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RESEARCH PAPER

Identification of natural antioxidants using GC-MS analysis from *Moringa oleifera* with meat preservative potential

Nadia Sardar[1], Yamin Bibi[1,2], Muhammad Arshad[1], Anwaar Ahmad[3], Hesham S. Almoallim[4], Mohammad Javed Ansari[5]

1 *Department of Botany, PMAS-Arid Agriculture University Rawalpindi, Rawalpindi 46300, Pakistan*
2 *Department of Botany, Rawalpindi Women University, Rawalpindi 46300, Pakistan*
3 *Institute of Food and Nutritional Sciences, PMAS-Arid Agriculture University Rawalpindi, Rawalpindi 46300, Pakistan*
4 *Department of Oral and Maxillofacial Surgery, College of Dentistry, King Saud University, PO Box-60169, Riyadh 11545, Saudi Arabia*
5 *Department of Botany, Hindu College Moradabad (Mahatma Jyotiba Phule Rohilkhand University Bareilly), Moradabad 244001, India*

Corresponding author: Yamin Bibi (yamin.bibi@f.rwu.edu.pk)

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Abstract

Antioxidants play a vital role in food preservation. They ensure that food keeps its taste and color and stays edible over a longer period. In this study, the oxidative stability of Frozen beef incorporated with *Moringa oleifera* leaves was evaluated. Antioxidant activities were investigated using DPPH (2,2-diphenyl-1-picrylhydrazyl) and Phosphomolybdate assay. Whereas the effect of tested extract on frozen beef quality was monitored using Thiobarbituric acid reactive substance (TBARS) lipid oxidation assay and organoleptic evaluation. Our results indicated that methanol extract of *Moringa oleifera* is the most active extract with IC_{50} of 22.52 µg/mL against DPPH free radical and 32.47 µg/mL against phosphomolybdate ion. Methanol extract also showed a considerable inhibition of lipid per oxidation along with some positive organoleptic effect, which indicates the presence of compounds with preservative effect on frozen beef. The GC-MS analysis of this extract resulted in the identification of 41 compounds. These identified compounds should be further purified and tested individually in order to indicate agents that have potential to be used commercially as natural meat preservatives.

Keywords

Moringa oleifera, ground beef, antioxidant, lipid oxidation

Introduction

Oxidation is one of the major factors in controlling the quality of meat products, as it affects meat properties such as taste, color, texture, and nutritional value. So, in order to achieve a better shelf life, various antioxidants were used i.e. butylated hydroxytoluene (BHT), butylated hydroxy anisole (BHA), tert-butyl hydroquinone (TBHQ), propyl gallate (PG, nitrites, sodium metabisulphite), sodium benzoate, sorbates, benzoate and many others (Ruiz-Hernández et al. 2023). These chemical preservatives have all along been used due to their affordability and effectiveness. But, instead of increasingly demanding legislation and control, several studies continue to highlight the toxicity that artificial additives pose to human health (Xu et al. 2023). Natural antioxidants in meat products are the best replacement to overcome this problem.

Plant polyphenols are considered a major natural source of bioactive compounds to be used as meat preservatives (Olvera-Aguirre et al. 2023). Many herbs and spices provide natural alternative source of preservatives that are readily acceptable by health-conscious consumers.

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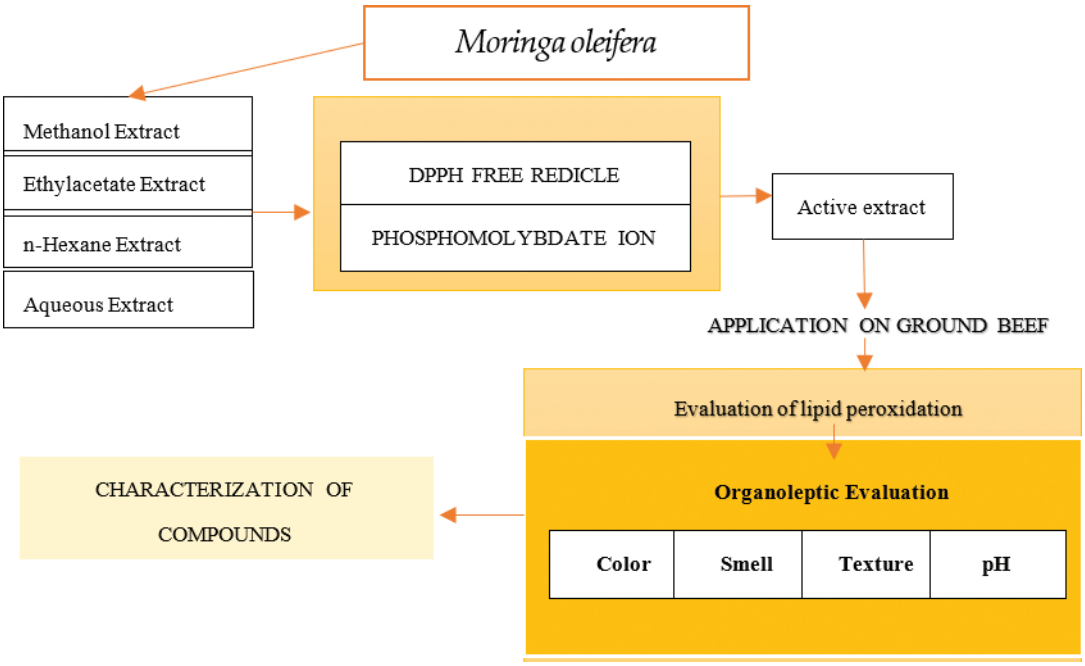

Figure 1. Graphical abstract.

They may be used in the food industry and considered as clean label products (Mojarradi et al. 2023).

Moringa oleifera, which is commonly known as horse radish tree or drumstick tree. Almost all parts of this plant such as roots, seeds, seed oils, leaves, and flowers have been used on a large-scale basis as food (Azlan et al. 2023). *Moringa oleifera* is a plant that has been known for its therapeutic potential. It is rich in bioactive compounds i.e. vitamins, saponins, alkaloids, and phenolic acids. Its leaves are used for the treatment of many chronic conditions, including insulin resistance, cancer, hypercholesterolemia, high blood pressure, diabetes, hepatic disease and inflammation (Rotella et al. 2023).

The high medicinal value of this plant makes it a potential candidate to be used as a natural preservative. In addition to discussing the legal framework, trends, constraints, and obstacles to the widespread use of plant-derived antioxidants by the meat industry, the present study aimed to provide an overview of the state of knowledge regarding compounds of *Moringa oleifera* and their applications in meat systems, with a focus on the mechanisms and spectrum of their antioxidant activity (Fig. 1).

Materials and methods

Collection and extract preparation

The leaves of *Moringa oleifera* were collected from PMAS-Arid Agriculture University Rawalpindi, Pakistan (Fig. 2) and dried under shade. Permission was taken from the administration unit for the collection of *Moringa oleifera* leaves. Voucher specimens were deposited in herbarium at PMAS-AAUR. Professor Dr. Rehmatullah Qureshi (plant taxonomist) identified the plant specimen. Experimental research and field studies on *Moringa oleifera* comply with institutional, national, and international guidelines. All the collected plants were ground into powder form in grinding mill separately and stored in airtight containers with label. For extract preparation cold maceration technique was performed (Zahara et al. 2022).

Preparation of ground meat

The fresh meat was cut into pieces of uniform weight for sensory, chemical, and microbiological analysis. Fresh minced meat was divided into twenty batches (twenty pieces (20 g) per batch). Four groups: control (without the addition of any additive), meat incorporated with artificial preservative (BHT), and meat incorporated with *Moringa oleifera* extract of different concentrations (0.5,1 and 1.5%). The whole experiment was replicated twice and measurements were carried out for each parameter studied in each replica. All the analyses were carried out on the 1st, 4th, 7th, and 10th day.

Analysis of antioxidant activity of extract

DPPH radical scavenging activity assay

Free radical scavenging bioassay was carried out by method described by Ishaque et al. (2018) and Hara et al. (2018) with few modifications. The antioxidant potential of crude extract was assessed by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) standard free radical scavenging method. The stock solution was prepared by dissolving DPPH in methanol. Using the stock solution samples were prepared and maintained at the absorbance of 517 nm by using a spectrophotometer. The sample solution was incubated for 15 min in the dark. Ascorbic acid and pure DMSO were used as positive and negative control. The scavenging activity were estimated by the following equation:

Figure 2. *Moringa oleifera* leaves, (**a**) and tree (**b, c**).

Scavenging effect (%)= [(control absorbance - sample absorbance) / control absorbance] × 100.

Percentage scavenging potential = Ac-As / Ac × 100

Phosphomolybdate assay

Using the procedure of Umamaheswari and Chatterjee (2008) sample solution was mixed with reagent solution (28 mM sodium phosphate, 0.6 M sulphuric acid and 4 mM ammonium molybdate). The test tubes were covered with silver foil and incubated in a water bath at 95 °C. Then it was allowed to attain room temperature. The absorbance was measured at 765 nm against a blank using spectrophotometer. The antioxidant capacity was estimated with the help of the following formula:

Antioxidant effect (%)=[(control absorbance-sample absorbance)/ control absorbance] × 100

Evaluation of lipid oxidation

Using the protocol given by Verardo et al. (2009) secondary lipid oxidation products were quantified with Thiobarbituric acid reactive substances (TBARS) distillation method and expressed as malondialdehyde (MDA) equivalents. A 20 g portion of each meat sample was homogenized with 50 mL of distilled–deionized water and 10 mL of tri-chloroacetic acid (15%, final concentration) using a stomacher for 2 min. The homogenate was centrifuged at 20,000 rpm for 5 min, and the supernatant was filtered through Whatman no. 1 filter paper. A 2 mL aliquot of 0.06 M thiobarbituric acid was added to 8 mL of the filtrate. The solution was vortexed for 15 s, placed in at 80 °C water bath for 90 min, and then cooled on ice. Absorption was measured at 532 nm using a UV–visible spectrophotometer. Results were expressed as mg malondialdehyde (MDA) equivalent/ kg of meat sample.

Organoleptic evaluation of ground beef treated with *Moringa oleifera* methanol extract

pH levels

The pH level was determined by following the method of Lee et al. (1996). 10 g sample were taken from all the treatment and put it in 50 mL of distilled water. Homogenized the sample in a homogenizer for 1 min. The homogenate obtained was used to check the pH. The pH was measured at 1st, 4th, 7th, and 10th day

Color

Color analysis was performed using Konica Minolta CR-400 Chroma Meter. The color was described by L* (lightness), a* (redness) and b* (yellowness) and were measured on the top surface and middle of ground beef on the 1st, 4th, 7th, and 10th day.

Smell and taste (aroma and flavor)

The odor of the meat samples was detected manually by panelists after every 1, 4, 7 and 10 days and evaluated as i.e. 1: good 2: better 3: slightly better 4: poor.

Texture and consistency (tenderness and juiciness)

In order to check texture, the meat was checked by texture analyzer after every 1, 4, 7 and 10 days. Organoleptic characterization of ground beef was done according to the protocol given by FAO and WHO (1999).

Characterization of compounds

The methanol fraction of *Moringa oleifera* was further characterized by using spectroscopic techniques (Gas Chromatography Mass spectrometry (GC-MS) according to the method followed by Nisa et al. (2022) with few modifications. The samples (1 µL aliquot) were injected in split mode. Agilent Technologies 7890B gas chromatography system coupled to an Agilent Technologies

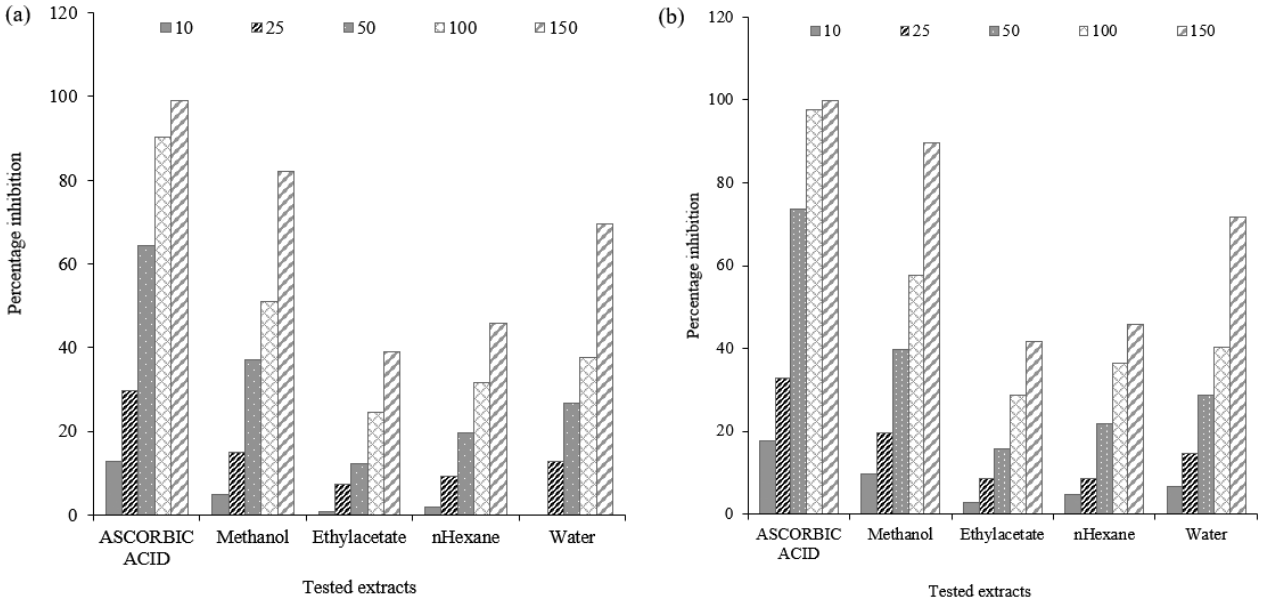

Figure 3. Antioxidant activity of *Moringa oleifera* extracts against: (**a**) DPPH; (**b**) phosphomolybdate free radicals.

8200 Accurate-Mass Q-ToF mass spectrometer (Agilent Technologies Deutschland GmbH-Germany) with 1:10 flow. Separation of the metabolites was performed on an HP-5MS capillary column coated with polyimide (20 m, 0.182 mm i.d., 0.18 µm film thickness; Agilent Technologies Deutschland GmbH-Germany) with 230 °C of the ion source. The mass spectral analysis was performed in scan mode with a quadruple temperature of 150 °C and a fragmentation voltage of 70 eV. Solvent delay was adjusted accordingly for the two tested GC oven programs.

Results

Antioxidant activity of tested extracts of *Moringa oleifera*

The antioxidant capacity of *Moringa oleifera* extracts i.e. methanol, ethyl acetate, n-hexane, and Aqueous was assessed using DPPH and phosphomolybdate assay i.e. Fig. 3. The results indicated that methanol extract of *Moringa oleifera* has maximum activity against both free radicals with percentage inhibition of 82% and 89.64% at 150 µg/mL concentration followed by aqueous extract i.e. 69% and 71.63%.

As shown in Table 1, the IC_{50} value of tested extracts was evaluated using graph pad prism. Against DPPH free radical the IC_{50} values were 22.52 with methanol, 30.73with ethylacetate, 24.52 with n-hexane and 27.83 µg/mL with aqueous extract. Similar trend was observed against phosphomolybdate ion i.e. 32.47, 57.78, 45.62 and 50.32 µg/mL respectively.

Lipid oxidation in ground beef is treated with *Moringa oleifera* methanol extract

For evaluation of lipid peroxidation Thiobarbituric acid reactive substances (TBARS) assay was used. This assay involves the reaction of lipid peroxidation products, primarily malondialdehyde (MDA), with thiobarbituric acid (TBA), which leads to the formation of MDA-TBA2 adducts called TBARS. Three different concentrations of *Moringa oleifera* methanol extract were tested (Fig. 4). As a control, a standard antioxidant Butylated hydroxytoluene (BHT) was used. BHT is an antioxidant that is used in various products to prevent free radical-mediated oxidation.

In our tested samples from day 0 to 10 with the increased concentration of methanol extract there was a decrease in TBARS value as compared to negative control, which indicates a low oxidative stress. The standard antioxidant i.e. BHT has shown on day 10 Thiobarbituric acid

Table 1. IC_{50} of *Moringa oleifera* at different concentration in different solvents.

Tested Extract	DPPH free radical	Phosphomolybdate ion
Methanol	22.5209	32.4764
Ethylacetate	30.7365	57.7855
n-Hexane	24.5202	45.6232
Aqueous	27.8357	50.3229

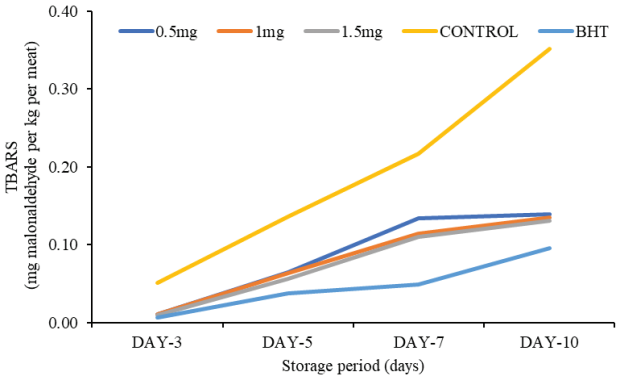

Figure 4. Lipid oxidation in ground beef is treated with *Moringa oleifera* methanol extract.

reactive substances value of 0.096 whereas in beef treated with methanol extract a contraction dependent values were 0.1333, 0.1336 and 0.105 respectively.

Organoleptic Evaluation of Ground Beef Treated with *Moringa oleifera* Methanol Extract

pH

The pH of the meat is one of the most significant determinants of meat quality. Meat pH level is a key sign of its safety, freshness and flavor. It is a measurement of the meat acidity or alkalinity. The pH value calculated at day 4 to determine the overall quality of meat at storage of 4 °C. The pH of ground beef in three different concentrations of *Moringa oleifera* leaves powder (Methanol extract) is shown in Fig. 5. BHT was added as a positive control and samples without plant extract or BHT as a negative control. The pH values were observed at day 1, day 4, day 7 and day 10. The mean pH was slightly different among the treated samples and control. The mean pH value increased gradually in all treatments during the storage period. On day 1, the pH of control sample was 5.43 while that of *Moringa oleifera* methanol extract was highest 5.45. By increasing the storage period the pH value increased in all treatments of plants and reached at 5.82 on day 10. The pH value lied between 5.36 to 5.78 in the first week of storage at lowest concentration of sample, whereas its value lied between 5.27 to 5.65 in first week of storage at higher concentration of sample.

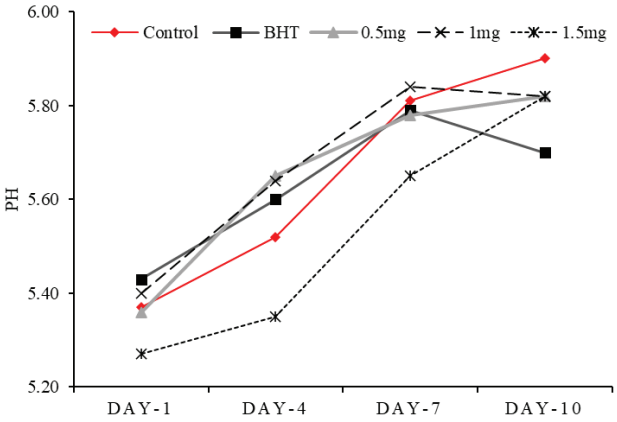

Figure 5. pH changes in ground beef after application of *Moringa oleifera* extract.

Color

Ground beef treated with *Moringa oleifera* methanol extract was monitored for ten days, during that period change in color was measured. As a control a standard antioxidant Butylated hydroxytoluene (BHT) was used. The beef was stored under refrigerated storage. Three color parameters i.e. lightness L*, redness a* and Yellowness b* were measured. As shown in Fig. 6 with increase in storage time, L* (lightness) and b* (yellowness) decrease for all the treatments. Whereas the a* (stability of the red color) values did not decrease significantly ($p < 0.05$) with the increase of *Moringa oleifera* extract concentration in treated ground beef samples. The color change during storage is related to oxidized myoglobin, met myoglobin formation and lipid oxidation of meat products.

Smell

Our results indicated that the aroma of treatments with low concentration of *Moringa oleifera* extract 0.5% (M-1) and 1% (M-2) was not good enough and its smell gone bad with the increase in storage day, specifically at day-7th and day-10th i.e. Fig. 7 whereas the aroma in with high concentration of *Moringa oleifera* M-3 (1.5%) and standard antioxidant (BHT) are comparable to each other with no significant difference in all storage days.

Texture

The texture was characterized between 1 to 4 (1: good 2: better 3: slightly better 4: poor). On day-1 all the treatment and BHT are at 4, but with increasing in storage time the texture of the treatment with low concentration in *Moringa oleifera* showed negative results and attained 1 i.e. Fig. 8. The treatment with higher concentration of *Moringa oleifera* (1.5%) results are significantly similar with standard antioxidant BHT in all storage days.

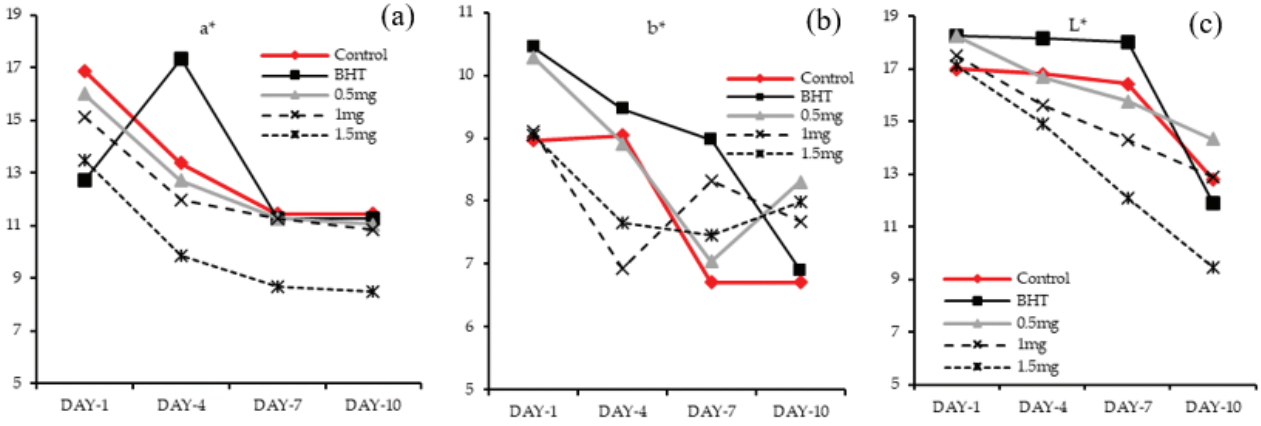

Figure 6. The color changes in ground beef after application of *Moringa oleifera* Methanol extract: (**a**) change in redness; (**b**) change in yellowness; (c**)** change in lightness.

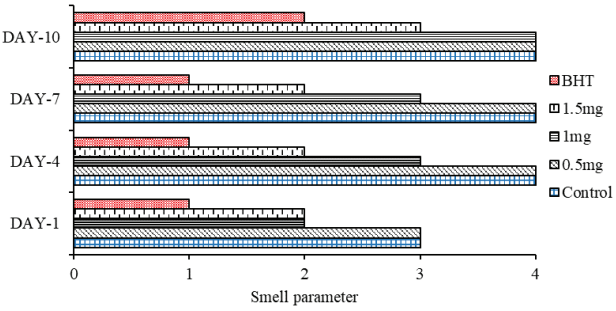

Figure 7. Change in smell of ground beef after application of *Moringa oleifera* Methanol extract. Parameters: 1-good, 2-better, 3-slightly better, 4-poor.

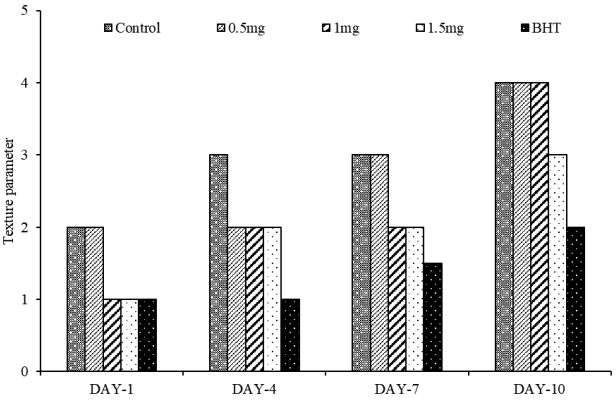

Figure 8. Change in Texture of ground beef after application of *Moringa oleifera* Methanol extract. Parameters: 1-good, 2-better, 3-slightly better, 4-poor.

GC-MS analysis of *Moringa oleifera* methanol extract

Upon GC-MS analysis of *Moringa oleifera* the chromatogram showed a large number of peaks, and each peak generates a unique mass spectrum used for identification of compounds i.e. Fig. 9. The list of phytochemicals identified in Methanol extract of *Moringa oleifera* leaves, their retention time, width, height, area, and base peak area reflects 41 peaks of biomolecules i.e. Table 2. Various compounds were identified i.e. Cyclopentasiloxane, decamethyl, 9-Octadecenamide, Undecane, 5,5-dimethy, Hexane, 3,3-dimethyl, (1-Methoxy-pentyl)-cyclopropane, Heptane, 4-ethyl-2,2,6,6-tetramethyland etc. In Table2. The phytocomponents, their retention time, peak area obtained from GC-MS analysis were enlisted.

Discussions

During antioxidant analysis, the highest IC_{50} value was observed by ethyl acetate extract whereas methanol and aqueous extract showed a low IC_{50} value. The low IC_{50} value of methanol extract indicated the presence of some bioactive compounds. Previously Barzan et al. (2024) suggested that the high antioxidant activity of *Moringa oleifera* extract is directly related to the increased content of phenolic compounds. In another study it was indicated

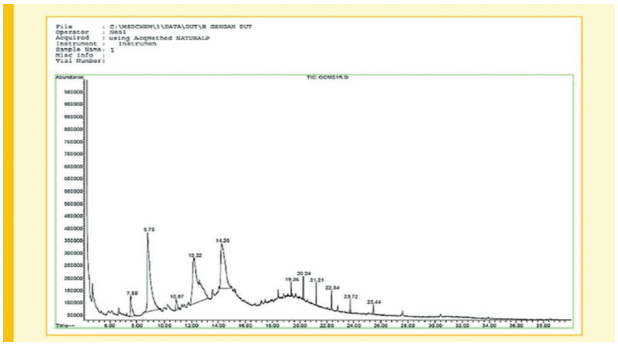

Figure 9. GC-MS Chromatogram of Methanol extract of *Moringa oleifera* leaves.

that *Moringa oleifera* leaf extract is a rich source of flavonoid and phenolic compounds, which are responsible for its strong antioxidant activity (Gharsallah et al. 2023; Xu et al. 2024a).

The methanol extract of *Moringa oleifera* leaves appeared to be the most active one thus this extract was applied on minced beef in three different concentrations i.e. 0.5 mg, 1 mg, and 1.5 mg. To evaluate preservative effect TBARS assay was performed to monitor lipid peroxidation. TBARS values indicated the level of lipid peroxidation in biological samples. Higher TBARS values indicated higher levels of lipid peroxidation and oxidative stress (Osyczka et al. 2023; Jia et al. 2024). In our tested samples from day 0 to 10 there is a decrease in TBARS value as compared to negative control, which indicated a low oxidative stress.

Several studies showed a progressive relationship between reduced lipid oxidation and polyphenol content. High values of TBARS in control sample throughout the storage days might be due to the association of polyunsaturated fatty acids with catalysts such as iron ions from the tissue of ground beef (Chen et al. 2023). Such storage will ultimately promote the breakdown of heme compounds, thereby liberating the low-molecular-weight iron compounds in ground beef that are assumed to be accountable for lipid oxidation. It was also observed that the thiobarbituric acid (TBA) value of cooked ground buffalo meat (GBM) treated with 1.5% crude extract of *M. oleifera* leaves was significantly lower than that of the control.

Moreover, similar results were observed with the inclusion of pre-blended carnosine. It decreased the TBARS values of ground buffalo meat during storage days (El-Sayed et al. 2023; Wang et al. 2022). The same authors informed that certain bacteria such as *Pseudomonas ovalis* as well as strains of *Streptomyces* also contribute to lipid oxidation by producing compounds such as peroxides, carbonyls aldehydes, and ketones, or other similar compounds.

The methanol extract was again subjected to organoleptic evaluation, which indicated that the higher concentration of *M. oleifera* methanol extract has considerable preservative effect. Thus spectroscopic technique (Gas Chromatography Mass spectrometry (GC-MS)) was performed, which identified 41 compounds Table 2.

Table 2. List of phytochemicals identified in Methanolic extract of *Moringa oleifera* leaves, their retention time, width, height, area and base peak area.

Sr. No.	Label	Retention time	Width	Height	Area	Base Peak
1.	Cyclopentasiloxane, decamethyl	7.024	0.1	4609	26578	266.989
2.	Cyclobutane, 1,1,2,3,3-pentamethyl	9.87	0.024	722	668	55.0704
3.	Undecane, 5,5-dimethyl	21.88	0.005	1199	6592	71.1015
4.	1-(4-Butoxy-2,6-dimethylphenyl)ethanone	15.82	0.053	580	2008	149.142
5.	9-Octadecenamide	20.34	0.074	4606	40679	72.1031
6.	Sulfurous acid, 2-ethylhexyl hexyl ester	18	0.077	1703	8444	57.0857
7.	9-Ethylfluorene	16.31	0.091	5151	11603	178.083
8.	Pentanoic acid, 1,1-dimethylpropyl ester	18.64	0.034	1455	3114	57.0857
9.	Octane, 2,6,6-trimethyl	11.75	0.033	1417	4989	57.0857
10.	Octane, 2,6,6-trimethyl	7.681	0.002	6245	40947	57.0857
11.	Sulfurous acid, 2-ethylhexyl hexyl ester	17.64	0.048	1137	3855	57.0857
12.	Octane, 1,1'-oxybis	16.75	0.055	4286	19497	57.0857
13.	Decane, 3,8-dimethyl	8.594	0.002	4181	21766	57.0857
14.	2,2-Dimethyl-3-heptanone	16	0.079	2815	9454	57.0857
15.	Valeric acid, 2-tetrahydrofurylmethyl ester	9.121	0.071	1646	9079	71.1015
16.	(1-Methoxy-pentyl)-cyclopropane	13.03	0.031	569	1349	85.1159
17.	17a-Aza-D-homoandrostan-17-one, (5.alpha.)	6.401	0.005	1159	1294	274.221
18.	Pentanoic acid, 1,1-dimethylpropyl ester	15.07	0.034	1683	3988	57.0857
19.	Sulfurous acid, nonyl 2-pentyl ester	8.818	0.071	7980	38922	71.1015
20	(1-Methoxy-pentyl)-cyclopropane	10.04	0.086	731	2150	71.1015
21.	Dodecane, 1-fluoro	11.21	0.077	3266	20521	57.0857
22.	Undecane, 5,5-dimethyl	14.2	0.071	2556	11267	57.0857
23.	Heptane, 4-ethyl-2,2,6,6-tetramethyl	13.77	0.038	2863	6652	57.0857
24.	Hexyl octyl ether	8.939	0.053	2899	13521	71.1015
25.	Hexane, 3,3-dimethyl	13.27	0.045	1508	6529	57.0857
26.	Hexane, 3,3-dimethyl	11.09	0.043	2726	9858	57.0857
27.	Sulfurous acid, 2-ethylhexyl hexyl ester	11.56	0.05	2721	10464	71.1015
28.	2-Methylthiolane, S,S-dioxide	14.83	0.053	894	3055	71.1015
29.	2,2-Dimethyl-propyl 2,2-dimethyl-propanesulfinyl sulfone	7.882	0.083	2041	11681	57.0857
30.	Nonane, 4,5-dimethyl	20.18	0.06	2941	15088	57.0857
31.	Nonane, 2,2,4,4,6,8,8-heptamethyl	11.68	0.046	4603	19738	57.0857
32.	Cyclooctasiloxane, hexadecamethyl	13.63	0.046	1303	6645	73.0622
33.	2-Acetyl-3-methylbenzo[b]thiophene	8.487	0.003	72221	243842	175.154
34.	Sulfurous acid, 2-ethylhexyl undecyl ester	18.35	0.072	4743	34184	71.1015
35.	Sulfurous acid, 2-ethylhexyl nonyl ester	14.58	0.06	3808	21037	57.0857
36.	Sulfurous acid, 2-ethylhexyl undecyl ester	11.63	0.053	9155	42194	57.0857
37.	Tridecanol, 2-ethyl-2-methyl	16.33	0.05	7231	44534	57.0857
38.	Dodecane, 1-fluoro	10.42	0.057	10048	46172	57.0857
39.	Benzaldehyde, 4-propyl	8.774	0.076	4914	25122	91.0684
40.	Cyclohexasiloxane, dodecamethy	9.454	0.064	8735	56662	340.994
41.	Phenol, 2,5-bis(1,1-dimethylethyl)	11.92	0.083	21306	67418	191.003

The first compound identified is cyclopentasiloxane, decamethyl. It is an organosilicon compound that is identified with molecular weight 370.77 with the formula [$(CH_3)_2$-SiO_5]. This compound is classified as a cyclomethicone. It is commonly utilized in cosmetics, for instance sunblock's, deodorants, hair sprays and skin care goods. Cyclobutane, 1,1,2,3,3-pentamethyl has previously been identified from *Salvia hispanica* and *Nigella sativa* (Borraja et al. 2022; Xu et al. 2024b) whereas Silacyclo-butane, 1,1,3-trimethy, an Organosilicon has been reported from *Bothriochloa bladhii*.

Undecane, 5,5-dimethyl with molecular weight 211.26 belonged to class of saturated aliphatics, has previously been identified from *Brassica rapa* L. (Al-Tameme et al. 2015), *Origanum vulgare* seed (Kumar et al. 2017; Zhang et al. 2023). 1-(4-Butoxy-2,6-dimethylphenyl) ethenone has previously been identified from seeds and fruit pulp of *Skimmia anquetilia* (Parkash et al. 2011). *Cassia obtusifolia* L. or *Cassia tora* L., (Li et al. 2012). 2,2-Dimethyl-propyl 2,2-dimethyl-propanesulfinyl sulfone with Molecular Weight has previously been identified from *Aristolochia indica* (Okuno et al. 2019).

9-Octadecenamide belonged to primary amides class of fatty acyls with molecular weight 281.5 has previously been identified from *Hildegardia barteri* and is reported to have antibacterial and antioxidant activity (Khan et al. 2019). Sulfurous acid, 2-ethylhexyl hexyl ester which has previously been identified from *Stachytarpheta indica* (Yuvaraj et al. 2019).

9-Ethylfluorene with molecular weight 194.27 has previously been identified from hazelnut shells (Abbasi 2015). Another compound identified is pentanoic acid, 1,1-dimethylpropyl ester with molecular weight 172.26 has previously been identified from methanol extract of *Trogoderma gra*narium (Ntalli et al. 2021). Octane, 2,6,6-trimethyl which is previously been identified from methanol leaf extract of *Toddalia asiatica* (L.) (Patil and Jadhav 2014) *Solanumn nigrum* L (Taherpour et al. 2017) and *Dracocephalum moldavica* L (Shuge et al. 2009). During GC/MS analysis two different isomers of nonane (alkane hydrocarbon) were identified I.e. 2,2,4,4,6,8,8, hepta methyl, nonane 1,5 do methyl. Nonane isomers have also been isolated from other plants i.e. *Pleioblastus amarus* (Patel and Metha 2021), *Solanum aculeastrum* (Normadndin and Boudreault 2021) and *Nuphar japonica* (Zhao et al. 2020; Zhang et al. 2024).

Valaric acid methyl ester is a clear colorless to yellowish oily liquid with molecular weight 116.16. This compound is commonly used in fragrances, beauty care, soap, laundry detergents at levels of 0.1–1%. Naturally it has been isolated from ether extracts of *Scapania verrucosa* Heeg (Guo et al. 2008), *Stachytarpheta indica* (Yuvaraj et al. 2019) and *Sambucus nigra* L. (Matlok et al. 2021). These identified compounds are recommended to be isolated, purified and evaluated individually for their antioxidant activity. Synergistic effect of compounds present in extract may be responsible for antioxidant and preservative effect. Similar results were described by Li et al. (2023) that combined effects of resveratrol (RS) and benzyl isothiocyanate (BITC) on *Listeria monocytogenes* dramatically increased the antibacterial action and stopped bacterial growth in chicken meat preservation.

Conclusions

This study concludes that *M. oleifera* leaves methanol extract provide antioxidant benefits to ground beef during cold storage (4 °C) and its effects were concentration dependent. Addition of *M. oleifera* leaves at higher concentration significantly retards lipid oxidation as well as reduces microbial growth. Therefore, it is suggested that its leaves - a natural plant material, could be a good option in food bio-preservation to extend the shelf-life of meat and meat products in comparison with artificial preservatives. In addition, the compounds identified from its extract should be further tested for microbial, antioxidant and cytotoxicity in order to develop a commercially used natural preservative.

Author Contribution

YAMIN BIBI and ANWAAR AHMAD designed the study. NADIA SARDAR performed the experiments. YAMIN BIBI helped in data curation and analysis of data. SALEH H. SALMEN and MOHAMMAD JAVED ANSARI collected literature reviews and helped in writing the original draft of the article. YAMIN BIBI and MUHAMMAD ARSHAD provided technical expertise to improve the article and helped in funding acquisition. All authors reviewed and edited the manuscript.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare no conflicts of interest.

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References

Abbasi MSA (2015) Annulation reactions of heterocyclic chlorovinyl-aldehydes. Synthesis of functionalized 6H-benzo [c] chromen-6-ones (biaryl lactones), 6H-benzo [c] chromenes and heteroannulated pyridines. PhD Thesis, Universität Rostock, Rostock, Germany.

Al-Tameme HJ, Hameed IH, Idan SA, Hadi, MY (2015) Biochemical analysis of *Origanum vulgare* seeds by fourier-transform infrared (FT-IR) spectroscopy and gas chromatography-mass spectrometry (GC-MS). Journal of Pharmacognosy and Phytotherapy 7(9): 221–237. https://doi.org/10.5897/JPP2015.0362

Azlan UK, Khairul Annuar NA, Mediani A, Aizat WM, Damanhuri HA, Tong X, Yanagisawa D, Tooyama I, Ngah WZW Hamezah HS (2023) An insight into the neuroprotective and anti-neuroinflammatory effects and mechanisms of *Moringa oleifera*. Frontiers in Pharmacology 13: 1035220. https://doi.org/10.3389/fphar.2022.1035220

Barzan G, Sacco A, Giovannozzi AM, Portesi C, Schiavone C, Salafranca J, Wrona M, Nerin C, Rossi AM (2024) Development of innovative antioxidant food packaging systems based on natural extracts from food industry waste and *Moringa oleifera* leaves. Food Chemistry 432: 137088. https://doi.org/10.1016/j.foodchem.2023.137088

Borrajo P, Karwowska M, Lorenzo JM (2022) The effect of *Salvia hispanica* and *Nigella sativa* seed on the volatile profile and sensory parameters related to volatile compounds of dry fermented sausage. Molecules 27(3): 652. https://doi.org/10.3390/molecules27030652

Chen XW, Li XX, Hu QH, Sun SD, Wan ZL (2023) Multifactorial revealing the association between components and lipid oxidation of edible vegetable oils in bulk and emulsion systems. LWT, 114909. https://doi.org/10.1016/j.lwt.2023.114909

El-Sayed MH, Alshammari FA, Sharaf MH (2023) Antagonistic potentiality of actinomycete-derived extract with anti-biofilm, antioxidant, and cytotoxic capabilities as a natural combating strategy for multidrug-resistant ESKAPE pathogens. Journal of Microbiology and Biotechnology 33(1): 61. https://doi.org/10.4014/jmb.2211.11026

FAO & World Health Organization (1999) Food safety issues associated with products from aquaculture report of a Joint FAO/NACA/WHO study group.

Gharsallah K, Rezig L, Rajoka MSR, Mehwish HM, Ali MA, Chew SC (2023) *Moringa oleifera*: Processing, phytochemical composition, and industrial application. South African Journal of Botany 160: 180–193. https://doi.org/10.1016/j.sajb.2023.07.008

Guo L, Wu JZ, Han T, Cao T, Rahman K, Qin LP (2008) Chemical composition, antifungal and antitumor properties of ether extracts of *Scapania verrucosa* Heeg. and its endophytic fungus *Chaetomium fusiforme*. Molecules 13(9): 2114–2125. https://doi.org/10.3390/molecules13092114

Hara K, Someya, T, Sano K, Sagane Y, Watanabe T, Wijesekara RGS (2018) Antioxidant activities of traditional plants in Srilanka by DPPH free radical-scavenging assay. Data in Brief 17: 870–875. https://doi.org/10.1016/j.dib.2018.02.013

Ishaque M, Bibi Y, Qayyum A, Rafiq MK, Arshad M, Naqvi SS, Nisa S, Jenks MA (2018) Antioxidant potential, total phenolic and flavonoid contents of three culinary medicinal plant species of Lesser Hamalya, Pakistan. Bulgarian Chemical Communications 50(3): 368–373.

Jia S, Shen H, Wang D, Liu S, Ding Y, Zhou X (2023) Novel NaCl reduction technologies for dry-cured meat products and their mechanisms: A comprehensive review. Food Chemistry 431: 137142. https://doi.org/10.1016/j.foodchem.2023.137142

Khan S, Kaur H, Jhamta R (2019) Evaluation of antioxidant potential and phytochemical characterization using GC-MS analysis of bioactive compounds of *Achillea filipendulina* (L.) leaves. Journal of Pharmacognosy and Phytochemistry 8(3): 258–265.

Kumar J, Paul B, Nebapure SM, Singh S (2017) Comparative GC-MS analysis of two *Brassica rapa* L. varieties for identification of volatile compounds. Chemical Science Review and Letters 6: 884–889.

Lee RJ, Wang S, Low PS (1996) Measurement of endosome pH following folate receptor-mediated endocytosis. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research 1312(3): 237–242. https://doi.org/10.1016/0167-4889(96)00041-9

Li YM (2012) Comparison of volatile components of *Semen cassiae* and semen seeds tea. Journal of Medicinal Plants Research 6(22): 3865–3869. https://doi.org/10.5897/JMPR12.343

Li Z, Wu H, Liu J, Hao H, Bi J, Hou H, Zhang G (2023) Synergistic effects of benzyl isothiocyanate and resveratrol against Listeria monocytogenes and their application in chicken meat preservation. Food Chemistry 419: 135984. https://doi.org/10.1016/j.foodchem.2023.135984

Matłok N, Kapusta I, Piechowiak T, Zardzewiały M, Gorzelany J, Balawejder M (2021) Characterisation of some phytochemicals extracted from black elder (*Sambucus nigra* L.) flowers subjected to ozone treatment. Molecules 26(18): 5548. https://doi.org/10.3390/molecules26185548

Mojarradi F, Bimakr M, Ganjloo A (2023) Effect of bio-edible coating based on *Lallemantia iberica* seed mucilage incorporated with *Malva sylvestris* leaf bioactive compounds on oxidative stability of turkey meat. Journal of Food Measurement and Characterization 18: 402–412. https://doi.org/10.1007/s11694-023-02160-8

Nisa S, Bibi Y, Masood S, Ali A, Alam S, Sabir M, Qayyum A, Ahmed W, Alharthi S, Santali EY, Alharthy SA, Bawazir WM, Almashjary MN (2022) Isolation, Characterization and Anticancer Activity of Two Bioactive Compounds from *Arisaema flavum* (Forssk.) Schott. Molecules 27(22): 7932. https://doi.org/10.3390/molecules27227932

Normandin C, Boudreault PL (2021) Concise large-scale synthesis of tomatidine, a potent antibiotic natural product. Molecules 26(19): 6008. https://doi.org/10.3390/molecules26196008

Ntalli N, Skourti A, Nika EP, Boukouvala MC, Kavallieratos NG (2021) Five natural compounds of botanical origin as wheat protectants against adults and larvae of *Tenebrio molitor* L. and *Trogoderma granarium* Everts. Environmental Science and Pollution Research 28: 42763–42775. https://doi.org/10.1007/s11356-021-13592-4

Okuno Y, Bonala R, Attaluri S, Johnson F, Grollman AP, Sidorenko VS, Oda Y (2019) Bioactivation mechanisms of N-hydroxyaristolactams: Nitroreduction metabolites of aristolochic acids. Environmental and Molecular Mutagenesis 60(9): 792–806. https://doi.org/10.1002/em.22321

Olvera-Aguirre G, Piñeiro-Vázquez ÁT, García JRS, Zárate AS, Ochoa-Flores AA, Campos MRS., Perez EVB, Chay-Canul AJ (2023) Using plant-based compounds as preservatives for meat products: A review. Heliyon. https://doi.org/10.1016/j.heliyon.2023.e17071

Osyczka P, Chowaniec K, Skubała K (2023) Membrane lipid peroxidation in lichens determined by the TBARS assay as a suitable biomarker for the prediction of elevated level of potentially toxic trace elements in soil. Ecological Indicators 146: 109910. https://doi.org/10.1016/j.ecolind.2023.109910

Patel M, Mehta P (2021) Bamboo a supplement to human health: a comprehensive review on its ethnopharmacology, phytochemistry, and pharmacological activity. The Natural Products Journal 11(2): 140–168. https://doi.org/10.2174/2210315510666200101092551

Patil A, Jadhav V (2014) GC-MS analysis of bioactive components from methanol leaf extract of *Toddalia asiatica* (L.). International Journal of Pharmaceutical Science Review and Research 29(1): 18–20.

Prakash O, Gondwal M, Pant AK (2011) Essential oils composition and antioxidant activity of water extract from seeds and fruit pulp of *Skimmia anquetilia* NP Taylor & Airy Shaw. Journal of Essential Oil Research 24(1): 83–90. https://doi.org/10.1080/10412905.2012.646034

Rotella R, Soriano JM, Llopis-González A, Morales-Suarez-Varela M (2023) The Impact of *Moringa oleifera* Supplementation on Anemia and other Variables during Pregnancy and Breastfeeding: A Narrative Review. Nutrients 15(12): 2674. https://doi.org/10.3390/nu15122674

Ruiz-Hernández K, Sosa-Morales ME, Cerón-García, Gómez-Salazar JA (2023) Physical, chemical and sensory changes in meat and meat products induced by the addition of essential oils: A concise review. Food Reviews International 39(4): 2027–2056. https://doi.org/10.1080/87559129.2021.1939369

Shuge T, Xiaoying Z, Fan Z, Dongqing A, Tao Y (2009) Essential oil composition of the *Dracocephalum moldavica* L from Xinjiang in China. Pharmacognosy Research 1(4).

Taherpour AA, Khodaei MM, Ameen BAH, Ghaitouli, M, Mahdizadeh N, Amjadian, HR, Larijani K (2017) Chemical composition analysis of the essential oil of *Solanumn nigrum* L. by HS/SPME method and calculation of the biochemical coefficients of the components. Arabian Journal of Chemistry 10: S2372–S2375. https://doi.org/10.1016/j.arabjc.2013.08.015

Umamaheswari M, Chatterjee TK (2008) In vitro antioxidant activities of the fractions of *Coccinia grandis* L. leaf extract. African Journal of Traditional, Complementary and Alternative Medicines 5(1): 61–73. https://doi.org/10.4314/ajtcam.v5i1.31258

Verardo V, Ferioli F, Riciputi Y, Iafelice G, Marconi E, Caboni MF (2009) Evaluation of lipid oxidation in spaghetti pasta enriched with long chain n–3 polyunsaturated fatty acids under different storage conditions. Food Chemistry 114(2): 472–477. https://doi.org/10.1016/j.foodchem.2008.09.074

Wang Q, Guo Q, Niu W, Wu L, Gong W, Yan S, Nishinari K, Zhao M (2022) The pH-responsive phase separation of type-A gelatin and dextran characterized with static multiple light scattering (S-MLS). Food Hydrocolloids 127: 107503. https://doi.org/10.1016/j.foodhyd.2022.107503

Xu L, Liu S, Cheng Y, Qian H (2023) The effect of aging on beef taste, aroma and texture, and the role of microorganisms: A review. Critical Reviews in Food Science and Nutrition 63(14): 2129–2140. https://doi.org/10.1080/10408398.2021.1971156

Xu M, Liu Q, Ni X, Chen C, Deng X, Fang Y, Wang X, Shen Q, Yu R (2024a) Lipidomics reveals the effect of hot-air drying on the quality characteristics and lipid oxidation of Tai Lake whitebait (*Neosalanx taihuensis* Chen). LWT - Food Science and Technology 197: 115942. https://doi.org/10.1016/j.lwt.2024.115942

Xu M, Ni X, Liu Q, Chen C, Deng X, Wang X, Yu R (2024b) Ultra-high pressure improved gelation and digestive properties of Tai Lake whitebait myofibrillar protein. Food Chemistry 21: 101061. https://doi.org/10.1016/j.fochx.2023.101061

Yuvaraj R, Rao MRK, Prabhu K, Sundram RL, Shil S, Kumar MS, Vijayalakshmi N (2019) The gas chromatography–mass spectrometry study of one medicinal plant, *Stachytarpheta indica*. Indian Journal of Pharmaceutical Science 12(8): 382–394.

Zahara K, Bibi Y, Masood S, Nisa S, Sher A, Ali N,Kumar S, Qayyum A, Ahmed W, Sami R, Al-Mushhin AAM, Aljahani AH (2022) Isolation and identification of bioactive compounds from *Bidens* spp. using HPLC-DAD and GC-MS analysis and their biological activity as anticancer molecules. Molecules 27(6): 1927. https://doi.org/10.3390/molecules27061927

Zhang H, Fu R, Zheng Q, Song X, Wu J, Ren Y (2024) Continuous mode of color and functionality construction for cotton by bacterial pigment based on nano-suspension system. Industrial Crops and Products 214: 118510. https://doi.org/10.1016/j.indcrop.2024.118510

Zhang T, Yu S, Pan Y, Li H, Liu X, Cao J (2023) Properties of texturized protein and performance of different protein sources in the extrusion process: A review. Food Research International 174(Part 1): 113588. https://doi.org/10.1016/j.foodres.2023.113588

Zhao WY, Yan JJ, Liu TT, Gao J, Huang HL, Sun CP, Huo XK, Deng S, Zhang BJ, Ma XC (2020) Natural sesquiterpenoid oligomers: A chemical perspective. European Journal of Medicinal Chemistry 203: 112622. https://doi.org/10.1016/j.ejmech.2020.112622