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Evaluation of *Ocimum basilicum* and *Moringa oleifera* as a Natural Preservatives of Canned Tuna Product

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Key words
Canned tuna, Sensory, *Ocimum basilicum*, *Moringa oleifera*

ABSTRACT

This study was conducted to evaluate the quality of local canned tuna products sold in supermarkets in Kafr El Sheikh governorate, through physicochemical, bacteriological and sensory parameters assessment in an attempt to improve the quality of local products using 1.5 and 3% *Ocimum basilicum* leaf extract (BLE) and 0.5 and 1% *Moringa oleifera* leaf extract (MLE) during refrigeration. The results showed that BLE 1.5 and 3% and MLE 0.5 and 1% improved pH, total volatile nitrogen (TVN) and thiobarbituric acid (TBA) values compared to control samples. The bacterial examinations indicated that BLE and MLE had a high percent of inhibition on *Staphulococcus aureus* and anaerobic bacteria. The bacterial count for *S. aureus* was reduced on the third day of storage for BLE 1.5 and 3% and MLE 0.5 and 1% by 36.2%, 53.1%, 68.1%, and 91.5%, respectively, increasing with storage time. While the reduction percentages were 61.5%, 84.6%, and 92.3%, respectively, on the 3rd day of storage for anaerobic bacteria with prolonged shelf life to the 6th day. Both BLE and MLE had no significant difference in sensory parameters and overall acceptability depending on the 9-hedonic score.

INTRODUCTION

Fish and canned fish products are helping to fill the gap in animal protein requirements, providing for about 16 percent of total animal protein taken by humans worldwide (FAO, 1997) and 6 percent of overall protein intake by humans (Copat *et al*., 2013). The demand for ready to eat foods has risen in today's world. People choose ready to eat foods because they are simple to cook, easy to buy from the market, and relatively inexpensive. Nonetheless, new consumption habits and modern lifestyles have had a significant impact on canned food consumption. Tuna is the first popular canned fish product available on markets (Hospido *et al*., 2006). The global demand for canned tuna will continue to rise and will reach an increase of roughly 34% from the current level during the next few years compared to the current level (Forleo *et al*., 2023).

Tuna fish in a can is a great source of nutrition because it is rich in high-quality digestible protein and vitamins such as A, B12, and D3 (Khedkar *et al*., 2003). It also contains small to moderate levels of minerals like calcium, phosphorus, potassium, and iodine, as well as microelements like selenium, fluorine, and zinc. It includes low saturated fat and long-chained polyunsaturated fatty acids, omega-3 fatty acids, which are beneficial to one's health (NMFS, 2020). They also have anti-arteriosclerosis properties (Usydus *et al*., 2008). As a result, it is recommended to be included in the human diet (Odiko and Obirenfoju, 2017), and even for public health causes, authorities in many nations promote the health benefits of fish and encourage the consumption of its products; such campaigns, with recent health issues in the meat and poultry industries, have likely contributed to the raised demand for fish products. In addition to the obvious health benefits of fish as a food, the possible risk of exposure to microbiological pollutants found in fish and fish-related products needs to be considered in the evaluation of the food's health quality (Usydus *et al*., 2008). Spoilage of fresh fish and fish products is mainly caused by microbial growth and/or activity, which results in changes in color, flavor, and texture, making the product organoleptically refused by consumption (Özogul *et al*., 2004). And because canned tuna fish is commonly consumed in Egypt, the challenge of this study was to evaluate the effects of using

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Ocimum basilicum (BLE 1.5% and 3%) and *Moringa oleifera* (MLE 0.5% and 1%) as natural preservatives on product quality. Consequently, their effects on shelf life through physicochemical evaluation (pH, TVN and TBA), bacteriological evaluation through assessment of their concentrations on reduction of *Staphylococcus aureus* and anaerobic bacterial count and sensory evaluation of the products.

MATERIALS AND METHODS

Collection and preparation of samples

A total number of 32 local canned tuna samples were purchased from different markets in Kafr El-Sheikh governorate, Egypt. The samples were transferred to the laboratory without delay and evacuated in sterile containers under complete aseptic conditions. Samples were divided into 5 groups; each group was represented by 5 samples and control group was represented by 12 samples. The first group was prepared as control (Untreated group) and the other 4 groups were treated with BLE (1.5% and 3%) and MLE (0.5% and 1%).

Treatment of samples with plant leaf extracts

Each treated sample was placed on sterile stainless wire mesh screen and dipped for 15 min in the extracts (1.5, 3 g of pure *Basil* leaf powder and 0.5, 1 g of *Moringa oleifera* leaf extract to 100 ml of sterile filtered water) then drained well for 5 min. The control group was similarly dipped in sterile distilled water. Samples were labelled and each sample was put separately in polyethylene bags. The experiment was conducted over 21 days of refrigerated storage at 2-4°C (Viji *et al*., 2017). All examined samples of such groups, whether treated or control, were evaluated for physiochemical, bacteriological and sensory. Assessments were made at zero time (within 2 h after treatment) then done repeatedly every 3 days till sample spoilage appeared in each group (zero, 3rd, 6th, 9th, 12th, 15th, 18th and 21st days). This scheme was replicated for 5 times.

Determination of pH

Approximately 10 g of the material were blended with 10 mL of neutralized distilled water. This mixture was shaken continuously for 10 min. at room temperature. An electrical pH meter (Bye model 6020, USA) was used to determine the pH value. Using two buffer solutions with known pH values (alkaline pH 7.01, acidic pH 4.01), calibrate a pH meter. After the temperature correction system had been calibrated, the pH electrode was first rinsed with neutralized water then inserted to the homogenate (Pearson, 2006).

Determination of total volatile nitrogen (TVN)

According to ES (2006), 10 g of the sample were inserted to 300 ml of distilled water in a clean distillation flask and carefully mixed with the polytron probe. Two grams of magnesium oxide and an antifoaming agent were added. The receiving flask was filled with 25 mL of 2 % boric acid and a few drops of an indicator. The receiving flask was positioned so that the receiver tube dropped below the boric acid solution. The distillation flask was gradually heated by ten min till boiling, which lasted for 25 min, followed 25 min of distillation. Titration of TVN delivered in boric acid by H_2 SO_4 n 0.1 was recorded. As a result, TVA was estimated using the formula below:

TVN/l00g = (mls H_2 SO_4 n 0.1 for sample – ml H_2 SO_4 n 0.1 for Blank) x 14.

Determination of thiobarbituric acid number (TBA)

According to ES (2006), the test is based on determining malonaldehyde (MDA) as a lipid peroxidation end product. A TBA number or value that is represented as milligrams of malonaldehyde equivalents per kilogram of the samples often indicates the degree of oxidative rancidity. When unsaturated fatty acids in flesh undergo oxidative destruction, free malondialdehyde is generated, producing the TBA- malondialdehyde complex. When malondialdehyde is released, it can be used as a lipid oxidation and food quality indicator. In fact, in a distillation flask, 10 g of canned tuna sample were transferred and mixed with 50 ml of distilled water then (2.5 ml of hydrochloric acid diluted in 47.5 ml of water) were added. Antifoaming agents were introduced in small pieces. Within 10 min of the start of boiling, the distillation flask was slowly heated for the distillation of 50 ml. In a tube with a cover, 5 ml of distilled solution was added, followed by 5 ml of prepared thiobarbituric acid. The tube had been covered and placed in the boiling water bath, where it heated for about 35 min and was put for ten min in the water to cool. The sample's absorbance was measured under wave length 538 using a Spectrophotometer (UNICAM969AA Spectronic, USA). TBA value= absorbance of sample x 7.8 (malonaldehyde (mg) /Kg).

Bacteriological examination

The pathogenic strain of *S. aureus* was provided by the Food Analysis Center, Faculty of Veterinary Medicine, Benha University, *S. aureus* (ATCC 8095). Bacteria were sub cultured in Brain–Heart Infusion (BHI) broth and given a 24 h incubation period at 37°C. Centrifugation (3000×*g*, 15 min) was used to harvest the cells, which were then twice-washed and re-suspended in saline (NaCl, 0.85%, w.v) (Wu and Cheng, 2014).

For inoculation of the tested tuna control or treated

groups, 1×10^6 CFU /g (6 log) of the dense suspension for each strain was employed.

For enumeration of the tested pathogens, 100µl of a suitable dilution of the bacteria were cultivated on Baired Parker agar plates. After incubation of the plates at 37°C for 48 h, enumeration was carried out.

For anaerobic count, one ml from each of the previously prepared serial dilutions was spread over anaerobic agar plates using a sterilized bent glass spreader. The inoculated plates had a two-day anaerobic incubation at 35°C. All total formed colonies were detected and recorded as total anaerobic count/g. Anaerobic count/g = average number of duplicate plates x dilution factor (ISO, 2013).

Sensory evaluation

Sensory evaluation was done by three Food Control Department Workers, Faculty of Veterinary Medicine, Kafrelsheikh University, according to the 9-point hedonic scale and hedonic ranking in food science. The samples were tested for taste, texture, smell, color and overall acceptability. They scored from 1 to 9; 1 identified as dislike extremely, 2 dislike very much, 3 dislike moderately, 4 dislike slightly, 5 neither like nor dislike, 6 like slightly, 7 like moderately, 8 like very much, and 9 like extremely, according to Svensson (2012).

Statistical analysis

All data were statistically examined using SPSS 25.0 for windows (SPSS Inc., Chicago, IL, USA) and analyzed using Microsoft Excel 365. Quantitative variables were expressed as the mean±SD and median (range). Comparing categorical data was done using the Chi-square test. To compare normally distributed quantitative variables between two subgroups, the T-test (t) test was used. Non-parametric Mann-Whitney U (M-W) test was used to compare non-normally distributed quantitative variables between 2 subgroups. At a confidence interval of 95% and a P-value <0.05; the applied test was regarded as statistically significant.

RESULTS AND DISCUSSION

Table I showed the effect of adding BLE (1.5% and 3%) and MLE (0.5% and 1%) to the examined local shredded tuna samples on the pH value during refrigeration at 2-4 °C. The results showed a significant difference between both Basil and Moringa compared to control, as well as a significant difference between treated samples with Basil versus Moringa. However, there was no recorded significant difference in the concentration of BLE and MLE. Control samples (without treatments) was not accepted from the 3rd day of storage according to the Egyptian of Standardization (EOS, 2005) which status that pH value should not be more than 6.7. An increase in the pH value of control samples indicates the activity of proteolytic enzymes that produce ammonia, resulting in changes in the degree of acidity during storage (Santoso *et al*., 1999). While samples treated with Basil leaf extract was still accepted until the 6th, 9th day for concentration 1.5% and 3%, respectively, and for 12th, 15th day of storage with MLE 0.5% and 1%, respectively. In basil treated groups with concentrations of 1.5 and 3%, the pH value tends to rise, with a pH ranging between 6.1±0.06 to 7.06±0.13, respectively. In Moringa treated groups 0.5 and 1% concentration, the pH value tends to rise,

Table I. Effect of *Ocimum basilicum* leaf extract (BLE) and *Moringa oleifera* leaf extract (MLE) on pH of treated canned tuna samples.

Days of storage	Control (n=12)	BLE		MLE		P value
		1.5 % (n=5)	3 % (n=5)	0.5% (n=5)	1 % (n=5)	
0	6.2±0.08	6.16±0.07	6.1±0.06	6.05±0.09	6.02±0.06	P1=0.11, P2=0.0001*, P3=0.011*, P4=0.21, P5=0.3
3	6.8±0.1	6.4±0.11	6.3±0.1	6.3±0.33	6.11±0.11	P1=0.001*, P2=0.0001*, P3=0.22, P4=0.16, P5=0.23
6	7.2±0.2	6.5±0.16	6.4±0.14	6.3±0.1	6.22±0.12	P1=0.001*, P2=0.0001*, P3=0.011*, P4=0.27, P5=0.09
9	D	6.9± 0.52	6.5 ± 0.12	6.27 ± 0.5	6.3 ±0.09	P3=0.025*, P4=0.21, P5=0.8
12	D	6.9± 0.14	6.8 ±0.15	6.66 ±0.14	6.5 ± 0.11	P3=0.01*, P4=0.19, P5=0.09
15	D	6.1 ± 2.2	6.8 ± 0.16	6.8 ± 0.12	6.59 ±0.14	P3=0.65, P4=0.45, P5=0.1
19	D	D	7.06 ±0.13	6.89 ±0.11	6.73 ±0.15	P3=0.007*, P5=0.09
21	D	D	D	D	6.69 ±0.13	N/A

D, decomposed; *, significant difference at (P<0.05). P1, BLE vs. control, P2, MLE vs. control; P3, BLE vs. MLE; P4, BLE concentrations 1.5% vs. 3%; P5, MLE concentrations 0.5% vs. 1%.

Table II. Effect of BLE and MLE on total volatile nitrogen (TVN) of treated canned tuna samples.

Days of storage	Control (n=12)	BLE		MLE		P value
		1.5 % (n=5)	3 % (n=5)	0.5% (n=5)	1 % (n=5)	
0	2.9±0.23	3.1±0.56	2.97±0.5	2.8±0.25	2.76±0.24	P1=0.62, P2=0.12, P3=0.18, P4=0.77, P5=0.92
3	14±0.7	6.3±0.7	5.8±0.6	5.6±0.66	5.2±0.68	P1=0.001*, P2=0.0001*, P3=0.045*, P4=0.27, P5=0.32
6	28±1.3	9±0.9	8.7±0.88	8.5±0.88	7.74±0.79	P1=0.001*, P2=0.0001*, P3=0.085, P4=0.59, P5=0.19
9	D	13.5 ± 1.4	12.9 ± 1.1	12.8 ± 1.34	11.8 ± 1.3	P3=0.16, P4=0.49, P5=0.27
12	D	18.12 ±0.9	17.1 ± 0.7	16.92 ± 1.15	15.38 ± 1.25	P3=0.015*, P4=0.007*, P5=0.08
15	D	22.6 ± 1.19	19.7± 1.3	19.38 ± 1.2	17.7 ±0 .7	P3=0.002*, P4=0.08, P5=0.027*
19	D	D	28.58± 1.49	27.6± 1.4	22.78 ± 0.9	P3=0.025*, P5=0.001*
21	D	D	D	D	27.1 ± 1.4	N/A

For abbreviations and statistical details see Table I.

Table III. Effect of BLE and MLE on thiobarbituric acid (TBA) of treated canned tuna samples.

Days of storage	Control (n=12)	BLE		MLE		P value
		1.5 % (n=5)	3 % (n=5)	0.5% (n=5)	1 % (n=5)	
0	0.14±.02	0.14±.02	0.14±.02	0.13±.02	0.12±.02	P1=0.73,P2=0.18, P3=0.34, P4=0.82,P5=0.6
3	2.4±0.2	0.4±0.14	0.4±0.13	0.35±0.13	0.3±0.1	P1=0.001*,P2=0.0001*, P3=0.064,P4=0.42,P5=0.5
6	4.8±0.23	0.9±.15	0.8±.08	0.7±.12	0.58±0.15	P1=0.001*,P2=0.000*, 3=0.002*,P4=0.07,P5=0.17
9	D	1.4± 0.163	1.2± 0.15	1.1 ± 0.15	0.75 ± 0.15	P3=0.0001*,P4=0.07, P5=0.007*
12	D	2.2 ± .11	1.9 ± 0.16	1.6 ± 0.13	1.2± 0.13	P3=0.0001*,P4=0.007*, P5=0.001*
15	D	3.3 ± 0.26	3.1± 0.23	2.64 ± 0.2	2.2 ± 0.2	P3=0.0001*,P4=0.18, P5=0.032*
19	D	D	3.8 ± 0.23	3.64± 0.2	2.7 ± 0.26	P3=0.014*,P5=0.0001*
21	D	D	D	D	3.9± 0.27	N/A

For abbreviations and statistical details see Table I.

with a pH ranging between 6.02±0.069 to 6.89 ± 0.11, respectively, which gives superiority to MLE. Because both Basil and Moringa leaf extract contain antimicrobial compounds that can reduce the bacterial growth in the canned tuna samples, so that the accumulation of ammonia can take place more slowly. TVN is regarded as a fish quality indicator as well as an index of freshness and spoilage degree (Wong and Gill, 1987) and consists of nitrogenous chemicals (such as amines and ammonia) as a result of the enzymatic hydrolysis of non-protein and protein nitrogenous compounds by spoilage microorganisms (Manat *et al*., 2005). The TBA index is a measurement of malonaldehyde level, which is a lipid hydroperoxide breakdown product produced during the oxidation of PUFAs (Gomes *et al*., 2003). Regarding the effect of BLE and MLE on TVN and TBA during storage of samples at zero day (Table II, III), there was no significant difference between any of the treated samples compared to the control, even within different concentrations of BLE and MLE, but a significant difference started to be recorded from the 3rd day of storage between treated samples with BLE and MLE versus control, as well as samples treated with BLE and MLE, this may be attributed to the fact that MLE has a high concentration of natural antioxidant components, such as α-tocopherol, ascorbic acid, carotenoids, polysaccharide, flavonoids, saponins, phenolics, tannins, and proanthocyanidins (Abuye *et al*., 2003; Moyo *et al*., 2012) and also *Ocimum basilicum* extracts possess a higher total phenolic acid content and greater antioxidant activity (Antonescu *et al*., 2021). Samples treated with BLE and MLE showed that TVN values of all the samples were found below the permissible limit according to the Egyptian organization of standardizations (EOS, 2005) of not more than 40 mg/l00g and within the acceptable limit (30–45 mg/100g) stated by EC (1995), but the control samples increased from values

of 2.9±0.23 to 28. ±1.4 mg N/100 g of fish at the end of the 6th day of storage.

From the results, it was found that BLE and MLE increased slowly the level of TBA of examined samples during the storage period in comparison to control samples and this could be regarded to the strong antioxidant activity of both BLE and MLE. According to the allowable TBA value in fish products (4.5 mg MDA/kg) recommended by ESO (2005), Results indicated also that TBA values of the canned tuna samples showed no differences between all samples at zero time. Meaning that treated samples with BLE or MLE require time to give their effect as their results change better than the untreated samples from zero day to the end of the storage period. These results were in argument with El-Rahman *et al*. (2019) as the TBA of luncheon meat during cold storage found that moringa leaves extract decreased the formation of TBA in luncheon samples during the cold storage for four weeks. Results indicated that TBA values of the luncheon samples showed no differences between all prepared samples at zero day.

Table IV. Effect of BLE and MLE on count (log_{10} cfu/g) and % reduction of *Staphylococcus aureus* of treated canned tuna samples.

Days of storage	Control (n=12)	BLE		MLE	
		1.5 % (n=10)	3 % (n=10)	0.5% (n=10)	1 % (n=10)
0	6.0±5.0	6.0±5.0	6.0±5.0	6.0±5.0	6.0±5.0
3	5.97±5.2	5.8±1.6 (36.2%)	5.6±4.8 (53.1%)	5.5±4.8 (68.1%)	4.9±4 (91.5%)
6	5.9±5.1	5.2±4 (77.2 %)	4.9±4.04 (89.4%)	3.95±3.14 (98.8%)	3.1±1.7 (99.8%)
9	5.7±4.5	4.9±4.04 (84%)	4.2±3.3 (97%)	2.7±1.5	ND
12	S	2.95±2.2	ND	ND	ND
15	S	2.3±1	ND	ND	ND

S, spoiled; ND, non-detected.
For abbreviations see Table I.

Handling and manipulation during preparation and/or processing steps might contaminate tuna. Furthermore, refrigeration for a long time before industry doesn't inhibit *S. aureus* count as it persists in frozen tuna cuts at -20°C for 4 weeks and increases population up to 3 log cfu/g when thawed (Wu and Cheng, 2014). Therefore, the incidence of *Staphylococcus aureus* presence in canned tuna can be due to handling manually of pre-cooked tuna fish before processing or during a faulty processing step of the canning operation. Also, enterotoxins from *S. aureus* have the potential to cause food poisoning. Table IV shows that the effect of adding BLE (1.5% and 3%) and MLE (0.5% and 1%) as natural preservatives in canned tuna samples on the *S. aureus* count revealed that there were reduction percentages of *S. aureus* on the third day of storage by 36.2%, 53.1%, 68.1%, and 91.5%, respectively. It is significant to be mentioned that the reduction percentage increases with increasing of storage time, reaching 77.2%, 89.4%, 98.8%, and 99.8% at the sixth day of storage, respectively. These results could be due to the antibacterial effect of both BLE and MLE against *Staphylococcus aureus*. The *Moringa oleifera* had a high amount of flavonoids and tannins which have shown potential antibacterial effect against great numbers of microbes including *S. aureus* (Yamunadevi *et al*., 2011). Omaima *et al*. (2007) identified gained isolates revealed 26.6 % S. *aureus* in each of tuna. These results agreed with Lawson (1970) who recorded that *Staphylococcus aureus* was rarely recovered from freshly caught fish, but after handling and processing reached from 10–30 %. Dhinesh *et al*. (2021) found that none of the canned fish samples that were examined were sterile and all of them included harmful bacteria such *Staphylococcus aureus*, *E. coli*, *Vibrio* spp., *Salmonella* spp., and *Listeria* spp. Basil leaf extract contains antimicrobial compounds such as essential oils, phytosterols, alkaloids, phenolic compounds, tannins, lignin, starches, saponins, flavonoids, terpenoids, and anthraquinones so that they can be used as natural preservatives (Anggraini, 2018). Where it causes disruption of bacterial activity. It binds to fish protein compounds, making it difficult to over haul either by enzymes from fish or bacteria (Shofiani *et al*., 2020). The antibacterial action of the Moringa extract was shown to be good against *S. aureus,* which was also mentioned in a number of other researches (Kudi *et al*., 1999; Awadh *et al*., 2001). The most effective concentration was MLE 1%, which showed its efficiency and optimal function to inhibit *S. aureus* so protect the people from food-borne diseases, thus improving food security and safety. The moringa leaves have antioxidants and antimicrobial properities (Sánchez-Machado *et al*., 2010) and recently have been reported to increase the shelf life of some meat products (Das *et al*., 2012; Jayawardana *et al*., 2015).

Table V showed the obtained results during the treatment of samples with BLE (1.5% and 3%) and MLE (0.5% and 1%) in a trial to reduce anaerobic bacterial count. The reduction percentage was 61.5%, 84.6%, and 92.3%, respectively, on the 3rd day of storage. Only samples treated with BLE 1.5% extended to the 6th day of storage with a reduction percentage of 97.1%. The prolonged shelf life could be a reason for the hydrophobic property of BLE and its main components, particularly linalool, as the bacterium's cell wall may be breached by

Table V. Effect of BLE and MLE on count (log_{10} cfu/g) and % reduction of anaerobic bacteria of treated canned tuna samples.

Days of storage	Control (n=12)	BLE		MLE	
		1.5 % (n=10)	3 % (n=10)	0.5% (n=10)	1 % (n=10)
0	2.8±1.7	2.8±1.7	2.8±1.7	2.8±1.7	2.8±1.7
3	3.11±2	2.7±1.5 (61.5%)	2.3±1 (84.6%)	2±1 (92.3%)	ND
6	3.4±2.3	2±1 (97.1%)	ND	ND	ND
9	3.9±2.9	ND	ND	ND	ND
12	S	ND	ND	ND	ND

S, spoiled; ND, non-detected.
For abbreviations and statistical details see Table I.

some compounds, most notably the oxygenated monoterpenoid linalool, which can attack the phospholipid bilayer found in cell membranes (Hussain *et al*., 2008). It may also interact with cell wall-based enzymes to generate enhanced permeability and cytoplasm leakage. According to Emirolu *et al*. (2010), linalool can either serve as a protein denaturing agent or a solvent dehydrating agent, which both contribute to its antibacterial activity.

The results obtained from Table VI revealed that all treated canned tuna samples using BLE (1.5% and 3%) and MLE (0.5% and 1%) had no significant difference in sensory parameters (taste, texture, smell, color and overall acceptability depending on the 9-hedonic score, but the rejection was declared when the score fell below 5.0 for each parameter and below 20.0 for overall acceptability. The control samples showed spoilage on the 9th day.

Table VI. Sensory evaluation of treated canned tuna samples using BLE and MLE.

Sensory attributes	Samples	0 day	3rd day	6th day	9th day	12th day	15th day	18th day	21st day
Taste (9 points)	Control	6.13±0.64	5.40±1	3.9±0.8	Decomposed	Decomposed	Decomposed	Decomposed	Decomposed
	1.5% BLE	6.07±0.7	5.3±0.62	4.1±0.8	2.80±.7	2.1±0.6	1.73±0.6	Decomposed	Decomposed
	3% BLE	6.3±0.7	5.5±0.99	4.2±0.8	2.87±.64	2±.756	1.87±.640	1.1±.352	Decomposed
	0.5% MLE	6.13±0.9	5.3±0.7	4.2±0.8	2.60±.63	2.1±0.6	1.60±.507	1.1±0.3	Decomposed
	1% MLE	6.13±0.7	5.3±0.6	4.1±0.6	2.67±.62	2.1±0.6	1.60±.632	1.1±.352	1.1±0.3
P-value (P1, P2, P3)		0.9, 0.9, 1	0.9,1,0.9	0.8,0.7,0.6	0.7, N/A, N/A	0.9,N/A, N/A	0.6, N/A,N/A	0.8, N/A, N/A	N/A
Texture (9 points)	Control	6.1±1	5.3±0.8	4.1±0.8	Decomposed	Decomposed	Decomposed	Decomposed	Decomposed
	1.5% BLE	6.3±0.7	5.5±0.7	4.2±0.9	2.80±.6	2.27±0.6	1.73±0.6	Decomposed	Decomposed
	3% BLE	6.20±1.01	4.9±0.7	4.5±0.6	2.67±0.5	2.1±.743	1.87±.640	1.1±0.3	Decomposed
	0.5% MLE	6.51±0.9	5.2±0.9	4±0.8	3±.655	2.4±.632	1.87±.516	1.1±0.3	Decomposed
	1% MLE	6.53±0.92	5.7±0.8	4.1±0.7	3.1±.7	2.4±.507	1.80±.561	1.2±0.4	1.1±.35
P-value (P1, P2, P3)		0.12,0.2,0.13	0.07,0.9,0.8	0.3,0.4,0.9	0.18,N/A,N/A	0.6,N/A, N/A	0.9,N/A, N/A	0.4, N/A, N/A	N/A
Smell (9 points)	Control	6.3±.458	5.3±0.7	4.5±0.6	Decomposed	Decomposed	Decomposed	Decomposed	Decomposed
	1.5% BLE	6.3±1.033	5.5±0.7	4.5±0.5	3.1±.64	2.4±0.5	1.73±0.6	Decomposed	Decomposed
	3% BLE	6.3±.884	5.5±1.1	4.6±0.5	3.20±.56	2.47±0.5	2.1±0.7	1.2±0.4	Decomposed
	0.5% MLE	6.2±1.014	5.1±0.7	4.3±0.6	3.07±0.3	2.3±0.6	1.67±0.5	1.2±0.4	Decomposed
	1% MLE	6.3±0.6	5.1±0.6	4.3±0.7	2.9±.5	2.1±0.6	1.73±0.6	1.2±0.4	1.1±0.3
P-Value (P1, P2, P3)		0.9,1,0.9	0.6,0.8,0.7	0.4,0.8,0.5	0.5, N/A, N/A	0.35,N/A,N/A	0.2, N/A,N/A	1, N/A, N/A	N/A
Color (9 points)	Control	6.6±.828	5.7±0.7	4.3±0.6	Decomposed	Decomposed	Decomposed	Decomposed	Decomposed
	1.5% BLE	6.53±.834	5.6±0.6	4.3±0.9	3.53±.52	2.27±0.6	1.67±0.5	Decomposed	Decomposed
	3% BLE	6.3±1.1	5.5±0.6	4.1±0.7	3.5±.516	2.3±0.5	1.93±0.7	1.1±.352	Decomposed
	0.5% MLE	6.47±.99	5.5±0.8	4.2±0.6	3.5±.834	2.3±0.5	1.80±.561	1.1±0.3	Decomposed
	1% MLE	6.4±.986	5 ±0.7	4.1±0.8	3.5±.834	2.6±.507	1.93±0.6	1.2±0.4	1.1±0.3
P-Value (P1, P2, P3)		0.9, 0.8,0.8	0.3,0.7 ,0.2	0.9,0.8,0.7	0.9, N/A, N/A	0.3, N/A, N/A	0.6, N/A,N/A	0.6, N/A, N/A	N/A

P1, Significance between the groups; P2, control Vs. BLE; P3, Control Vs. MLE.
For abbreviations and statistical details see Table I.

This may be due to rapid microbial growth and oxidation of lipids, which affect the sensory characteristics. All samples, either treated or not treated, gave an unacceptable score on the 6th day during storage at 2-4 °C also, the treated leaves extract groups show an increase in shelf life but give an unacceptable score to consumers. MLE 1% could effectively slow down chemical deterioration, reduce bacterial growth and prolong the shelf life of canned tuna samples by 21 days during storage at 2-4 °C. Therefore, MLE 1% can be used commercially and safely as an organic preservative to increase the shelf life of canned tuna products. MLE could be very suitable as a natural additive or substituted material in the production of many foodstuffs. Adeyemi *et al*. (2013) recorded that the sensory scores for color, flavor, juiciness, and overall acceptability of the fish samples suggested that a marinade made from Moringa oleifera might be utilized to preserve the quality characteristics of smoke-dried African catfish that had been kept for two months.

CONCLUSION

For conclusion, the addition of moringa (*M. oleifera*) leaf and basil (*O. basilicum*) leaf *extracts* at concentrations of (1.5% and 3%) and (0.5% and 1%), respectively, for the preservation of canned tuna had great significant positive effect. Generally, this investigation showed the potential value of BLE and MLE as a great natural source of phenolic compounds and antioxidants. The antibacterial activity of BLE and MLE was observed to be great for the reduction of *S. aureus* and anaerobic bacteria, leading to extension of the shelf life of the canned tuna product. They also had no effect on the sensory parameters. Considering the results of this study, it could be useful for characterization of BLE and MLE in canned tuna products for industrial utilization during low temperature storage.

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IRB approval

The experimental methods, procedure were approved by the Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt.

Ethics statement

The rules for the samples collection used in the experiments were authorized by the Research Ethical Committee of the Faculty of Veterinary Medicine at the University of Kafr El-Sheikh, Egypt.

Statement of conflict of interest

The authors have declared no conflict of interest.

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