculated by inoculating a 1 ml dilution onto Petrifilm (3M, Pakistan). According to Alqurashi & Aldossary (2021), the Petrifilm plates were stored in a refrigerator at 4°C after being heated at 37°C for 0, 3, 5, and 7 days. Additionally, to evaluate the impact of C.B samples on *Escherichia coli* and *Staphylococcus*, 1 ml dilutions were put onto certain agar media. Macconkey agar and Baired-Parker medium were the agar media utilized, and the plates were cultured for 24 hours at 37°C. Following 0, 3, 5, and 7 days of storage at 4°C in a refrigerator, microbial analysis was carried out. The number of microbial colonies on the plates was calculated and is shown as (log cfu/g).

2.8. Estimation of pH change

The pH values were calculated by following the method reported by Sarker et al. (2021). In brief, 1 g of samples containing WMOP and MOPE at two concentrations of 1% and 2%, as well as the antioxidant-free control group, were mixed entirely and standardized in 10 ml of distilled water. The samples were filtered through filter paper No. 1 before measuring the pH. The pH of each sample was determined using a pH meter prior to analysis, and buffer solutions with pH values of 5.0, 7.0, and 9.0 were used to calibrate the instrument.

2.9. Sensory evaluation

A panel of fifteen judges conducted a sensory evaluation at the University of Education, Lahore. These judges possessed expertise in assessing the characteristics of chicken products. Flavour, taste, texture, colour, and general acceptability were among the sensory aspects that were assessed as described by Shen et al. (2022) on a 6-point descriptive scale, where 6 meant "Like extremely," and 1 meant "Dislike extremely." On a grill, the chicken burgers were grilled for 10 minutes. The chicken burgers were broiled in a microwave for fifty seconds before dishing. The panellists assessed each sample after washing their palates with water in between samples. The evaluation included the control group (antioxidant-free chicken burgers) in addition to the MOPE and WMOP items.

2.10. Statistical analysis

Every experimental result was displayed as the standard error (SE) and the average of 3 replicate experiments. The SPSS software was used to conduct the statistical analysis. ANOVA was used to compare all samples, consisting of both control and MO treatment samples (WMOP and MOPE). A significant level of 5% ($p < 0.05$) to evaluate the impact of various treatments on chicken burgers was used.

3.0. RESULTS AND DISCUSSION

3.1. Determination of TPC and TFC

The total phenolic (TPC) and flavonoid content (TFC) of control C.B treated with WMOP and MOPE at various concentrations (1% and 2%) are summarized in **Table 2.** C.B treated with MOPE or WMOP (1% and 2%) exhibited substantially greater TPC and TFC values compared to control samples. TPC (65.5 GAE mg/g) was tested in 2% WMOP, and 2% MOPE revealed 83 GAE mg/g, TF of 33 CE mg/g in 2% WMOP, and lower values in control, while antioxidant activity was 71% after applying 2% MOPE and 0.02% in control. Furthermore, C.B treated with MOPE exhibited prominently greater TPC and TFC compared to those treated with WMOP ($p < 0.05$). This difference could be attributed to the polarity of the solvent employed for extracting polyphenols from MO leaves, leading to enhanced incorporation of antioxidants into C.B. This increase in antioxidant content likely contributed to the observed improvements in antimicrobial activity (Alqurashi & Aldossary, 2021; Hadadi et al., 2020).

Table 2: Antioxidant activity % , total flavonoid content (CE mg/g), total phenolic content (GAE mg/g) of *Moringa oleifera* leaves in chicken burgers

Sample	Antioxidant activity	TFC	TPC
Control	0.02 ± 0.05	0.02 ± 0.69	0.01 ± 0.02
1% WMOP	49 ± 1.16	16.3 ± 0.87	36.7 ± 1.12
2% WMOP	71 ± 1.25	33.7 ± 1.29	65.5 ± 1.37
1% MOPE	55 ± 1.71	14.6 ± 1.57	39.7 ± 1.04
2% MOPE	71.2 ± 1.54	19.8 ± 1.4	83 ± 1.01

Control (without antioxidant), 1%, 2% WMOP (burgers with 1% & 2% whole MO powder), 1%, 2% MOPE (burgers with 1% & 2% MO polyphenol extract)

3.2. Antioxidant activity of MO leaves

DPPH serves as a free radical that is employed to assess the capacity of plant extracts to scavenge free radicals (Hussen & Endalew, 2023). In this research, we evaluated the antioxidant potential of C.B treated with WMPO and MOPE. The treated sample exhibited more antioxidant potential than the control. The antioxidant activity of the 1% and 2% WMOP and MOPE samples differed considerably ($p < 0.05$) from that of the control (Table 2). The analysis of antioxidant activity showed that MOPE had a strong ability to scavenge radicals in chicken burgers, and this effect became stronger when the concentration was raised: 2% MOPE addition (83% ± 1.01) and 1% MOPE addition (55% ± 1.71). This activity may be attributed to elevating the levels of TPC and TFC in MOPE, which are known to contribute to enhanced antioxidant capacity. Recent studies have highlighted the potential of antioxidants as natural additives in various meat products to extend shelf life, improve safety, and enhance product quality (Badawy et al., 2020; Hajji et al., 2021; Ramli et al., 2021). Additionally, foods enriched with these natural bioactive ingredients offer various health benefits and are explored as natural, efficient nutrients, attracting growing attention from the food industry. (Gutiérrez-Del-río et al., 2021).

3.3. Changes in pH during preservation

After being refrigerated at 4 °C for 0, 3, 5, and 7 days, the pH values of the control and MO-treated chicken burgers were determined, as depicted in **Table (3)**. Our findings reveal a prominent increase in pH levels across all samples with preservation duration (0, 3, 5, and 7 days). Specifically, pH values rose from 6.19 ± 0.02 to 7.24±0.02 in the control group, from 6.13±0.02 to 6.98±0.02 with 1% MOPE treatment, from 6.27±0.01 to 6.74±0.02 with 2% MOPE treatment, from 5.89±0.01 to 6.85±0.01 with 1% WMOP treatment, and from 5.71±0.02 to 6.85±0.02 with 2% WMOP treatment. This rise in pH may be attributed to microbial metabolism and enzymatic reactions within the samples with time (Duan et al., 2020; Wang et al., 2020). Moreover, our results demonstrated A substantial decrease in pH levels in MO-treated chicken burgers (both WMOP and MOPE) compared to control samples during different storage periods (0, 3, 5, and 7 days) ($p< 0.05$). This observation could be related to the elevated levels of antioxidants and bioactive ingredients present in MO leaves, which were incorporated into the C.B. Similar trends have been observed in previous studies, where using MO flowers and leaves resulted in increased pH levels in chicken meat nuggets and sausages over various storage periods (Gomes et al., 2023; Verma et al., 2020).

Table 3: Change of pH with different preservation time

Treatment	Preservation Time (Days)			
	0	**3**	**5**	**7**

Control	6.19 ± 0.02	6.37 ± 0.01	6.91 ± 0.02	7.24 ± 0.02
1% WMOP	5.89 ± 0.01	6.16 ± 0.01	6.37 ± 0.02	6.85 ± 0.01
2% WMOP	5.71 ± 0.02	6.12 ± 0.02	6.24 ± 0.01	6.49 ± 0.02
1% MOPE	6.13 ± 0.02	6.36 ± 0.03	6.67 ± 0.01	6.98 ± 0.02
2% MOPE	6.27 ± 0.01	6.40 ± 0.02	6.49 ± 0.01	6.74 ± 0.02

Control (without antioxidant), 1%, 2% WMOP (burgers with 1% & 2% whole MO powder), 1%, 2% MOPE (burgers with 1% & 2% MO polyphenol extract)

3.4. Antimicrobial activity of MO leaves

The total plate count (TPC) values of control and MO-treated (WMOP and MOPE) C.B at varying concentrations (1% and 2%) following 0, 3, 5, and 7 days of storage at 4 °C are shown in **Table (4)**. As the storage duration increased, there was a substantial rise in the bacterial action count in the control, WMOP, and MOPE groups. Specifically, TPC in control burgers (no antioxidant contents) exhibited an increase from 4.59 ± 0.02 $\log^{10}$ cfu/g (0 day) to 126.83 ± 0.17 $\log^{10}$ cfu/g (7 days). When comparing WMOP and MOPE samples at concentrations of 1% and 2% to control samples (MOPE and WMOP) after 3, 5, and 7 days of storage, a prominent reduction in total phenolic content ($p < 0.05$) was noted. This study has shown that higher amounts of bioactive chemicals and antioxidant capacity can help reduce bacterial action in meat products, extending their shelf-life (Rebezov et al., 2022; Wu et al., 2023; Ahmed et al 2023). As a result, these factors may be responsible for this reduction. Moreover, after 7 days of storage, C.B treated with MO (1% and 2% concentrations of MOPE and WMOP) showed considerably less TPCs than the control. This observation aligns with previous reports indicating that polyphenols and bioactive ingredients in MO leaves possess antimicrobial properties against various pathogenic bacteria (Ramli et al., 2020 & 2021; Abdallah et al., 2023). Several bioactive ingredients, like pterygospermin found in MO leaves, exhibit antimicrobial capacities that can mitigate the levels of harmful bacteria in meat products, thus contributing to their prolonged shelf life and serving as natural food preservatives. Previous studies have highlighted that high levels of phenolic components from various plant sources can effectively reduce microorganism levels in poultry and meat products (Fraqueza et al., 2021). Phenolic chemicals may interfere with protein translocation, affect DNA and RNA synthesis, and damage cell walls, among other potential mechanisms (Alqurashi & Aldossary, 2021). In addition, our research concluded that *Staphylococcus* or *E. coli* was not found in any of the samples (data not shown), which is probably because Pakistan applies strict food safety and hygiene regulations to chicken and meat products.

Table 4: Total plate count (TPC; log ^{10} cfu/g) of chicken burgers

Treatment	Preservation Time (Days)			
	0	**3**	**5**	**7**
Control	4.59 ± 0.02	36.15 ± 0.21	89.37 ± 0.52	126.83 ± 0.17
1% WMOP	3.19 ± 0.07	9.76 ± 0.05	42.27 ± 0.02	89.47 ± 0.02
2% WMOP	4.75 ± 0.03	6.95 ± 0.07	17.65 ± 0.02	35.48 ± 0.02
1% MOPE	3.09 ± 0.12	18.74 ± 0.03	24.67 ± 0.1	71.38 ± 0.02
2% MOPE	2.06 ± 0.05	12.67 ± 0.03	58.19 ± 0.08	81.57 ± 0.06

Control (without antioxidant), 1%, 2% WMOP (burgers with 1% & 2% whole MO powder), 1%, 2% MOPE (burgers with 1% & 2% MO polyphenol extract)

3.5. Sensory analysis

To determine the optimum quantity of MO leaf (WMOP and MOPE) added to chicken burgers, a sensory evaluation was conducted using different concentrations (1% & 2%) in addition to the control. The sensory attributes of all C.B were assessed, and no significant differences were observed **(Table 5).** Across all concentrations, including the control, MOPE, and WMOP chicken burger, there were no notable changes in flavour, colour, texture, taste, or overall acceptability ($p > 0.05$). This suggests that the incorporation of MO leaf extracts (WMOP and MOPE) at both 1% and 2% concentrations did not adversely affect the sensory characteristics of the chicken burgers. These findings imply that MO leaf extracts can be effectively added to chicken burgers as natural additives without compromising their sensory qualities, thereby enhancing their nutritional value and potential health benefits.

Table 5: Effect on sensory attributes burgers

Treatment	Overall acceptance	Flavor	Colour	Texture	Taste
Control	8.21± 1.12	8.29 ± 1.43	8.29 ± 1.27	7.87 ± 1.09	7.49 ± 1.45
1% WMOP	7.61 ± 1.06	7.31 ± 1.25	7.88 ± 1.48	8.18 ± 1.37	7.62 ± 1.98
2% WMOP	6.29 ± 1.37	6.38 ± 1.17	7.58 ± 1.35	7.81 ± 1.63	7.29 ± 1.31
1% MOPE	7.81 ± 1.16	7.89 ± 1.53	7.98 ± 1.73	7.21 ± 1.06	7.78 ± 1.19
2% MOPE	6.70 ± 1.49	7.61 ± 1.12	7.52 ± 1.22	7.01 ± 1.68	6.53 ± 1.34

Control (without antioxidant), 1%, 2% WMOP (burgers with 1% & 2% whole MO powder), 1%, 2% MOPE (burgers with 1% & 2% MO polyphenol extract)

Conclusion:

This is for the first time to examine the antimicrobial and antioxidant properties of *Moringa oleifera* (MO) leaves obtained from Al-Rahman farm in Kabirwala, Punjab, Pakistan, and how they might be used in chicken burgers. Our findings demonstrate that MO leaves serve as an essential reservoir of polyphenols and show excellent antioxidant activity. These antimicrobial properties contribute to extending the shelf-life of chicken burgers, with refrigerated storage at 4°C showing potential for up to 7 days. The utilization of MOPE and WMOP as natural food preservatives in chicken burgers presents a promising avenue for the food industry. Beyond enhancing shelf-life, these natural additives offer valuable antioxidant activities, providing potential health benefits to consumers. Our results further highlight the importance and applicability of MOPE and WMOP in food preservation procedures by indicating that they may be essential in increasing the shelf-life of different meat products.

Acknowledgment: Authors are thankful to the Department of Chemistry, Division of Science and Technology, University of Education, Lahore, Faisalabad campus 37300 Pakistan. Government College University, Faisalabad for providing research facilities.

Conflict of Interest: It is declared that there no conflict of interest among all Authors

Reference:

Abdallah, R., Mostafa, N. Y., Kirrella, G. A. K., Gaballah, I., Imre, K., Morar, A., Herman, V., Sallam, K. I., & Elshebrawy, H. A. (2023). Antimicrobial Effect of Moringa oleifera Leaves Extract on Foodborne Pathogens in Ground Beef. *Foods*, *12*(4), 1–13. https://doi.org/10.3390/foods12040766

Abdel-Latif, H. M. R., Abdel-Daim, M. M., Shukry, M., Nowosad, J., & Kucharczyk, D. (2022). Benefits and applications of Moringa oleifera as a plant protein source in Aquafeed: A review. *Aquaculture*, *547*(January 2021), 737369. https://doi.org/10.1016/j.aquaculture.2021.737369

Ahmad, A., Mahmood, N., Hussain, M., Aiman, U., Al-Mijalli, S. H., Raza, M. A., & Al Jbawi, E. (2023). Improvement in oxidative stability and quality characteristics of functional chicken meat product supplemented with aqueous coriander extract. *International Journal of Food Properties*, *26*(1), 855–865. https://doi.org/10.1080/10942912.2023.2189086

Ahmad, N., Arslan, M., Kashif, A. R., & Abbas, A. (2022). *Nutraceutical and biological attributes of wild Withania coagulans fruits*. *8*(3), 106–113.

Alqurashi, R. M., & Aldossary, H. M. (2021). In vitro antioxidant and antimicrobial activity of Moringa oleifera leaf as a natural food preservative in chicken burgers. *Emirates Journal of Food and Agriculture*, *33*(6), 450–457. https://doi.org/10.9755/ejfa.2021.v33.i6.2711

Arora, S., & Arora, S. (2021). Nutritional significance and therapeutic potential of Moringa oleifera: The wonder plant. *Journal of Food Biochemistry*, *45*(10), 1–24. https://doi.org/10.1111/jfbc.13933

Assanti, E., Karabagias, V. K., Karabagias, I. K., Badeka, A., & Kontominas, M. G. (2021). Shelf life evaluation of fresh chicken burgers based on the combination of chitosan dip and vacuum packaging under refrigerated storage. *Journal of Food Science and Technology*, *58*(3), 870–883. https://doi.org/10.1007/s13197-020-04601-4

Badawy, M. E. I., Lotfy, T. M. R., & Shawir, S. M. S. (2020). Facile synthesis and characterizations of antibacterial and antioxidant of chitosan monoterpene nanoparticles and their applications in preserving minced meat. *International Journal of Biological Macromolecules*, *156*, 127–136. https://doi.org/10.1016/j.ijbiomac.2020.04.044

Boeira, C. P., Alves, J. dos S., Flores, D. C. B., de Moura, M. R., Melo, P. T. S., & da Rosa, C. S. (2021). Antioxidant and antimicrobial effect of an innovative active film containing corn stigma residue extract for refrigerated meat conservation. *Journal of Food Processing and Preservation*, *45*(9), 1–14. https://doi.org/10.1111/jfpp.15721

Chhikara, N., Kaur, A., Mann, S., Garg, M. K., Sofi, S. A., & Panghal, A. (2021). Bioactive compounds, associated health benefits and safety considerations of Moringa oleifera L.: an updated review. *Nutrition and Food Science*, *51*(2), 255–277. https://doi.org/10.1108/NFS-03-2020-0087

Duan, M., Zhang, Y., Zhou, B., Qin, Z., Wu, J., Wang, Q., & Yin, Y. (2020). Effects of Bacillus subtilis on carbon components and microbial functional metabolism during cow manure–straw composting. *Bioresource Technology*, *303*(November 2019), 122868. https://doi.org/10.1016/j.biortech.2020.122868

Fraqueza, M. J., Laranjo, M., Elias, M., & Patarata, L. (2021). Microbiological hazards associated with salt and nitrite reduction in cured meat products: control strategies based on antimicrobial effect of natural ingredients and protective microbiota. *Current Opinion in Food Science*, *38*, 32–39. https://doi.org/10.1016/j.cofs.2020.10.027

Gomes, S. M., Leitão, A., Alves, A., & Santos, L. (2023). Incorporation of Moringa oleifera Leaf Extract in Yoghurts to

Mitigate Children's Malnutrition in Developing Countries. *Molecules*, *28*(6), 1–20. https://doi.org/10.3390/molecules28062526

Gómez, I., Janardhanan, R., Ibañez, F. C., & Beriain, M. J. (2020). The effects of processing and preservation technologies on meat quality: Sensory and nutritional aspects. *Foods*, *9*(10), 1–30. https://doi.org/10.3390/foods9101416

Gutiérrez-Del-río, I., López-Ibáñez, S., Magadán-Corpas, P., Fernández-Calleja, L., Pérez-Valero, Á., Tuñón-Granda, M., Miguélez, E. M., Villar, C. J., & Lombó, F. (2021). Terpenoids and polyphenols as natural antioxidant agents in food preservation. *Antioxidants*, *10*(8). https://doi.org/10.3390/antiox10081264

Hadadi, Z., Nematzadeh, G. A., & Ghahari, S. (2020). A study on the antioxidant and antimicrobial activities in the chloroformic and methanolic extracts of 6 important medicinal plants collected from North of Iran. *BMC Chemistry*, *14*(1), 1–11. https://doi.org/10.1186/s13065-020-00683-5

Hajji, M., Falcimaigne-Gordin, A., Ksouda, G., Merlier, F., Thomasset, B., & Nasri, M. (2021). A water-soluble polysaccharide from Anethum graveolens seeds: Structural characterization, antioxidant activity and potential use as meat preservative. *International Journal of Biological Macromolecules*, *167*, 516–527. https://doi.org/10.1016/j.ijbiomac.2020.12.006

Hussen, E. M., & Endalew, S. A. (2023). In vitro antioxidant and free-radical scavenging activities of polar leaf extracts of Vernonia amygdalina. *BMC Complementary Medicine and Therapies*, *23*(1), 1–12. https://doi.org/10.1186/s12906-023-03923-y

Islam, Z., Islam, S. M. R., Hossen, F., Mahtab-Ul-Islam, K., Hasan, M. R., & Karim, R. (2021). Moringa oleifera is a Prominent Source of Nutrients with Potential Health Benefits. *International Journal of Food Science*, *2021*(June 2013). https://doi.org/10.1155/2021/6627265

Liu, R., Liu, J., Huang, Q., Liu, S., & Jiang, Y. (2022). Moringa oleifera: a systematic review of its botany, traditional uses, phytochemistry, pharmacology and toxicity. *Journal of Pharmacy and Pharmacology*, *74*(3), 296–320. https://doi.org/10.1093/jpp/rgab131

Lorenzo, J. M., Munekata, P. E. S., Pateiro, M., Domínguez, R., Abdulrazzaq Alaghbari, M., & Tomasevic, I. (2021). Preservation of meat products with natural antioxidants from rosemary. *IOP Conference Series: Earth and Environmental Science*, *854*(1). https://doi.org/10.1088/1755-1315/854/1/012053

Manessis, G., Kalogianni, A. I., Lazou, T., Moschovas, M., Bossis, I., & Gelasakis, A. I. (2020). Plant-derived natural antioxidants in meat and meat products. *Antioxidants*, *9*(12), 1–30. https://doi.org/10.3390/antiox9121215

Martillanes, S., Ramírez, R., Amaro-Blanco, G., Ayuso-Yuste, M. C., Gil, M. V., & Delgado-Adámez, J. (2020). Effect of rice bran extract on the preservation of pork burger treated with high pressure processing. *Journal of Food Processing and Preservation*, *44*(1), 1–11. https://doi.org/10.1111/jfpp.14313

Meireles, D., Gomes, J., Lopes, L., Hinzmann, M., & Machado, J. (2020). A review of properties, nutritional and pharmaceutical applications of Moringa oleifera: integrative approach on conventional and traditional Asian medicine. *Advances in Traditional Medicine*, *20*(4), 495–515. https://doi.org/10.1007/s13596-020-00468-0

Milla, P. G., Peñalver, R., & Nieto, G. (2021). Health benefits of uses and applications of moringa oleifera in bakery products. *Plants*, *10*(2), 1–17. https://doi.org/10.3390/plants10020318

Mutar, Y. S., Al-Rawi, K. F., & Mohammed, M. T. (2021). Moringa oleifera: Nutritive importance and its medicinal application, as a Review. *Egyptian Journal of Chemistry*, *64*(11), 6827–6834. https://doi.org/10.21608/EJCHEM.2021.78212.3823

Padayachee, B., & Baijnath, H. (2020). An updated comprehensive review of the medicinal, phytochemical and pharmacological properties of Moringa oleifera. *South African Journal of Botany*, *129*, 304–316. https://doi.org/10.1016/j.sajb.2019.08.021

Ramli, A. N. M., Manap, N. W. A., Bhuyar, P., & Azelee, N. I. W. (2020). Passion fruit (Passiflora edulis) peel powder extract and its application towards antibacterial and antioxidant activity on the preserved meat products. *SN Applied Sciences*, *2*(10), 1–11. https://doi.org/10.1007/s42452-020-03550-z

Ramli, A. N. M., Sukri, N. A. M., Azelee, N. I. W., & Bhuyar, P. (2021). Exploration of antibacterial and antioxidative activity of seed/peel extracts of Southeast Asian fruit Durian (Durio zibethinus) for effective shelf-life enhancement of preserved meat. *Journal of Food Processing and Preservation*, *45*(9), 1–10. https://doi.org/10.1111/jfpp.15662

Rebezov, M., Chughtai, M. F. J., Mehmood, T., Khaliq, A., Tanweer, S., Semenova, A., Khayrullin, M., Dydykin, A., Burlankov, S., Thiruvengadam, M., Shariati, M. A., & Lorenzo, J. M. (2022). Novel techniques for microbiological safety in meat and fish industries. *Applied Sciences (Switzerland)*, *12*(1). https://doi.org/10.3390/app12010319

Rudy, M., Kucharyk, S., Duma-Kocan, P., Stanislawczyk, R., & Gil, M. (2020). Unconventional methods of preserving meat products and their impact on health and the environment. *Sustainability (Switzerland)*, *12*(15).

https://doi.org/10.3390/SU12155948

Sarker, M., Hashem, M., Azad, M., Ali, M., & Rahman, M. (2021). Food grade vinegar acts as an effective tool for short-term meat preservation. *Meat Research*, *1*(1), 1–9. https://doi.org/10.55002/mr.1.1.5

Shen, Y., Hong, S., Du, Z., Chao, M., O'Quinn, T., & Li, Y. (2022). Effect of adding modified pea protein as functional extender on the physical and sensory properties of beef patties. *Lwt*, *154*, 112774. https://doi.org/10.1016/j.lwt.2021.112774

Tayengwa, T., Chikwanha, O. C., Gouws, P., Dugan, M. E. R., Mutsvangwa, T., & Mapiye, C. (2020). Dietary citrus pulp and grape pomace as potential natural preservatives for extending beef shelf life. *Meat Science*, *162*(September 2019), 108029. https://doi.org/10.1016/j.meatsci.2019.108029

Verma, A. K., Rajkumar, V., Kumar, M. S., & Jayant, S. K. (2020). Antioxidative effect of drumstick (Moringa oleifera L.) flower on the quality and stability of goat meat nuggets. *Nutrition and Food Science*, *50*(1), 84–95. https://doi.org/10.1108/NFS-12-2018-0348

Wang, S. P., Rubio, L. A., Duncan, S. H., Donachie, G. E., Holtrop, G., Lo, G., Farquharson, F. M., Wagner, J., Parkhill, J., Louis, P., Walker, A. W., & Flint, H. J. (2020). Pivotal Roles for pH, Lactate, and Lactate-Utilizing Bacteria in the Stability of a Human Colonic Microbial Ecosystem. *MSystems*, *5*(5). https://doi.org/10.1128/msystems.00645-20

Wu, J., Zang, M., Wang, S., Zhao, B., Bai, J., Xu, C., Shi, Y., & Qiao, X. (2023). Nisin: From a structural and meat preservation perspective. *Food Microbiology*, *111*(September 2022), 104207. https://doi.org/10.1016/j.fm.2022.104207

Received: Febraury 8th 2024 **Accepted: March 21th 2024**