The effect of adding Moringa oleifera leaf extracts in prolonging the shelf life of chilled fish meat (*Epinephelus coioidis*)

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Abstract
The study aimed to use Moringa oleifera leaf extracts(MOLP) to improve the nutritional propertiesand inhibit lipid oxidation and the proliferation of microorganisms in fish meat. The results of the peroxide number of fish meat samples of the Epinephelus coioidis type stored in cold storage to which extracts were added indicated that the alcoholic extract at both concentrations was significantly excelled on the synthetic antioxidant (BHT) compared to the aqueous extract and the control sample. The alcoholic extract at a concentration of 400PPM recorded the lowest values of the peroxide number (1.11, 1.32, 3.12). , 6.16, 7.51, 8.71, 9.89, 10.03, 10.23) Meq/kg during a period of (0, 1, 3, 5, 7, 9, 11, 13, 15) days, respectively, compared to the rest of the treatments and the control sample. The results of thiobarbituric acid values for cold-stored Epinephelus coioidis fish meat samples to which extracts were added showed that the alcoholic extract with a concentration of 400PPM was significantly excelled on the synthetic antioxidant (BHT) compared to the rest of the treatments and the control sample. The alcoholic extract with a concentration of 400PPM recorded the lowest values for thiobarbituric acid (0.32, 0.34, 0.69, 0.87, 0.92, 1.42, 1.72, 1.89, 2.13) mg malone aldehyde/kg over a period of (0, 1, 3, 5, 7, 9, 11, 13, 15) days, respectively, compared to the rest of the treatments and the control sample The addition of Moringa oleifera leaf extract can be used in other food applications.
Keywords: Moringa oleifera; fish meat; plant extracts ; lipid oxidation

Introduction

The use of food additives, such as preservatives and antioxidants, is one of the main methods to maintain the meat quality and meat products during processing and storage [16]. Oxidative reactions of fats and proteins are the main causes of deterioration in the quality of animal tissues, including, which leads to an undesirable change in odor, color change, decreased shelf life and the accumulation of toxic compounds that pose a threat to the health of consumers [14]. Plants are considered the most important source of natural antioxidants because they contain biologically active compounds, such as flavonoids, carotenoids, tocopherols, and polyphenolic substances. These contribute to maintaining and improving the quality of meat and its products [15]Plant extracts have been used since ancient times in folk medicine, and over time they have been used to preserve food materials. Vegetable oils are among the most important of these extracts and are considered secondary metabolites of plants [7]. Moringa Oleifera is the most widely cultivated species of the Moringaceae family and is native to the sub-Himalayan regions of India, Pakistan, Bangladesh and Afghanistan. It is a perennial softwood tree with low-quality timber and has been used for many aspects such as food processing, medical and traditional industrial matters [18], lipid

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oxidation is a major cause of spoilage of fish meat that leads to off-flavors, unpleasant odors, change in texture, color change and decrease in nutritional value. To retain the quality traits for a longer period and extend the shelf life during storage of chilled and frozen fish, using . A summary of the harms and negatives of artificial antioxidants and the positives and importance of natural antioxidants on the health of the body.are widely used. [12 The study aimed to prolong the shelf life of marine fish meat by treating marine fish meat with extracts of Moringa oleifera functional ingredient to enhance the quality attributes of fish meat leaves and knowing the role of this Extracts help reduce microbial spoilage and oxidation reactions.

Materials and methods

Plant leaves used in the study and their source:

The leaves of Moringa oleifera plant were obtained from some people interested in growing rare and medicinal trees in Babylon province, and the tree was confirmed and diagnosed with the help of Professor Dr. Ibrahim Radhi - Al-Furat Al-Awsat Technical University - Al-Musauib College of Technology - Department of Plant Production Technologies - Fasalja plant (a specialist in this field) collected the leaves, washed them well, then dried them naturally, then ground and sieved them to obtain a fine powder, then kept them in the refrigerator by polyethylene bags until use.

2-1-Preparation of plant extracts:

2-1-1 Alcoholic extract:

The alcoholic and aqueous extract s of the plant was prepared according to the method of [1]where 20 g of Moringa oleifera leaf powder was mixed with 400 ml of 98% ethyl alcohol and with 400 ml of distilled water the mixture was left for 24 hours on a magnetic stirrer at a temperature of 25°C, then filtered by Filter paper (Whatman No. 1). The filtrate was concentrated using a rotary vacuum evaporator, then the material was incubated at refrigerator temperature until use.

2-1-2Water Extract

Prepare the aqueous extract as(2-1-1) stated in the method, replacing the alcohol with water

2-2Preparing marine fish meat samples

In this study, Epinephelus coioidis Orange-spotted grouper (h) fish was used. The fish was brought from local markets in Najaf. The number of fish used in the study was (15) and its total weight was (13,400) g. A physical inventory was carried out by removing the head, tail and fins. The internal viscera and bones were removed and washed with distilled water. After that, the fish was cut into slices weighing (30-50) g and measuring approximately (5 x 10) cm, as in the [2]. The quantity was divided into :

•Treatment T1 (samples kept in refrigerator without adding extract(

•Treatment T2: Samples stored in cold storage with the addition of (aqueous) extract at concentrations (200 PPM (

•Treatment T3: Samples stored in cold storage with the addition of (aqueous) extract at concentrations (400 PPM.(

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•Treatment T4: Samples stored in cold storage with the addition of (alcoholic) extract at concentrations (200 PPM.(

•Treatment T5: Samples stored in cold storage with the addition of (alcoholic) extract at a concentration of 400 PPM.

•Treatment T6: Samples were kept cold with the addition of the synthetic antioxidant BHT at a concentration of 200 PPM.

The samples were placed in dishes and were measured over time periods (0,1,3,5,7,9,11,15) days, and chemical tests were performed (P.V value and TBA value)

2-3Specific chemical tests: Chemical Quality Tests

2-3-1Peroxide Value (PV)

The peroxide value was measured based on the method of [13] where 5 g of samples were weighed and 30 ml of a mixture containing three parts of glacial acetic acid, two parts of chloroform, 5 ml of saturated potassium iodide, 20 ml of distilled water, and a few drops of starch indicator were added to it. The mixture was wiped with a 0.001 sodium thiosulphate solution until the blue color disappeared, and the peroxide value was calculated based on the following equation:

$$\text{Peroxide number (Meq/kg)} = \frac{\text{volume of sodium thiosulphate consumed} * \text{titres}}{\text{Sample weight (g)}} \text{ x } 1000$$

2-3-2Estimation of the value of thiobarbituric acid (TBA)

It followed the method mentioned in (Pearsson (1970), by weighing 10 g of the sample and mixing it with 50 ml of distilled water for two minutes, after which 47.5 ml of distilled water was added to it, then 2.5 ml of hydrochloric acid (4 mm) was added to it to lower the pH to 1.5, then An anti-foaming agent was added to it along with some glass balls, then the distillation device was connected and heated on an electric heater. 50 ml of the dripping liquid was collected within 10 minutes. 5 ml of the dripping liquid was taken in a test tube with a tight stopper and 5 ml of TBA reagent was added to it The preparation was (0.2883 g/100 ml of 90% glacial acetic acid). The control sample was prepared by adding 5 ml of the reagent. The tubes were closed well and placed in a water bath at boiling temperature for 35 minutes. The tubes were then cooled for 10 minutes, then the absorbance was measured with a spectrophotometer at a wavelength. 538 nm and the TBA number was calculated as follows:

TBA (mg malondehyde/kg oil) = [50× sample absorbance reading – control treatment absorbance reading] / sample weight (mg)

3-Statistical Analysis and Design

Statistical analysis of the data was conducted using a Complete Randomized Design (CRD) with three replications, and the Least Significant Difference (LSD) test was used to compare the means at a probability level of 0.05 [3].

4- Results and Discussion

4-1The effect of adding different proportions of alcoholic and aqueous extracts of Moringa oleifera leaves and synthetic antioxidants on

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the peroxide number of fish meat (Epinephelus coioidis) stored at 4°C.

Table (1) shows the values of the peroxide number (Meq/kg) measured in the meat of Epinephelus coioidis fish treated with different concentrations (200, 400 PPM) of the aqueous and alcoholic extracts, Moringa oleifera leaves, and the synthetic antioxidant in concentration and stored at a refrigeration temperature of 4°C and a storage period (0,1 ,3 ,5 ,7 ,9 ,11,13,15) days .The results of the treatment of the aqueous extract at a concentration of 200 PPM were (1.11, 2.14, 4.60, 7.09, 9.22, 11.77, 12.11, 12.34) Meq/kg, respectively, and the treatment of the aqueous extract at a concentration of 400 PPM was (1.11, 1.95, 3.91, 6.42, 8.21, 9 .89, 10.26, 10.80, 11.09) Meq/kg, respectively, and the alcoholic extract treatment at a concentration of 200 PPM (1.11, 1.56, 4.01, 5.94, 7.44, 9.01, 10.09, 10.41, 10.64) Meq/kg, respectively, and the alcoholic extract treatment at a concentration 400 PPM (1.11, 1.21, 3.10, 5.81, 6.10, 7.12, 8.41, 9.69, 10.07) Meq/kg, respectively, and the synthetic antioxidant treatment (BHT) was 200 PPM (1.11, 1.32, 3.12, 6.16, 7.51, 8.71 9 .89 , 10.03, 10.23) Meq/kg, respectively, while the control treatment recorded (1.11, 2.54, 4.81, 7.20, 9.90, 11.81, 12.11, 13.20, 13.90) Meq/kg, respectively.

The results of the statistical analysis showed that there were no significant differences immediately after manufacturing (day zero) for the control sample and the samples treated with Moringa oleifera leaf extracts and (BHT) and for all concentrations used, due to the fish meat not being exposed to the process of oxidation (self-oxidation) by enzymes due to its lack of exposure to oxygen. The bacteria were still inactive, as the peroxide value reached 1.11 Meq/kg, after which the peroxide number value began to increase significantly with increasing storage period. On the first day, the control sample recorded (1.11) Meq/kg and increased until it reached (13.90) Meq/kg. kg on the fifteenth day of refrigerated storage, while the treatments of the alcoholic extract at two concentrations (200 and 400) PPM and the synthetic antioxidant (200) PPM were significantly superior to the rest of the treatments, as the lowest values of peroxide number were recorded on the fifteenth day of refrigerated storage (10.64, 10.07, 10.23).) Meq/kg, respectively. Note that the permissible limit is within [4,10] which indicated that oil or fat becomes unacceptable when the peroxide number exceeds 10 Meq/kg.

As for the storage period, significant differences were found between the periods, as a significant increase in the peroxide value was observed over the storage period, as the peroxide value was at its lowest value in the (0) day period, reaching (1.11) Meq/kg, then it began to increase as the storage periods progressed. Until it reached its highest value on day 15, recording (13.90) Meq/kg[8] found, when he studied adding different concentrations of moringa and olive leaf extracts to manufactured chicken broth and comparing it with (BHT) for 20 days, that the moringa leaf extract was more effective as a natural antioxidant than the olive leaf extract and (BHT), and it had [17] indicated in his study the effect of adding a mixture of curry leaf extract and Moringa oleifera leaf extract in different concentrations to shrimp for a storage period that extended to 15 days, and it reduced The increase in the peroxide number

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compared to the control sample to which the extracts were not added and which recorded the highest peroxide number. It was shown that these extracts contain different chemicals with multiple functions that enable them to be alternatives to industrial additives.

The purpose of performing the peroxide number test is to determine the beginning of the oxidation process and that the antioxidant activity occurs as a result of the presence of active compounds found in plant extracts, which have a high ability to suppress the free radicals formed during the auto-oxidation process of fats, and also have the ability to hinder the activity of some enzymes [5].

It was noted from the results obtained in this study that there was a significant superiority of the samples to which Morinha oleifera leaf extracts were added. This may be due to the fact that Morinha oleifera leaves contain a high level of antioxidant activity and this is due to its components of the active compounds of phenols and flavonoids.

Table (1) shows the effect of adding different proportions of the alcoholic and aqueous extract of Moringa oleifera leaves and the synthetic antioxidant on the peroxide number of fish meat Epinephelus coioidis stored at 4°C.

Store/day										
15	13	11	9	7	5	3	1	0	**concentration PPM**	**Extract**
12.34	**12.11**	**11.77**	**11.30**	**9.22**	**7.09**	**4.60**	**2.14**	**1.11**	**200**	**Aqueous**
11.09	**10.80**	**10.26**	**9.89**	**8.21**	**6.42**	**3.91**	**1.95**	**1.11**	**400**	
10.64	**10.41**	**10.09**	**9.01**	**7.44**	**5.94**	**4.01**	**1.56**	**1.11**	**200**	**Alcoholic**
10.07	**9.69**	**8.41**	**7.12**	**6.10**	**5.81**	**3.10**	**1.21**	**1.11**	**400**	
10.23	**10.03**	**9.89**	**8.71**	**7.51**	**6.16**	**3.12**	**1.32**	**1.11**	**BHT 200**	
13.90	**13.20**	**12.11**	**11.81**	**9.90**	**7.20**	**4.81**	**2.54**	**1.11**	**control**	
LSD = 0.6431										

2-4 The effect of adding different proportions of the alcoholic and aqueous extract of Moringa oleifera leaves and the synthetic antioxidant (TBA) to the meat of Epinephelus coioidis fish stored at a temperature of 4°C.

Table (2) shows the effect of the interaction between treatments and storage duration on the values of thiobarbutic acid (TBA) for Epinephelus coioidis fish steaks, which were treated with aqueous and alcoholic extracts of

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Moringa oleifera leaves at concentrations of 200 and 400 PPM and the synthetic antioxidant BHT at a concentration of 200 PPM and under refrigerated storage at 4°C for storage periods (0, 1, 3, 5, 7, 9, 11, 13, 15) days ,

The results were the treatment of the aqueous extract at a concentration of 200 PPM (0.32, 0.47, 1.11, 1.44, 2.01, 2.51, 2.83, 3.01, 3.47) mg malone aldehyde/kg meat, and the treatment of the aqueous extract at a concentration of 400 PPM (0.32, 0.44, 0.91, 1.2). 7, 1.88, 2.17, 2.33, 2.53, 2.) mg malon aldehyde/kg meat and treatment of alcoholic extract at a concentration of 200 PPM (0.32, 0.36, 0.79, 1.01, 1.34, 1.90, 2.24, 2.44, 2.64) mg malon aldehyde/kg Gum of meat and treatment with alcoholic emulsion concentration 400 PPM (0.32, 0.34, 0.69, 0.87, 0.92, 1.42, 1.72, 1.89, 2.13) mg malon aldehyde/kg meat and treated with synthetic antioxidant BHT at a concentration of 200 PPM (0.32, 0.35, 0.75, 0.9). 0, 1.01, 1.50, 1.87, 1.97, 2.21) mg malondehyde/kg meat, while the control treatment recorded (0.32, 0.49, 1.10, 1.52, 2.20, 2.77, 3.10, 3.49, 3.91) mg malondehyde/kg meat. While the FSIS (2000) confirmed that the maximum permissible limit for thiobarbutic acid values is 2 mg malondehyde/kg meat .

The results of the statistical analysis showed that there were significant differences for Epinephelus coioidis fish meat samples treated with moringa leaf extracts at both concentrations and the synthetic antioxidant compared to the control treatment. It is also noted that the alcoholic extract treatment at a concentration of 400 PPM and the industrial antioxidant treatment were excelled on the aqueous extract treatments On the other hand, the aqueous extract treatments were significantly superior to the control treatment. The control that recorded the highest value of TBA was (3.91) mg malondehyde/kg meat on the fifteenth day, and the alcoholic extract treatment with a concentration of 400 PPM recorded the lowest value of TBA on the fifteenth day and amounted to (2.13) mg malondehyde/kg meat.

From Table (2) we notice that there are no significant differences immediately after manufacturing, as the TBA value reached (0.32) mg malone aldehyde/kg meat, and it was a lower value, but as the storage period increased, there was a significant increase in the TBA value, as it was on the fifteenth day of the aqueous extract. At both concentrations (3.47, 2.82) mg malondehyde/kg meat, respectively, while for the alcoholic extract it was (2.64, 2.13) mg malondehyde/kg meat, respectively, while the synthetic antioxidant treatment recorded (2.21) mg malondehyde/kg meat. While the control factors amounted to (3.91) mg malondehyde/kg meat. The growth of microbes and the activity of enzymes that decompose fats and proteins cause an increase in the percentage of TBA. This is due to the increase in the concentration of malondehyde, which is formed secondary to the oxidation of fats and the breakdown of peroxides, and its production increases as the storage period advances .

The difference in TBA values is due to the role of the active antioxidant compounds (phenolic compounds) present in these extracts, as well as the content of these extracts from these compounds, and this is consistent with what was indicated by (Falowo et al., 2017) that the reason for the decrease in the TBA value when using extracts Moringa

oleifera plant because it contains active compounds (phenols and flavonoids) that have an important role as antioxidants by suppressing free radicals by converting them to a stable state and thus ending oxidation reactions (Andres et al., 2004 .(

These results were consistent with the findings of [6] who indicated that Moringa oleifera leaf extract contributed to protecting goat meat patties from fat oxidation during refrigerated storage with a higher percentage of the synthetic antioxidant (BHT). [9] Extracts of Moringa oleifera leaves, olive leaves, and green tea leaves played a major role in reducing the acid value of processed and chilled tuna fish fingers, because these leaves contain effective compounds with important biological functions.

Table (2) The effect of adding different proportions of alcoholic and aqueous extract of Moringa oleifera leaves and synthetic antioxidants on the TBA value of Epinephelus coioidis fish meat stored at 4°C.

Store/day									concentration ppm	Extract
15	13	11	9	7	5	3	1	0		
3.47	**3.01**	**2.83**	**2.51**	**2.01**	**1.44**	**1.11**	**0.47**	**0.32**	**200**	**Aqueous**
2.82	**2.53**	**2.33**	**2.17**	**1.88**	**1.27**	**0.91**	**0.4 4**	**0.32**	**400**	
2.64	**2.44**	**2.24**	**1.90**	**1.34**	**1.01**	**0.79**	**0.36**	**0.32**	**200**	**Alcoholic**
2.13	**1.89**	**1.72**	**1.42**	**0.92**	**0.87**	**0.69**	**0.34**	**0.32**	**400**	
2.21	**1.97**	**1.87**	**1.50**	**1.01**	**0.90**	**0.75**	**0.35**	**0.32**	**BHT 200**	
3.91	**3.49**	**3.11**	**2.77**	**2.20**	**1.52**	**1.10**	**0.49**	**0.32**	**control**	
LSD = 0.3271										

Conclusions

Adding alcoholic and aqueous extracts of Moringa oleifera leaves to grouper meat steaks at different concentrations led to a reduction in the increase in the values of both the peroxide number and thiobarbituric acid and thus prolonging the shelf life of the grouper meat steaks compared to the control sample. The alcoholic extract with a concentration of 400 PPM was the best in reducing chemical indicators. Moringa oleifera leaf extract can be used as an alternative to industrial antioxidants due to the high effectiveness of these extracts as antioxidants in addition to being a natural plant substance with high nutritional value and no health harms to humans.

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