Received: 14 September 2023, Accepted: 10 October 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 10, 1944-1948

Evaluation of the Effect of *Moringa oleifera* Leaf Extract and Powder on the Shelf Life and Safety of Oriental Sausage

Fatma A. Mohamed[1*], Soad A.S. Ismail[2], Hosny A. Abdelrahman[2]

[1]Central Lab., Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.

[2]Food Hygiene and Control Department, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.

***Correspondence**
Corresponding author: Fatma A. Mohamed
E-mail address: Dr_fatma_atef@yahoo.com

Abstract

This novel study was carried out to evaluate the effect of *Moringa oleifera* leaf extract and leaf powder on the shelf life and safety of fresh oriental sausage. Therefore, 30 fresh oriental sausage samples were made and divided into 3 portions (10 samples of each), one as a control, while the other two portions were prepared with 1% of *M. oleifera* leaf ethanolic extract and 2.5% *M. oleifera* leaf powder. The prepared samples were then stuffed in a natural casing collected from sheep and kept at 4°C to be analyzed for chemical parameters (pH, TVN, and TBA) and bacteriological evaluation (psychrotrophic and *Enterobacteriaceae*) at the 1st, 3rd, 6th, 9th and 12th days of storage at 4°C. The addition of different concentrations of *M. oleifera* significantly ($p < 0.05$) decreased the chemical parameters and bacterial indices. This study concluded that the addition of 2.5% *M. oleifera* leaf powder to the fresh oriental sausage has a significant impact on its chemical and bacterial quality, which leads to prolonging the shelf life of the fresh oriental sausage until the 9th day of chilling storage at 4°C.

KEYWORDS
Moringa oleifera, Bacteriological evaluation, Chemical evaluation, Oriental sausage.

INTRODUCTION

Egyptian oriental sausage is a popular meat product due to its simplicity of manufacturing and reasonable cost. It has a high nutritional value due to the presence of meat in its composition as a source of protein; it contains meat product enhancer additives and a variety of spices (Elsayed and Abdelrahman, 2021). Oriental sausage may become contaminated by various species of bacteria; their quantity is influenced by the quality of the raw material, additives, casing, and hygienic conditions during production and handling. At cold temperatures, a number of spoilage microorganisms can grow and reproduce, which could eventually result in sausage spoilage (Muschiolik, 1991). The shelf life of meat products is the recommended maximum duration for which they can be stored while maintaining the specified quality under expected or stipulated distribution, storage, and display conditions. Microbial growth, color change, and lipid oxidation are factors that affect shelf life and, as a result, customer acceptance (Gyeszly, 1991; Zhao *et al*., 1994). Due to the nutritional, medical, and industrial benefits of *Moringa oleifera* leafs "Miracle tree", it has become increasingly important in recent years due to the presence of high quantities of protein, vitamins, minerals, tocopherols, carotenoids, polyphenols, alkaloids, and flavonoids. Its leaves have recently been recognized as a natural plant supplement for extending the shelf life of food products. It has the potential to be employed as a new plant food material in the future to replace artificial additives and improve food safety. The main effects of adding *Moringa oleifera* leafs are to improve food shelf life due to their antioxidative activity, which prevents the growth of pathogenic and spoilage microorganisms (Siddhuraju and Becker, 2003; Lako *et al*., 2007; Yang *et al*., 2023). Therefore, this is a novel study that was carried out to evaluate the effect of *Moringa oleifera* leaf extract and leaf powder on the shelf life and safety of oriental sausage in the 1st, 3rd, 6th, 9th and 12th days at 4±1°C through the following items: Evaluation of the effect of different concentrations of the prepared extracts on chemical parameters (pH, TBA, and TVN) and their effect on Psychrotrophic and *Enterobacteriaceae* counts.

MATERIALS AND METHODS

Preparation of the plant extract

Fresh *Moringa oleifera* leaves were collected and washed with distilled water, then dried at room temperature. The dried leaves were ground by an electrical grinder, sieved through a fine mesh, and kept in polyethylene bags at 4°C. The fine *M. oleifera* leaf powder (MOLP) was divided into two portions; the first portion was incorporated into the oriental sausage, and the second portion was used for preparing an ethanolic extract according to the method of Vongsak *et al.* (2013).

Oriental sausage preparation for the experimental design

Oriental sausage was prepared according to the method described by Zaika *et al*. (1978) and in accordance with Egyptian standard specifications (EOS, 1972/2005), where it was formulated with 60% lean meat and 30% fat, 16–18 g of table salt, and a

ISSN: 2090-6277/2090-6269/

1% spice mixture. Meat and fat tissues were cut into small portions and ground to particles of about rice size (2x4 mm), and then the ingredients were blended to prepare the sausage mixture. A total of 30 sausage samples were made; the first portion was used as a control (n = 10), while the other portions were mixed with the prepared concentrations of 1% *M. oleifera* leaf ethanolic extract (MOLE) (n = 10) and 2.5% *M. oleifera* leaf powder (n = 10). The prepared oriental sausage samples were then stuffed in natural casing collected from sheep and kept at 4°C to be analyzed for chemical and bacteriological evaluation in the 1st, 3rd, 6th, 9th, and 12th days of storage at 4±1°C.

Chemical evaluation: All chemical analysis was carried out according to the methods recommended by AOAC (2005).

Determination of pH: It was determined by homogenizing 10 g of the prepared sample with 90 ml of distilled water at 25°C and the pH was measuring with a digital pH meter (model 220 Denver instrument) calibrated at pH buffers (4.00 - 7.00 - 10.00).

Determination of TVN: Ten grams of the prepared sample were added to a heated flask containing 300 ml distilled water plus 2 g magnesium oxide and an antifoaming agent. In the receiving flask, 25 ml of boric acid (2%), and a few drops of methyl red indicator were added. The two flasks (heating and receiving) were connected to the evaporator, and the water bath was managed. After 25 minutes, distillation was stopped. The content of the receiving flask was transferred to another flask and titrated to the end point by the very weak acid 0.1 (H_2SO_4). The percentage of the total volatile nitrogen was determined as follows: TVN (mg/100 g of prepared sample) = (volume (ml) of H_2SO_4 used for titration of the distilled - volume (ml) of H_2SO4 used in control sample)×14.

Determination of TBA: 10 g of the prepared sample was mixed with 50 ml of distillated water in a flask, and then 47.5 ml of distillated water and 2.5 ml of hydrochloric acid (4 N) were added to bring the pH to 1.5, with a few glass beads were added. The flask was heated to collect 50 ml of distillate for 10 minutes from the time boiling commences. 5 ml of distillate were collected into tubes with a stopper, and 5 ml of TBA reagent (0.2883 g of 2-Thiobarbituric acid /100 ml of 90 percent glacial acetic acid) was added to them , then tubes were closed with stopper, shaked and then heated in boiling water for 35 min. After that, the tubes were cooled in water for 10 min and the absorbance was measured against the blank containing 5 ml distilled water and 5 ml TBA reagent at wavelength 538 nm using 1 cm cells in spectrophotometer (Specord 250, Germany). TBA number= Power of light absorption x7.8 (results expressed as mg malondialdehyde per kg of prepared sample).

Bacteriological evaluation

Ten grams of oriental sausage samples homogenate with 225 buffered peptone 1% and serial decimal dilutions were prepared (APHA, 2002).

Determination of Psychrotrophic count (APHA, 2002)

One ml of each serial dilution was separately inoculated into duplicate plates of tempered standard plate count agar (44–46°C) and added to each inoculated plate, then thoroughly and uniformly mixed until solidification, and then incubated at 7°C for 10 days. The $\log_{10}$ of the total colony count /g was calculated.

Determination of Enterobacteriaceae count (ISO21528-2, 2004)

1 ml of each previously prepared serial dilution was inoculated into sterile duplicate Petri dishes. About 12 to 15 ml of sterile tempered molten violet red bile glucose agar medium (VRBG) cooled at 50°C were poured into each inoculated Petri dish, then thoroughly and uniformly mixed the inoculums and left to solidify. After complete solidification, the inoculated Petri dish was incubated aerobically at 37±1°C for 24±2 h. The suspected purplish-red colonies (sometimes surrounded by a reddish zone of precipitated bile) were considered typical colonies. The countable colonies in the selected duplicate plates of the same dilution were enumerated to obtain the total *Enterobacteriaceae* count per gram.

Statistical analysis

The statistical analysis was performed using (SPSS, 2007). The data were analyzed for significant differences between control and treated samples. Data were subjected to a one-way ANOVA followed by Duncan's multiple comparisons test. Differences in means between control and treatment were carried out by using Duncan's multiple range (DMRTs) test at the 0.05 level, whereas any significant differences ($P < 0.05$).

RESULTS

Effect of 1% MOLE and 2.5% MOLP on pH values

pH values (mean±S.E) of control oriental sausage samples in the 1st, 3rd, 6th, 9th and 12th days at 4±1°C were 5.480±0.0054, 5.671±0.0057; 6.271±0.0059, 6.571±0.0057 and 7.471±0.0057, respectively, while pH values of samples treated with 1% MOLE were 5.320±0.0059; 5.548±0.0055; 6.148±0.0051, 6.448±0.0055 and 7.348±0.0055, respectively. pH values (mean±S.E) of samples treated with 2.5% MOLP were 5.260±0.0053, 5.523±0.0053, 6.123±0.0056, 6.423±0.0053 and 7.323±0.0051, respectively (Table 1).

Table 1. Statistical analytical results of the effect of addition of *M. oleifera*** concentrations on pH values in oriental sausage samples during storage periods.

Storage Days / 4°C	*M. oleifera* Concentration	Statistical Analysis		
		Min.	Max.	Mean±S.E.
1st	Control	5.47	5.49	5.480^{a}±0.0054
	MOLE (1%)	5.31	5.33	5.320^{b}±0.0059
	MOLP (2.5%)	5.25	5.27	5.260^{c}±0.0053
3rd	Control	5.66	5.68	5.671^{a}±0.0057
	MOLE (1%)	5.54	5.56	5.548^{b}±0.0055
	MOLP (2.5%)	5.52	5.53	5.523^{c}±0.0053
6th	Control	6.26	6.28	6.271^{a}±0.0059
	MOLE (1%)	6.14	6.16	6.148^{b}±0.0051
	MOLP (2.5%)	6.12	6.13	6.123^{c}±0.0056
9th	Control	6.56	6.58	6.571^{a}±0.0057
	MOLE (1%)	6.44	6.46	6.448^{b}±0.0055
	MOLP (2.5%)	6.42	6.43	6.423 ^{c}±0.0053
12th	Control	7.46	7.48	7.471 ^{a}±0.0057
	MOLE (1%)	7.34	7.36	7.348 ^{b}±0.0055
	MOLP 2.5%)	7.32	7.33	7.323 ^{c}±0.0051

Mean values in the same column that are not followed by the same letter are significantly different ($p< 0.05$). **MOLE 1%: *Moringa oleifera* Leaf Extract. **MOLP 2.5%: *Moringa oleifera* Leaf Powder.

Effect of 1% MOLE and 2.5% MOLP on Thiobarbituric acid

TBA (mg-MDA/kg) values (mean±S.E) of control orien-

tal sausage samples in the 1st, 3rd, 6th, 9th and 12th days at 4±1oC were 0.0964±0.0048, 0.4564±0.0049, 0.8164±0.0052, 1.1764±0.0054 and 2.3546±0.0055, respectively. While TBA values (mean±S.E) of samples treated with 1% MOLE were 0.0951±0.0046, 0.3951±0.0048, 0.6951±0.0049, 0.9951±0.0051, and 1.6180±0.0046, respectively. TBA values (mean±S.E.) of treated samples with 2.5% MOLP were 0.0874±0.0041; 0.2885±0.0045; 0.4885±0.0047, 0.7885±0.0048, and 1.1023±0.0032, respectively (Table 2).

Table 2. Statistical analytical results of the effect of addition of *M. oleifera*** concentrations on TBA (mg-MDA/kg) values in oriental sausage samples during storage periods.

Storage Days / 4°C	*M. oleifera* Concentration	Statistical Analysis		
		Min.	Max.	Mean±S.E.
1st	Control	0.10	0.10	0.0964[a]±0.0048
	MOLE (1%)	0.09	0.10	0.0951 [a]±0.0046
	MOLP (2.5%)	0.08	0.09	0.0874 [b]±0.0041
3rd	Control	0.46	0.46	0.4564 [a]±0.0049
	MOLE (1%)	0.39	0.40	0.3951 [b]±0.0048
	MOLP (2.5%)	0.29	0.29	0.2885 [c]±0.0045
6th	Control	0.82	0.82	0.8164 [a]±0.0052
	MOLE (1%)	0.69	0.70	0.6951 [b]±0.0049
	MOLP (2.5%)	0.49	0.49	0.4885 [c]±0.0047
9th	Control	1.18	1.18	1.1764 [a]±0.0054
	MOLE (1%)	0.99	0.10	0.9000 [b]±0.0051
	MOLP (2.5%)	0.79	0.79	0.7885 [c]±0.0048
12th	Control	2.35	2.36	2.3546 [a]±0.0055
	MOLE (1%)	1.62	1.62	1.6180 [b]±0.0046
	MOLP (2.5%)	1.10	1.10	1.1023 [c]±0.0032

*TBA Level not more than (0.9 mg malondialdehyde/ kg) (EOS, 1972/2005)

Effect of 1% MOLE and 2.5% MOLP on Total Volatile Nitrogen

TVN (mg/100 g) values (mean±S.E) of control oriental sausage samples in the 1st, 3rd, 6th, 9th and 12th days at 4±1oC were 9.20±0.00589, 13.01±0.0059, 16.50±0.00598, 20.20±0.00595 and, 34.00±0.5912 respectively. While TVN values (mean±S.E) of samples treated with 1% MOLE were 8.60±0.00585, 11.30±0.0058, 13.40±0.00596, 15.50±0.00593 and 24.00±0.5970, respectively. TVN values (mean±S.E) of samples treated with 2.5% MOLP were 7.60±0.00581, 10.20±0.00587, 11.00±0.00591, 12.30±0.0059 and 20.00±0.5932, respectively (Table 3).

Effect of 1% MOLE and 2.5% MOLP on Total Psychrotrophic count

The mean values±SE of TPC in control oriental sausage samples in the 1st, 3rd, 6th, 9th and 12th days at 4±1oC were 3.66±0.011, 5.43±0.019, 6.08±0.042, 6.26±0.075, and 7.28±0.027 (log_{10} CFU/g) , respectively, while, these counts in samples treated with 1% MOLE were 3.32±0.024, 5.07±0.046, 5.80±0.080, 5.95±0.028, and 7.17±0.034 (log_{10} CFU/g), respectively. In addition, these counts in samples treated with 2.5% MOLP were 2.26±0.139, 3.90±0.159, 4.96±0.139, 5.65±0.090, and 6.99±0.051 (log_{10} CFU/g), respectively (Table 4).

Effect of 1% MOLE and 2.5% MOLP on Total Enterobacteriaceae count

The mean value±SE of TEC in control oriental sausage samples in the 1st, 3rd, 6th, 9th and 12th days at 4±1oC were 2.89±0.064, 3.39±0.051, 4.00±0.025, 4.56±0.139, and 5.96±0.005 (log_{10} CFU/g), respectively, while, these counts in samples treated with 1% MOLE were 2.26±0.139, 2.95±0.057, 3.39±0.206, 3.76±0.087, and 4.95±0.056 (log_{10} CFU/g), respectively. In addition, these counts in samples treated with 2.5% MOLP were 2.67±0.107, 3.14±0.036, 3.69±0.051, 4.00±0.046, and 5.36±0.0219 (log_{10} CFU/g), respectively (Table 5).

Table 3. Statistical analytical results of the effect of addition of *M. oleifera*** concentrations on *TVN (mg / 100 gm) values in oriental sausage samples during storage periods.

Storage Days / 4°C	*M. oleifera* Concentration	Statistical Analysis		
		Min.	Max.	Mean±S.E.
1st	Control	9.19	9.21	9.20[a]±0.00589
	MOLE (1%)	8.59	8.61	8.60 [b]±0.00585
	MOLP (2.5%)	7.59	7.61	7.60 [c]±0.00581
3rd	Control	12.99	13.03	13.01 [a]±0.0059
	MOLE (1%)	11.29	11.31	11.30 [b]±0.0058
	MOLP (2.5%)	10.19	10.21	10.20 [c]±0.00587
6th	Control	16.49	16.51	16.50 [a]±0.00598
	MOLE (1%)	13.39	13.41	13.40 [b]±0.00596
	MOLP (2.5%)	10.99	11.01	11.00 [c]±0.00591
9th	Control	20.19	20.21	20.20 [a]±0.00595
	MOLE (1%)	15.49	15.51	15.50 [b]±0.00593
	MOLP (2.5%)	12.29	12.31	12.30 [c]±0.0059
12th	Control	33	35	34.00 [a]±0.5912
	MOLE (1%)	23	25	24.00[b]±0.5970
	MOLP (2.5%)	19	21	20.00 [c]±0.5932

*TVN Level not more than (20 mg/100 g) (EOS, 1972/2005)

Table 4. Statistical analytical results of the effect of addition of *M. oleifera*** concentrations on log10 Psychrotrophic count in oriental sausage samples during storage periods.

Storage Days / 4°C	*M. oleifera* Concentration	Statistical Analysis		
		Min.	Max.	Mean±S.E.
1st	Control	3.64	3.68	3.66 [a]±0.011
	MOLE (1%)	3.28	3.36	3.32 [b]±0.024
	MOLP (2.5%)	2	2.48	2.26 [c]±0.139
3rd	Control	5.4	5.46	5.43 [a]±0.019
	MOLE (1%)	4.98	5.13	5.07 [b]±0.046
	MOLP (2.5%)	3.6	4.15	3.90 [c]±0.159
6th	Control	6	6.15	6.08 [a]±0.042
	MOLE (1%)	5.65	5.93	5.80 [a]±0.080
	MOLP (2.5%)	4.7	5.18	4.96 [b]±0.139
9th	Control	6.11	6.36	6.26 [a]±0.075
	MOLE (1%)	5.9	6	5.95 [b]±0.028
	MOLP (2.5%)	5.48	5.78	5.65 [c]±0.090
12th	Control	7.23	7.32	7.28 [a]±0.027
	MOLE (1%)	7.11	7.23	7.17 [a]±0.034
	MOLP (2.5%)	6.9	7.08	6.99 [b]±0.051

DISCUSSION

Sausage has high levels of moisture, nitrogenous substances, minerals, growth factors, fermentable carbohydrates (glycogen), and a favorable pH, which creates an ideal environment for the growth of food-borne diseases and common meat-spoiling bacteria (Aymerich *et al*., 2008). Meat products such as sausage may become contaminated with pathogens as a result of poor hygiene standards in meat processing operations, posing a major

Table 5. Statistical analytical results of the effect of addition of *M. oleifera*** concentrations on log10 Total *Enterobacteriaceae* count in oriental sausage samples during storage periods.

Storage Days / 4°C	*M. oleifera* concentration	Statistical Analysis		
		Min.	Max.	Mean±S.E.
1st	Control	2.78	3	2.89 ^{a}±0.064
	MOLE (1%)	2	2.48	2.26 ^{b}±0.139
	MOLP (2.5%)	2.48	2.85	2.67 ^{a}±0.107
3rd	Control	3.3	3.48	3.39 ^{a}±0.051
	MOLE (1%)	2.85	3.04	2.95 ^{c}±0.057
	MOLP (2.5%)	3.08	3.2	3.14 ^{b}±0.036
6th	Control	3.95	4.04	4.00 ^{a}±0.025
	MOLE (1%)	3	3.7	3.39 ^{b}±0.206
	MOLP (2.5%)	3.6	3.78	3.69 ^{ab}±0.051
9th	Control	4.3	4.78	4.56 ^{a}±0.139
	MOLE (1%)	3.6	3.9	3.76 ^{b}±0.087
	MOLP (2.5%)	3.95	4.11	4.00 ^{b}±0.046
12th	Control	5.95	5.97	5.96^{a}±0.005
	MOLE (1%)	4.85	5.04	4.95 ^{c}±0.056
	MOLP (2.5%)	5.32	5.4	5.36 ^{b}±0.0219

risk to human health (Yang *et al*., 2012).

Meat pH has a significant role in determining its freshness, flavor, and general quality. The pH value is utilized as an indicator of the keeping quality of products. Whereas the pH level can have a significant impact on the growth of microorganisms and the product's shelf life (Hathout and Aly, 2010).

Treated oriental sausage samples with 1% MOLE and 2.5% MOLP significantly ($p < 0.05$) decreased the pH when compared with control samples throughout the storage period these results were compatible with research studies on other meat products (Jayawardana *et al*., 2015; Elhadi *et al*., 2017; Ezzat *et al*., 2020; Nisar *et al*., 2020; Mashau *et al*., 2021).

It was noticed that the control samples spoiled on the 9th day of refrigeration storage, while the pH value of the treated samples remained within the permissible limit 5.6 - 6.4 according to ICMSF (1988) until the 9th day of chilling at 4±1°C. This may be attributable to the antibacterial components included in MOLE and MOLP having an inhibitory impact, which inhibited the growth and spread of spoilage microorganisms that utilized basic nitrogen molecules.

The TBA value is regarded as a chemical indicator of product deterioration and spoilage through the detection of lipid hydroperoxide, which is produced by the oxidation of unsaturated fatty acids (Nawar, 1996).

Treated oriental sausage samples with 1% MOLE and 2.5% MOLP significantly ($p < 0.05$) decreased the TBA when compared with control samples until the end of storage period. Nearly similar results in other meat products were attained (Jayawardana *et al*., 2015; Falowo *et al*., 2017; El-Rahman *et al*., 2019; Ezzat *et al*., 2020; Hamada *et al*., 2021; Mashau *et al*., 2021). This may be because *M. oleifera* concentrations prolong the shelf life of oriental sausage by retarding rancidity through their antioxidant activity or by acting as bacteriostatic and bactericidal agents.

Also, the treated oriental sausage samples remained within the permissible limit according to EOS (1972/2005) (0.9 mg malondialdehyde/kg) until the 9th day of chilling storage because the leaves of Moringa are great sources of natural antioxidants and safe bioactive substances like phenols. Phenols are crucial in decreasing fat oxidation, which extends the shelf life of widely consumed sausage.

Total volatile nitrogen (TVN), also known as organic amines, accumulates as a result of the breakdown of proteins and other nitrogen (N)-containing compounds as a result of spoiling mechanisms. These toxic substances alter the color and flavor of meat products significantly, which lowers their acceptability. Meat's TVB-N level rises while it is stored, and frequently, its pattern of accumulation parallels that of other biomarkers of decomposition such as microbial count and changes in sensory acceptability (Bekhit *et al*., 2021).

Treated oriental sausage samples with 1% MOLE and 2.5% MOLP significantly ($p < 0.05$) decreased the TVN when compared with control samples until the end of storage period. These results were compatible with Ezzat *et al*. (2020); Nisar *et al*. (2020) and Hamada *et al*. (2021).

The treated oriental sausage samples remained within the permissible limit according to EOS (1972/2005) (20mg/100g) until the 9th day of chilling storage because *M. oleifera* is an ideal candidate for preventing proteolysis through its protease inhibitor.

The treated oriental sausage samples with 1% MOLE and 2.5% MOLP significantly ($p < 0.05$) decreased the Total Psychrotrophic count (log_{10} CFU/g) when compared with control samples until the end of storage period. These results were nearly in other meat products similar to Jayawardana *et al*. (2015); Elhadi *et al*. (2017); Mashau *et al*. (2021) and Abdallah *et al*. (2023). It was obvious from the results that the Psychrotrophic count of control samples was higher than the corresponding counts of treated samples; this may be caused by the antibacterial effect of Moringa leaves due to the presence of a short peptide 4 ('-L-rhamnosyloxy) benzyl-isothiocyanate in moringa leafs that impedes microorganism growth by disrupting cell membrane synthesis or key enzymes.

Treated oriental sausage samples with 1% MOLE and 2.5% MOLP significantly ($p < 0.05$) decreased the *Enterobacteriaceae* count (log_{10} CFU/g) when compared with control samples at the end of the storage period. Nearly similar results in other meat products were attained by El-Moez *et al*. (2014) and Abdallah *et al*. (2023). It appeared from previous results that Total *Enterobacteriaceae* counts in control samples were higher than those in treated samples with 1% MOLE and 2.5% MOLP. This result could be explained by the high content of phenolic compounds in Moringa leafs, which lead to breakdown of the cell wall, rupturing of the cytoplasmic membrane, leakage of cellular components, alteration of fatty acid and phospholipid contents, interference with the formation of nucleic acids, and destruction of protein translocation, thus inhibiting *Enterobacteriaceae* growth.

CONCLUSION

This study concluded that the addition of 2.5% *M. oleifera* leaf powder to fresh oriental sausage has a significant impact on its chemical and bacterial quality. They demonstrate strong antimicrobial activity, which significantly ($p < 0.05$) lowers the numbers of the tested Psychrotrophic and *Enterobacteriaceae* count and improves the chemical quality which leads to prolonging the shelf-life of the fresh raw oriental sausage until the 9th day of chilling storage.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this study.

REFERENCES

Abdallah, R., Mostafa, N.Y., Kirrella, G.A., 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, 1-13.

AOAC, 2005. Official Methods of Analysis, 18th edn. Association of Official Analytical Chemists, Washington, D.C.

APHA, 2002. Compendium of methods for the microbiological examination of foods. Washington, DC: American Public Health Association.

Aymerich, T., Picouet, P.A., Monfort, J.M., 2008. Decontamination technologies for meat products. Meat Science 78, 114-129.

Bekhit, A.E.-D.A., Holman, B.W., Giteru, S.G., Hopkins, D.L., 2021. Total

volatile basic nitrogen (TVB-N) and its role in meat spoilage: A review. Trends in Food Science Technology. 109, 280-302.

El-Moez, S., El-Badawi, A., Omer, H., 2014. Assessment of antimicrobial effect of moringa: in vitro and in vivo evaluation. African Journal of Microbiology Research. 8, 3630-3638.

El-Rahman, A., Eman, S., Abdulla, G., El-Araby, G.M., El-Nemr, S., El-Shourbagy, G.A., 2019. Effect of Moringa leaves (*Moringa oleifera* Lam.) extract addition on luncheon meat quality. Zagazig Journal of Agricultural Research 46, 2307-2316.

Elhadi, D.A., Elgasim, E.A., Mohamed Ahmed, I.A., 2017. Microbial and oxidation characteristics of refrigerated chicken patty incorporated with moringa (*Moringa oleifera*) Leaf powder. CyTA-Journal of Food 15, 234-240.

Elsayed, E., Abdelrahman, H., 2021. Studies on the Nutritive Value and Chemical Quality of Different Commercial Grades of Oriental Sausage. Suez Canal Veterinary Medical Journal. SCVMJ. 26, 143-153.

EOS, 2005. Egyptian Organization for Standardization and quality control. Frozen Sausage. Arab republic of Egypt, ICS: 67.120.10.

Ezzat, G.A., Mohamed, M.A., Emara, M.M., Yassien, N.A., Mansour, N.K.M., 2020. Effect of *Moringa oleifera* Aqueous Extracts on the Physicochemical Characteristics, Microbiological quality and Biogenic Amines of Semi-dry Fermented Sausage. International Journal of Veterinary Science 9, 285-290.

Falowo, A., Muchenje, V., Hugo, A., Aiyegoro, O., Fayemi, P., 2017. Antioxidant activities of *Moringa oleifera* L. and *Bidens pilosa* L. Leaf extracts and their effects on oxidative stability of ground raw beef during refrigeration storage. CyTA-Journal of Food 15, 249-256.

Gyeszly, S.W., 1991. Total system approach to predict shelf life of packaged food products. The 1st edition, ASTM International.

Hamada, M., Eldaim, M.A., Fathalla, S.I., Abalkhail, A., Behiry, A.E., Alkafafy, M., 2021. The Potential Impact of *Moringa oleifera* for Diminishing the Microbial Contamination and Prolonging the Quality and Shelf-Life of Chilled Meat. Journal of Pure and Applied Microbiology 15, 826-838.

Hathout, A.S., Aly, S.E., 2010. Role of lactic acid bacteria as a biopreservative agent of Talbina. Journal of American Science 6, 889-898.

ICMSF (International Commission on Microbiological Safety of Foods), 2002. Microbiological Testing in Food Safety Management. New York: Kluwer Academic/ Plenum Publishers. Vol. 7.

ISO (International Organization for Standardization 21528-2), 2004. Microbiology of Food and Animal Feeding stuffs - Horizontal Methods for the Detection and Enumeration of *Enterobacteriaceae* - Part 2: Colony Count Method. London: BSI.

Jayawardana, B.C., Liyanage, R., Lalantha, N., Iddamalgoda, S., Weththasinghe, P., 2015. Antioxidant and antimicrobial activity of drumstick (*Moringa oleifera*) leaves in herbal chicken sausages. LWT-Food Science and Technology 64, 1204-1208.

Lako, J., Trenerry, V.C., Wahlqvist, M., Wattanapenpaiboon, N., Sotheeswaran, S., Premier, R., 2007. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. Food Chemistry 101, 1727-1741.

Mashau, M.E., Ramatsetse, K.E., Ramashia, S.E., 2021. Effects of Adding *Moringa oleifera* Leavs Powder on the Nutritional Properties, Lipid Oxidation and Microbial Growth in Ground Beef during Cold Storage. Applied Sciences 11, 1-16.

Muschiolik, G., 1991. Food Freezing: Today and Tomorrow. Herausgegeben von W. B. Bald. 203 Seiten, 54 Abb. Springer-Verlag, London, Berlin, Heidelberg, Preis: 148,-DM. Food / Nahrung. 35, 1040-1040.

Nawar, W., 1996. Lipids. In: Fennema, OR (Ed.) Food Chemistry. Marcel Dekker, Inc., New York, USA.

Nisar, M.F., Arshad, M.S., Yasin, M., Khan, M.K., Afzaal, M., Sattar, S., Suleria, H.A.R., 2020. Evaluation of gamma irradiation and moringa Leaf powder on quality characteristics of meat balls under different packaging materials. Journal of Food Processing and Preservation 44, 1-11.

Siddhuraju, P., Becker, K., 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. Journal of Agricultural and Food Chemistry 51, 2144-2155.

SPSS, 2007. Statistical Package for Social Science Ver. 16 for Windows. SPSS.

Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y., Gritsanapan, W., 2013. Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* Leaf extract by the appropriate extraction method. Industrial Crops and Products 44, 566-571.

Yang, L., Liu, Y., Wu, H., Song, Z., Høiby, N., Molin, S., Givskov, M., 2012. Combating biofilms. FEMS Immunology and Medical Microbiology 65, 146-157.

Yang, M., Tao, L., Kang, X.-R., Wang, Z.-L., Su, L.-Y., Li, L.-F., Gu, F., Zhao, C.-C., Sheng, J., Tian, Y., 2023. *Moringa oleifera* Lam. Leaves as new raw food material: A review of its nutritional composition, functional properties, and comprehensive application. Trends in Food Science and Technology 138, 399-416.

Zaika, L.L., Zell, T.E., Palumbo, S., Smith, J., 1978. Effect of spices and salt on fermentation of Lebanon bologna-type sausage. Journal of Food Science 43, 186-189.

Zhao, Y., Wells, J.H., Mcmillin, K.W., 1994. Applications of dynamic modified atmosphere packaging systems for fresh red meats: Review 3. Journal of Muscle Foods 5, 299-328.