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Evaluation of the Quality of Fish Burger Formulated with *Moringa oleifera* Leaves During Frozen Storage

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ABSTRACT
This research investigated the stability of the Nile perch fish burgers preserved at -18°C for 28 days after treatment with *Moringa oleifera* leaves (MOL) at concentrations of 1 and 2%. Samples were evaluated through a sensory assessment and analyzed physiochemically and microbiologically. The treated groups had significantly lower mean total bacterial, psychrophilic, yeast, and mold counts during storage compared to the control group ($P < 0.05$). Moreover, no statistically significant difference appeared in the proximal analysis between the treated groups. There were no significant differences in pH values between the groups. However, compared to the group that received MOL, the control group showed a significant increase in both the cooking loss and water-holding capacity of the fish burgers. The control group on day 0 and day 28 had mean peroxide values (nmol/g) of 0.67 ± 0.03 and 2.38 ± 0.02, while the group treated with MOL 1% had mean values of 0.64 ± 0.02 and 1.13 ± 0.02, and the group treated with MOL 2% had mean values of 0.64 ± 0.02 and 1.02 ± 0.02, respectively. Additionally, at the end of the preservation time, the values were 0.92 ± 0.03 and 12.27 ± 0.35. The values for the group treated with MOL 1% were 0.76 ± 0.07 and 8.35 ± 0.02, and for the group treated with MOL 2%, they were 0.71 ± 0.03 and 7.66 ± 0.05, respectively. In addition, the group that was exposed to MOL 1% demonstrated a remarkable overall acceptance throughout storage. In conclusion, if used at lower concentrations, MOL's antibacterial activity can preserve food without negatively affecting its sensory qualities.

INTRODUCTION

Fish and their products provide easily digested high-quality proteins, health-promoting polyunsaturated fatty acids, and other essential components for human nutrition including minerals and vitamins (**Maulu *et al.,* 2021**). The development of

novel food products is crucial for two reasons: firstly, to expand the assortment of available items and maintain customer interest, and secondly, to utilize improved manufacturing techniques or novel raw material varieties in response to evolving consumer preferences and dietary patterns **(El-Lahamy *et al.,* 2019)**. More focus is being placed on enhancing the product value through specialized processing as a result of the growth and increased production of fish farming; therefore, countless novel seafood items are thus coming onto the market **(Nunoo *et al.,* 2019)**. A straightforward and economical way to raise consumer acceptance of fish and to develop diversified fishery products is to convert fish flesh into value-added products such as burgers **(Lithi *et al.,* 2020)**.

Fish burgers exemplify acceptable fast food options that are gaining prominence and have undergone significant development in the global food market; their quality has been the subject of numerous studies **(Talab *et al.,* 2023)**. Fish burgers are a popular ready-to-eat food among consumers due to their ease of preparation and high nutritional content and have been prepared from a variety of fish species by researchers around the world, including mackerel **(Ucak*et al.,* 2011)**, tuna **(Angiolillo *et al.,* 2017)**, African catfish **(Daengprok *et al.,* 2021, Talab*et al.,* 2023)**, the Nile tilapia (**Mahmoud, 2021**), and striped catfish and salmon mince **(Ditudompo *et al.,* 2022)**.

Preserving methods frequently employed involve the application of synthetic compounds and chemical constituents. Despite the established efficacy of these treatments, apprehensions have been raised regarding potential adverse health effects and environmental repercussions. Within the parameters of this specific methodology, the examination of natural preservatives like *Moringa oleifera* leaves (MOL) presents a convincing substitute that successfully tackles the junction of food safety, nutritional value, and environmental sustainability **(Mwankunda *et al.,* 2023)**. MOL is one of the plants that contain active antibacterial chemicals that can be utilized to prevent fish quality deterioration and can operate as a natural preservative **(Wahyuni *et al.,* 2018)**. MOL is widely recognized as a significant natural source of antimicrobial and antioxidant properties which contains antimicrobial compounds that damage the cell membranes of bacteria **(Rialita *et al.,* 2015)**, such as saponins, steroids, triterpenoids, flavonoids, tannins, and alkaloids **(Putri *et al.,* 2023)**. Additionally, the lipid oxidation process in fish decay can be impeded by the essential oils and flavonoids found in MOL **(Putri *et al.,* 2023)**. The current research involved the preparation of fish burgers using the Nile perch fish (*Lates niloticus*) in combination with MOL 1 and 2% powder. The microbiological, physicochemical, and organoleptic aspects of the prepared fish burger were studied while frozen for 28 days.

MATERIALS AND METHODS

Samples collection

The fresh Nile perch specimens were acquired from Aswan fish markets in Egypt. A total of 10kg of fish were gathered, with an average length of 20–26cm and a weight of 320g per fish. Fish were stored on ice and immediately brought to the Food Hygiene

Laboratory, Faculty of Veterinary Medicine, Aswan University, where they were thoroughly cleaned with tap water, beheaded, gutted, and filleted before being rewashed and drained. Fresh MOL was obtained from the Toshka station of the Desert Research Center in Aswan, Egypt.

Moringa oleifera powder preparation

After thoroughly washing and drying the MOL at room temperature for 24h, the leaves were pulverized into a powder using a Moulinex food blender, filtered through an aluminum sieve with a 2mm aperture, and sealed in glass bottles with secure lids and labels (**Adeyemi *et al.,* 2013**).

Fish burger preparation

The received fish were cleaned with fresh water after being beheaded and eviscerated. The fillets and skins were then removed using a pointed knife, and the fish was deboned and minced in a cold environment using a meat mixer at the Food Science and Technology Laboratory, Faculty of Agriculture and Natural Resources, Aswan University. **Lithi *et al.* (2020)** described that the Nile perch fish mince (65-75%) was combined with the following ingredients: maize flour (4.40%), egg (6.0%), salt (3.00%), sugar (1%), green chili (4.80%), black pepper (0.90%), onion (8.40%), ginger (3.45%), garlic (3.85%), and tasting salt (0.45%). With some adjustments, *M. oleifera* leaves were incorporated into the fish burger recipe at proportions of 0% (C), 1% (T1), and 2% (T2) by weight. Using a kitchen blender, the ingredients were well combined, weighed (75–80g per piece), and then formed and shaped using a standard burger press (8.5cm in diameter and 1cm in thickness). Following a sensory assessment of the product, all inspected groups were sealed in polyethylene bags, kept at -18°C for 28 days, and tested for physiochemical and microbiological deteriorative criteria on days 0, 7, 15, 21, and 28.

Sensory evaluation of fish burger

Using a five-point hedonic scale to rate the sensory qualities of taste, color, texture, smell, and overall approval, with 5 representingexcellent, 4 very good, 3 good, 2 fair, and 1 bad, twenty-one panelists, scientists, and postgraduate students of the Food Hygiene Department, Faculty of Veterinary Medicine, Aswan University, evaluated the sensory parameters. A fish burger sample was randomly removed from freezer storage, and kept at 4ºC to thaw for each sensory study. Following that, a burger patty fried in sunflower oil was shown to each panelist to identify each characteristic (**Elbarbary *et al.,* 2024**).

Microbiological analysis

10g of fish flesh were mixed for 2min in a sterile mortar with 90ml of sterile peptone water 0.1% to create a serial dilution of the samples. Plate count agar (HiMedia, M091) was incubated at $37\pm1°C$ for 48h for total plate count and $4\pm1°C$ for psychrophilic count (**APHA, 2001**), while potato dextrose agar (HiMedia, M096) was incubated at

25ºC for 5-7 days for yeast and mold count **(ISO 21527-2, 2008)**. All studies were carried out in triplicate on days 0, 7, 15, and 21, and the counts were represented as log cfu/g**(Elbarbary *et al.,* 2023)**.

Physical analysis

1. pH value

In a blender, 10g of the prepared fish burger and 20ml of distilled water were blended for one min. pH meter (HANNA HI9125 instruments, Romania) with a glass electrode was used to determine the pH. All measurements were taken in triplicate **(Khairy *et al.,* 2023)**.

2. Cooking loss

The initial and final weights of each sample were calculated by modifying the technique designated by **Khairy *et al.* (2023)** before and following heating. Using aluminum foil, every sample was cooked at 150°C for 30min. The burgers were allowed to cool for thirty minutes after cooking. The following formula was used for each sample to determine the percentage of cooking loss:

$$\text{Cooking loss \%} = \frac{Wa - Wb}{Wa} \times 100$$

W_a= raw sample weight; W_b= cooked sample weight

3. Water holding capacity (WHC)

A portion of skinless fish back meat approximately 1 x 1cm was taken, and the weight was recorded as W_1. The sample was placed in a centrifuge tube and wrapped with a double-layer filter paper before centrifugation at 3000rpm for 10 minutes at 4°C. The fillets were removed, weighed, and recorded as W_2 at the end of centrifugation **(Hafezparast-Moadab*et al.,* 2018)**. The following computations were employed to determine the water-holding capacity:

$$\text{Water holding \%} = \frac{W1 - W2}{W1} \times 100$$

Chemical analysis

1. Proximate composition

The official standard method of **AOAC (2016)** was used, employing standard proximate analysis to calculate the moisture, protein, fat, and ash contents.

2. Thiobarbituric acid (TBA) value determination

A standard solution of 0.37% TBA, 15%TCA,and 0.25N HCl was gradually heated in a water bath to 75°C. A volume of 2ml of the stock solution was mixed with 1ml of the homogenized sample, and the combination was cooked for 15min in a boiling water bath to produce a pink color. The absorbance of the supernatant was calculated at 532nm with

a spectrophotometer (model UNICO UV-2100) after chilling with tap water and centrifuging at 2000rpm for 15min. TBA assessment was given as mg malondialdehyde/ kg **(AOAC, 2016)**.

3. Peroxide value (PV) determination

3g of each sample was heated for 3min at 60°C in a water bath before being rapidly agitated with 30ml of acetic acid-chloroform solution (3:2, v/v) for 3min and 1ml of potassium iodide solution. After 5min in the dark, the mixture was titrated with a standard sodium thiosulfate solution (25g/L) **(AOCS, 1997)**. By the following equation, the PV was determined as meq/ kg: PV (meq kg) = (S×N) × 100 / W (kg); W: is the sample weight (kg); N: normality of sodium thiosulfate solution (N = 0.01), and S: titration volume (ml).

4. Total volatile basic nitrogen (TVB-N) determination

10g of fish burgers were mixed with magnesium oxide and distilled water in a Kjeldal flask before being attached to the Kjeldal system. The transferred fluid was collected in an Erlenmeyer flask holding boric acid (2%) and methyl-red indicator, using 10 N H_2SO_4till the color changed from green to light red **(AOAC, 1995)**. TVB-N was calculated as follows:

$$TVB–N \ (mg \ N/100 \ g) = 1.4 × used \ H_2SO_4 × 100 × amount \ of \ sample/1000mg$$

Statistical analysis

The data were subjected to a mixed examination of difference (ANOVA), with burger processing being regarded as a random effect and treatments as a fixed effect. Tukey's test was employed to compare pairs of data. Each analysis was conducted with a significance level of $P< 0.05$.

RESULTS

Table (1) reveals the mean value of microbial load (log cfu/g) of fish burger formulated with MOL during frozen storage. The total plate count exhibited a significant variation over the storage period between all groups, in which the initial count for the control group (C) and the treated groups with MOL 1% (T1) and 2% (T2) was 3.43±0.88, 3.40±0.54, and 3.40±0.66 log cfu/ g; while at the end of storage period, it was 4.13±1.27, 3.01±0.72 and 2.86±0.52 log cfu/ g, respectively. Furthermore, a significant variation ($P< 0.05$) was recorded in the psychrophilic count between the control group and treated ones, while no significant variation was observed between the treated groups. The count on day 0 and day 28 of storage for C was 3.21±0.73 and 4.10±1.48 log cfu/ g, while for T1 and T2, values were 3.11±0.26 and 3.13±0.61 log cfu/g on day 0, but on day 28 of storage values were 2.53±0.38 and 2.51±0.48 log cfu/g, respectively. Concerning yeast and mold count, a significant variation ($P< 0.05$) was detected between all examined groups. The

count at the end of the storage period was 2.97±1.08, 1.81±0.59, and 1.72±0.28 log cfu/g for C, T1, and T2. In addition, the mean value of proximate composition (%) of fish burgers formulated with MOL over 28 days of frozen storage is shown in Table (2). Furthermore, a significant variation (*P*< 0.05) was recorded between the control group and the treated ones, while no significant variation was observed between the treated groups.

Table 1. Mean value of microbial load (log cfu/g) of fish burger formulated with MOL during frozen storage

Microbial count	Group	Storage period (day)				
		0	**7**	**15**	**21**	**28**
Total plate	C	3.43 ± 0.88^a	3.62 ± 0.71^a	3.64 ± 0.76^a	3.83 ± 0.68^a	4.13 ± 1.27^a
	T1	3.40 ± 0.54^b	3.33 ± 0.82^b	3.23 ± 0.46^b	3.13 ± 0.83^b	3.01 ± 0.72^b
	T2	3.40 ± 0.66^b	3.31 ± 0.34^b	3.02 ± 0.63^c	2.94 ± 0.77^c	2.86 ± 0.52^c
Psychrophilic	C	3.21 ± 0.73^a	3.54 ± 0.55^a	3.81 ± 0.18^a	3.84 ± 0.43^a	4.10 ± 1.48^a
	T1	3.11 ± 0.26^b	2.95 ± 0.17^b	2.83 ± 0.27^b	2.65 ± 0.18^b	2.53 ± 0.38^b
	T2	3.13 ± 0.61^b	2.91 ± 0.36^b	2.81 ± 0.65^b	2.62 ± 0.33^b	2.51 ± 0.48^b
Yeast and mold	C	2.31 ± 0.33^a	2.55 ± 0.59^a	2.81 ± 0.47^a	2.91 ± 0.18^a	2.97 ± 1.08^a
	T1	2.25 ± 0.42^b	2.16 ± 0.25^b	2.01 ± 0.17^b	1.91 ± 0.81^b	1.81 ± 0.59^b
	T2	2.23 ± 0.32^b	2.21 ± 0.33^c	1.97 ± 0.14^b	1.81 ± 0.67^c	1.72 ± 0.28^c

C: Control samples, T1: Fish burger treated with MOL1%, T2: Samples treated with MOL 2%. Means in the same column with varying superscripts are significantly different at *P*<0.05.

Fig. (1) illustrates that the pH of the examined fish burger formulated with different percentages of MOL was not significantly different (*P*> 0.05) during 28 days of frozen storage. The pH of C was 5.97±0.05, 6.27±0.03, 6.61±0.04, 6.83±0.03,   and   7.33±0.02. Moreover, for T1 it was 5.87±0.04, 5.89±0.02, 6.11±0.02, 6.19±0.03, and 6.25±0.05, and for T2 was 5.88±0.03, 5.87±0.03, 6.03±0.04, 6.11±0.04, and 6.16±0.03. Furthermore, cooking loss (%) presented in Fig. (2) revealed values of 7.67±1.63, 8.32±1.17, 10.48±1.55, 13.18±1.14, and 17.42±1.22 for C, 6.74±1.46, 7.17±1.51, 7.27±1.56, 8.41±1.21, and 11.77±1.65 for T1, and 6.22±1.33, 7.87±1.64, 7.85±1.19, 8.78±1.43, and 10.56±1.73 for T2, with a significant variation (*P*< 0.05) between all studied groups. Meanwhile, Fig.(3) shows that the water-holding capacity (%) of the inspected groups was significantly different (*P*< 0.05), with mean values of 18.82±2.54, 20.57±1.37, 24.38±1.83, 27.48±1.53, and 29.54±1.23 for C; 12.78±2.54, 13.62±1.15, 15.19±1.33, 17.67±1.22, and 18.38±1.12 for T1; and 11.46±2.54, 12.59±1.86, 14.96±1.87, 16.68±1.18, and 18.85±1.15 for T2.

On the other hand, the TBA value displayed in Fig.(4) reveals no significant variation between the inspected groups on day 0, while a significant variation was recorded between the control and treated groups after that. The mean values of TBA

values (mg MDA/kg) for C, T1, and T2 were 0.61±0.21, 0.57±0.54, and 0.57±0.28 on day 0; 0.67±0.42, 0.61±0.02, and 0.59±0.04 on day 7; 0.71±0.51, 0.64±0.08, and 0.61±0.02 on day 15; 0.79±0.08, 0.71±0.03, and 0.69±0.02 on day 21;and 0.92±0.03, 0.76±0.07, and 0.71±0.03 on day 28, respectively.

Table 2. Mean value (±SD) of proximate composition (%) of fish burger formulated with MOL during frozen storage

Analysis	Group	Storage period (day)				
		0	**7**	**15**	**21**	**28**
Moisture	C	71.36±1.24[a]	70.82±1.64[a]	69.52±1.81[a]	68.31±1.34[a]	67.22±1.52[a]
	T1	68.22±1.22[b]	66.37±1.52[b]	65.83±1.37[b]	64.21±1.62[b]	64.07±1.23[b]
	T2	67.88±1.55[b]	65.38±1.22[b]	65.33±1.35[b]	63.76±1.42[b]	63.22±1.05[b]
Protein	C	16.75±1.24[a]	16.52±1.27[b]	16.08±1.75[b]	15.68±2.14[b]	13.48±2.02[b]
	T1	16.89±1.52[a]	16.87±1.51[a]	16.37±1.36[a]	16.31±1.71[a]	16.27±1.62[a]
	T2	16.93±1.73[a]	16.87±1.4[a]	16.75±1.09[a]	16.68±1.63[a]	16.54±1.03[a]
Fat	C	3.28±0.67[a]	3.67±0.37[a]	3.93±0.83[a]	4.18±0.53[a]	4.54±0.23[a]
	T1	3.18±0.27[a]	3.32±0.15[a]	3.43±0.33[b]	3.58±0.22[b]	3.78±0.12[b]
	T2	3.22±0.43[a]	3.31±0.86[a]	3.38±0.87[b]	3.46±0.18[b]	3.57±0.15[b]
Ash	C	2.67±0.22[a]	2.84±0.13[a]	3.19±0.15[a]	3.37±0.37[a]	3.47±0.45[a]
	T1	2.63±0.34[a]	2.66±0.01[b]	2.68±0.10[b]	2.72±0.04[b]	2.75±0.04[b]
	T2	2.57±0.62[a]	2.62±0.01[b]	2.62±0.06[b]	2.65±0.08[b]	2.66±0.04[b]

C: Control samples, T1: Fish burger treated with MOL1%, T2: Samples treated with MOL 2%. Means in the same column with varying superscripts are significantly different at *P*<0.05.
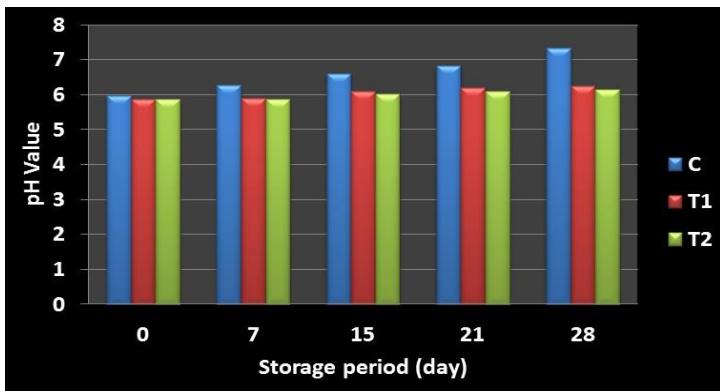

Fig. 1. pH value of the fish burger formulated with MOL during frozen storage. C: Control samples, T1: Fish burger treated with MOL 1%, T2: Samples treated with MOL 2%
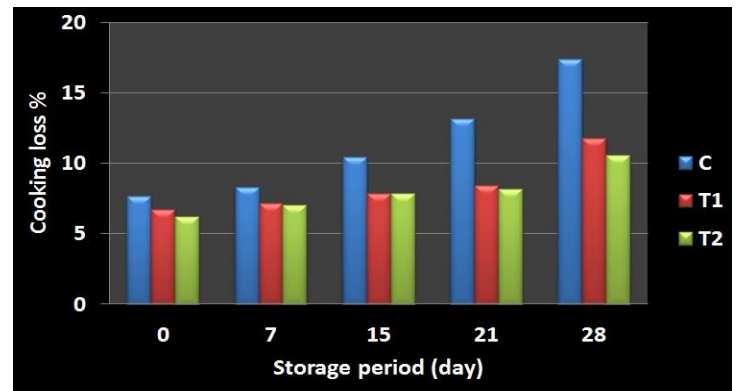

Fig. 2. Cooking loss of the fish burger formulated with MOL during frozen storage. C: Control samples, T1: Fish burger treated with MOL 1%, T2: Samples treated with MOL 2%
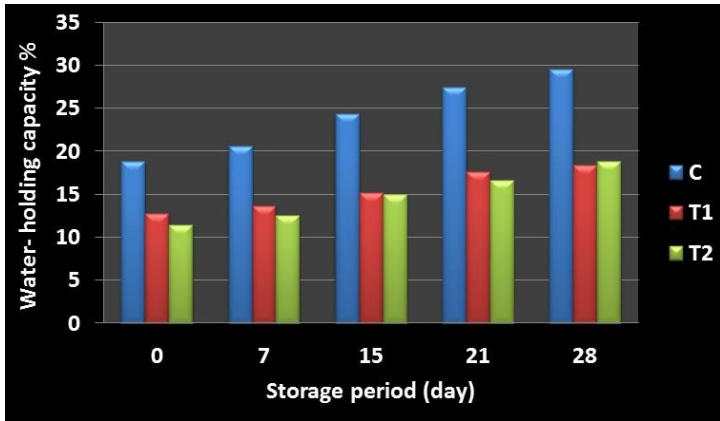
Fig. 3. Water-holding capacity of the fish burger formulated with MOL during frozen storage. C: Control samples, T1: Fish burger treated with MOL 1%, T2: Samples treated with MOL 2%
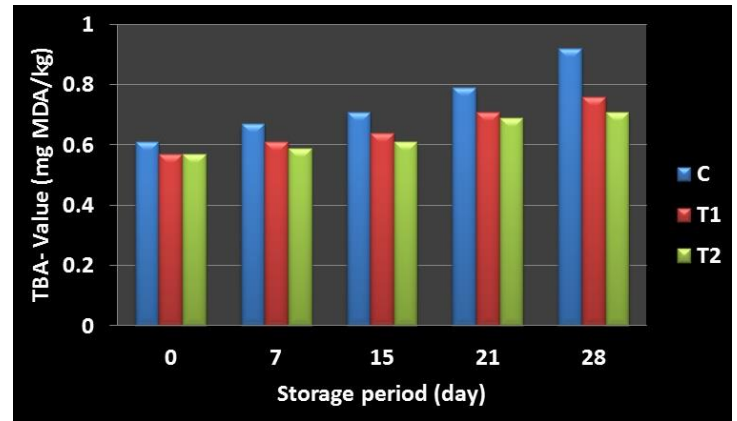

Fig. 4. TBA value (mg MDA/kg) of fish burger formulated with MOL during frozen storage. C: Control samples, T1: Fish burger treated with MOL 1%, T2: Samples treated with MOL 2%

Fig. 5. Peroxide value (nmol/g) of fish burger formulated with MOL during frozen storage. C: Control samples, T1: Fish burger treated with MOL 1%, T2: Samples treated with MOL 2%

Fig. 6. TVB–N (mg/100g) of fish burger formulated with MOL during frozen storage. C: Control samples, T1: Fish burger treated with MOL 1%, T2: Samples treated with MOL 2%

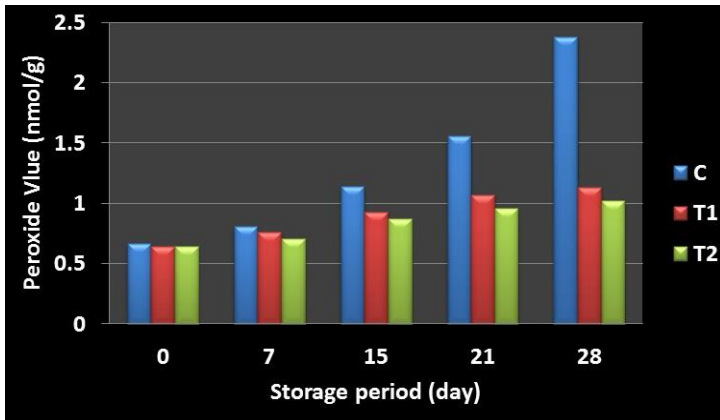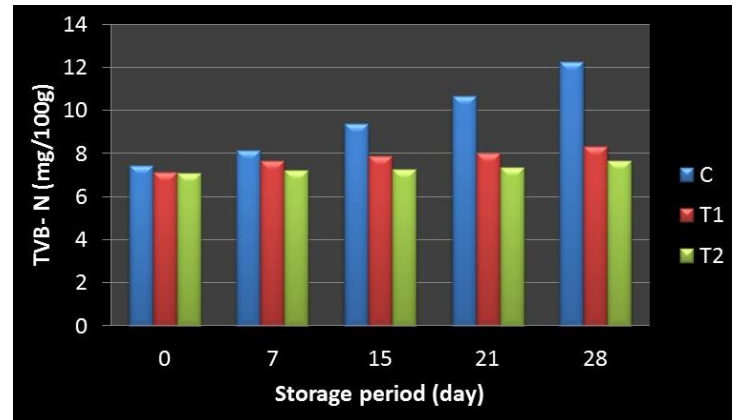Concerning the peroxide value (nmol/g) of the inspected samples demonstrated in Fig.(5) over the 28- day storage, the C group recorded values of 0.67 ± 0.03, 0.81 ± 0.03, 1.14 ± 0.02, 1.56 ± 0.02, and 2.38 ± 0.02. while T1and T2 displayed vaues of 0.64 ± 0.02 for each on day 0; 0.76 ± 0.02 and 0.71 ± 0.02 on day 7; 0.93 ± 0.02 and 0.87 ± 0.02 on day 15; 1.07 ± 0.02 and 0.96 ± 0.02 on day 21; and 1.13 ± 0.02 and 1.02 ± 0.02 on day 28, and no significant variation was detected between the inspected groups on day 0 and day 7; however, the significant variation was observed on days 15, 21, and 28. Furthermore, a significant variation for TVB–N (Fig. 6) was recorded between the inspected groups on days 7, 15, 21, and 28, while there was no significant variation between the control and treated groups on day 0. The TVB–N value (mg/100g) was recorded with values 7.43 ± 0.57, 8.14 ± 0.12, 9.39 ± 0.15, 10.67 ± 0.07, and 12.27 ± 0.35 for group C; 7.13 ± 0.84,

7.66±0.03, 7.87±0.10, 8.02±0.04, and 8.35±0.02 for T1 group; 7.09±0.22, 7.22±0.03, 7.29±0.05, 7.35±0.03, and 7.66±0.05 for T2 group, respectively.

Table (3) shows a significant variation ($P< 0.05$) in the organoleptic properties of fish burgers formulated with different percentages of MOL during the frozen storage. T1 exhibited an excellent general acceptance over the storage period, while T2 and C groups revealed a good general acceptance.

Table 3. Organoleptic properties of fish burger formulated with MOL during frozen storage

Group	Organoleptic qualities				
	Taste	Color	Texture	Smell	General acceptance
C	3[b]	3[b]	4[a]	3[b]	3[b] (Good)
T1	5[a]	4[a]	4[a]	5[a]	5[a] (Excellent)
T2	3[b]	4[a]	4[a]	3[b]	3[b] (Good)

Different superscript letters indicate that values within the same column are significantly different ($P<0.05$). C: Control samples, T1: Fish burger treated with MOL1%, T2: Samples treated with MOL 2%.

DISCUSSION

Fish-based foods are crucial to human nutrition; **Mwankunda *et al.* (2023)** identified the enhancement of shelf-life and food safety as the primary concerns of the contemporary food processing industry. The existing fish treatment technology necessitates the development of novel fish mince-based products that are stable, palatable, and nutritious **(Mwankunda *et al.,* 2023)**. Fish burgers are prevalent and well-received fast foods that have achieved a global distribution **(Parvizi & Moosavi-Nasab, 2021)**, thus numerous researchers have explored the potential of plant extracts in fish products to function as natural antioxidants **(Hentati *et al.,* 2019; Abdel-Latif *et al.,* 2021; Abou-Taleb, 2022; Mwankunda *et al.,* 2023)**.

The quality and microbiological safety of seafood and fish products are of the utmost importance to consumers, retailers, and processors. Fish are inherently vulnerable to contamination by pathogens and additional microorganisms **(Mwankunda *et al.,* 2023)**. The present investigation revealed a progressive rise in the overall bacterial, psychrophilic, yeast, and mold populations of the control Nile perch burger. At the end of the preserved time, these counts reached 4.13±1.27, 4.10±1.48, and 2.97±1.08 log cfu/g, respectively. Conversely, the data obtained indicate that the microbial count of the alternative Nile perch burger prepared with 1and 2% MOL decreased gradually throughout the storage period. A negative correlation exists between the concentration of MOL and the rate of microorganism proliferation in the samples reported throughout the

storage and analysis phases. MOL exhibited an efficacy against a diverse array of bacteria and fungi that cause food deterioration **(Ayirezang *et al.,* 2023)**.

Agreeing with **Ayirezang *et al.* (2023)**, extracts derived from MOL can be utilized as an antimicrobial component in food to impede the development of bacteria, given their acceptable safety margins. The impact of MOL on the microbial burden can be ascribed to the leaf's abundance of phytochemical compounds, including polyphenols (Favonoids, tannins, and phenolic acids) and phenolic acids, tannins, and glucosinolates **(Ma *et al.,* 2020)**. Polyphenols are recognized to have a broad-spectrum antibacterial action contrary to microorganisms by disrupting the bacterial cell membrane **(Manso*et al.,* 2021)**. Consistent findings have been documented by **Hentati *et al.* (2019)**, **Abdel-Latif *et al.* (2021)**, **Abou-Taleb (2022)**, **Ayirezang *et al.* (2023)** and **Mwankunda *et al.* (2023)**. Food-derived polyphenols are natural preservatives that have fewer adverse effects than synthetic preservatives **(Rahman *et al.,* 2020)**. In addition to this, MOL may potentially be utilized in the pre-freezing treatment of fish products, potentially inhibiting the growth of psychrophilic and psychrotrophic microbes throughout the freezing and thawing processes before consumption **(Manso *et al.,* 2021)**.

Regarding the quality and preservation of fish burgers, the pH level is critical. Initially, the inclusion of MOL had no noticeable effect on the pH of the fish burgers. Nevertheless, over time, the pH of each sample exhibits a marginal rise, suggesting a progressive transition toward alkalinity. Notably, the control sample demonstrates the highest pH values at subsequent time intervals (7.33±0.02), potentially attributable to lactic acid formation or microbial and enzymatic processes **(Mwankunda *et al.,* 2023)**. Meanwhile, MOL may assist in maintaining desired pH levels and improving the shelf life of fish burgers while frozen**; Parvizi and Moosavi-Nasab (2021)**, **Rasak*et al.* (2021)** and **Mwankunda *et al.* (2023)** corroborate these findings.

The findings of this research demonstrated that the incorporation of MOL significantly reduced ($P< 0.05$) the heating loss of the fish burger that was prepared. The effects of 1% and 2% MOL supplementation on cooking loss were not significantly different from those of the control but became substantially different as the frozen storage period progressed. The observed reduction in culinary loss when MOL was added to the prepared fish burger suggests that MOL is involved in the process of water binding. According to **Abustam*et al.* (2020)**, MOL possesses the ability to bond water from processed fish products, thereby reducing the cooking loss of fish burgers. This investigation coincides with the findings of **Ibrahim *et al.* (2017)**, **Parvizi and Moosavi-Nasab (2021)**, and **Rasak*et al.* (2021)** who found that, adding MOL reduced cooking loss compared to the control sample. In theory, cooking losses can be decreased by enhancing the binding ability of raw meat **(Rasak *et al.,* 2021)**.

The water-holding capacity (WHC) is the capability of meat to hold all or part of its water and is one of the most important characteristics of meat quality **(Watanabe *et al.,* 2018)**. Furthermore, the study's findings revealed that the capacity of the control

group to retain water during storage was considerably lesser than that of the group supplemented with MOL-containing fish burgers. Based on the observed components, it is possible to postulate that the MOL supplementation may have facilitated the maintenance of water conservation within the muscle cell by stabilizing the muscular membrane via the activation of antioxidants and prevention of free radicals, as well as by reducing protein denaturation, and consequently maintaining the volume of myofibrils **(Rehman *et al.,* 2018)**. This finding concurs with the outcomes of **Auirema *et al.* (2019)** and **Rasak *et al.* (2023)**.

Additionally, the moisture, protein, lipid, and ash percentages of the prepared fish burger with MOL were all greater than those of the control group. However, except for ash content, all these parameters exhibited a progressive decrease throughout the frozen storage period. The decrease in total moisture content across all groups was a result of dehydration occurring during the frozen storage. This also led to a reduction in the solubility of proteins, which in turn diminished the water-holding capacity of the fish burger samples. Moreover, a substantial reduction in protein content was observed in various prepared fish burger samples as the storage time progressed ($P < 0.05$). The protein level of the control group decreased significantly more than that of the groups treated with MOL. The protein content decline that was noted during the period of preservation may be attributed to the depletion of water-bound amino substances, both soluble and volatile, which are protein-linked in the fish burger samples **(Abou-Taleb, 2022)**. Furthermore, at the beginning of the time of storage, the fat level of the prepared fish burger was not substantially altered by the addition of MOL at various concentrations. A significant rise in fat content ($P < 0.05$) was observed among various prepared fish burgers as the duration of storage extended. The potential cause for the observed increase in the amounts of fat among preserved fish burgers is the breakdown of lipoprotein to lipid and protein, which led to an increase in ether extract. Conversely, the addition of varied quantities of MOL to the prepared fish burger had a substantial effect on the ash percentage. The ash level of many prepared fish burgers increased significantly ($P < 0.05$) with an increase in preserved time especially in the control one. The results matched the findings of **Abdel-Latif *et al.* (2021)**, **Abou-Taleb (2022)**, and **Mwankunda *et al.* (2023)**.

Lipid oxidation in meat products is primarily attributed to the non-enzymatic lipid oxidation. Thiobarbituric acid (TBA) levels are employed as a lipid oxidation index in several foods **(Papuc *et al.,* 2017)**. Throughout the entire storage time, the TBA value of the fish burger increased, particularly in the control samples, indicating a secondary lipid oxidation. The groups treated with MOL exhibited a notably reduced TBA value compared to the control group throughout the storage duration. This suggests that the MOL may have a beneficial effect on lipid oxidation, as supported by the samples' elevated phenolic content **(Ayirezang*et al.,* 2023)**. MOL is composed of potent antioxidant compounds including flavonoids (specifically quercetin and beta carotene),

which possess substantial quantities of donated H atoms. These H atoms facilitate the neutralization of oxidants, thereby endowing MOL with a notable antioxidant activity **(Tambe *et al.,* 2022)**. The same outcomes were achieved by **Abou–Tor and Abouel–Yazeed (2019)** and **Abedelmaksoud*et al.* (2023)**. According to the Egyptian regulations, the authorized upper limit of TBA as an indicator of fish quality during fish preservation is 4.5mg MDA/kg fish meat **(ES, 3494/2005)**. The statistics show that none of the investigated samples surpassed the restrictions.

Moreover, the peroxide value (PV) determined the hydroperoxides, which are the principal products of auto-oxidation and are odorless. On the contrary, the process of decomposing food results in the production of various products such as furans, hydrocarbons, and carbonyl compounds, which are accountable for the rancid flavor that, is associated with putrefying food **(Yanishlieva & Marinova, 2001)**. Throughout the storage period, the PV of various treatments increased in this study. However, during storage, the PV of the groups treated with MOL was significantly ($P < 0.05$) lower than that of the control. The observed outcomes may be attributed to the polyphenolic nature of MOL, which is linked to its antioxidant properties **(Ma *et al.,* 2020)**. According to **Turhan *et al.* (2009)**, phenolic antioxidants do not function as oxygen absorbers; instead, they inhibit the production of fatty acid-free radicals, which are responsible for oxygen absorption or interaction during auto-oxidation. This effect delays the commencement of the adipose autooxidative process **(Abdollahi*et al.,* 2014)**. This finding is consistent with the results of **Bavitha *et al.* (2016)** and **Parvizi and Moosavi-Nasab (2021)**.

Additionally, the total volatile nitrogen (TVB-N) is a by-product of bacterial spoilage and the action of endogenous enzymes; its concentration is frequently used as an indicator to determine the quality and shelf- life of products **(EEC, 1995)** and to quantify tissue protein degradation in meat products, resulting from proteolytic enzymes and microbial activities during storage **(Wang *et al.,* 2020)**. According to **ES (4177/2005)**, the maximum allowable concentration of TVB-N in meat products is 20mg/100g. Additionally, **Lakshmanan (2000)** substantiated that fish muscle containing 35 to 40mg TVB-N/100g is commonly considered expired. During storage, the TVB-N concentration of the examined groups increased significantly and progressively ($P < 0.05$), but remained within the acceptable range for all inspected groups. The TVB-N production increases in response to an increase in enzymatic activities, specifically, the protease enzyme generated by specific microorganisms, which facilitates the degradation of the sausage's protein structures **(Huang *et al.,* 2014)**. Nevertheless, throughout the storage period, the TVB-N level of groups treated with MOL was noticeably reduced in contrast to the control group ($P < 0.05$). One possible explanation for the reduced TVB-N level in the fish burger treated with MOL could be its antibacterial properties. The TVB-N production can be diminished by antibacterial compounds such as MOL, which do so by reducing the capacity of bacteria to oxidatively deaminate non-protein nitrogen substances or both **(Banks *et al.,* 1980)**. **Abou–Tor and Abouel-Yazeed (2019)**, **Lithi *et**

al. **(2020)**, **Abdel-Latif** *et al.* **(2021)** and **Abou-Taleb (2022)**, all reported comparable findings.

Regarding an additional issue, the sensory evaluation serves as a significant indicator of the preferences of prospective consumers. Notwithstanding its limitations, it will continue to be the pre-eminent method of evaluating the grade of meat products and other foods **(Abou-Taleb, 2022)**. The present study assessed the sensory qualities, taste, color, texture, smell, and overall acceptability of prepared fish burgers having various proportions of MOL. The findings revealed that the inclusion of MOL at a 1% concentration significantly enhanced the sensory qualities of the fish burger product (P<0.05), and additionally, it increased their acceptability throughout the storage period. From the beginning to the end of frozen preservation, the fish burger formulated with MOL 1% achieved a higher score than the group treated with MOL 2% or the control group. Consistent with **Rahman** *et al.* **(2020)**, **Abdel-Latif** *et al.* **(2021)**, **Abou-Taleb (2022)** and **Mwankunda** *et al.* **(2023)**, the results of this investigation support one another. Due to the demonstrated minimal toxicity of MOL, increased concentrations may be employed to effectively combat food spoilage bacteria while maintaining safety standards **(Ayirezang** *et al.,* **2023)**. Intake has been proved safe at supra-supplementation levels ≤1,000mg/kg, significantly below the quantity studied in this investigation, when used as a nutraceutical **(Asare** *et al.,* **2012)**. However, consumers report that higher MOL concentrations (2.0%) result in worse sensory ratings across all criteria, implying that excessive concentrations may hurt the sensory quality of the fish burger.

CONCLUSION

The findings obtained demonstrated that the incorporation of 1 and 2% *Moringa oleifera* leaves into fish burgers has antimicrobial properties during the preservation period, without negatively impacting sensory qualities. In addition, it demonstrated a significant antioxidant impact that prolonged the storage life of frozen fish burgers and improved its stability. The potential efficacy of the plant extract may encourage the utilization of *Moringa oleifera* as a potent preservative in the preservation of fish products using natural products. However, further research is necessary to corroborate the findings regarding additional plants.

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