

# The efficacy of *Rosmarinus officinalis* L. extract in keeping quality of cold fish fillet

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**Abstract-** The effect of rosemary extract on the shelf stability and organoleptic quality of fish stored at 2°C was determined after 0, 3, 6, 9, 12 and 15 days of refrigerated storage. Fish fillet (*Seriola Dumeriri*) treated with 1% (aqueous solution) of rosemary extract had significantly ( $P < 0.05$ ) lower thiobarbituric acid (TBA) and Total Volatile Nitrogen (TV-N) values until 12 day of refrigerated storage compared to control. The result revealed that samples treated with rosemary had lower total bacterial count and psychrophilic count than those of untreated samples. The organoleptic results showed that fish fillets treated with rosemary had a good acceptance throughout storage and rosemary treatment extended the stability of fish fillets to 12 days during refrigerated storage in comparison with the untreated samples.

**Index Terms-** "Fish preservation, *Rosmarinus officinalis* L., Fish fillets, Natural preservatives "

## I. INTRODUCTION

Fish is a highly demanded and nutritious food product, yet perishability remains the biggest challenge for its preservation. The deterioration of fresh fish during storage is attributed to different damage mechanisms, like microbiological spoilage, autolytic degradation, and lipid oxidation [Prabhakar et al., 2020]. Fish is a good source of protein and is low in total fat. It has the added advantage that it is high in fatty acids that protect against heart disease and to some extent is useful for protecting against angina pectoris. On the other hand fish is a sensitive and perishable food tending to fast spoilage. Fish is a complex process in which microorganisms interact with physical and chemical changes. Usually, enzyme activities in fish tissues and chemical reactions are responsible for the loss of freshness of fish, whereas the microbial metabolic activities are involved in the overall spoilage (Khan et al., 2006). After fish are caught, spoilage starts rapidly, and rigor mortis is responsible for changes in the fish after its death. A breakdown of various components and the formation of new compounds are responsible for the alterations in odor, flavor, and texture that happen throughout the spoilage process, and deterioration occurs very quickly due to various mechanisms triggered by the metabolic activity of microorganisms, endogenous enzymatic activity (autolysis), and by the chemical oxidation of lipids (Prabhakar et al., 2020; Gram and Huss, 1996). Lipid oxidation can occur either enzymatically or non-enzymatically in fish. In the enzymatic hydrolysis (lipolysis) process, glycerides are split by lipases, forming free fatty acids that are responsible for the common off-flavor (rancidity) and from the denaturation of sarcoplasmic and myofibrillar proteins (Huis In't Veld, 1996). Lipid oxidation can occur either enzymatically or non-enzymatically in fish. In the enzymatic hydrolysis (lipolysis) process, glycerides are split by lipases, forming free fatty acids that are responsible for the common off-flavor (rancidity) and from the denaturation of sarcoplasmic and myofibrillar proteins (Huis In't Veld, 1996). The use of synthetic and natural antioxidants to control lipid oxidation in seafood is a necessary measure. Synthetic antioxidants such as butylhydroxytoluene (BHT) and butylhydroxyanisole (TBHQ) and others are used to solve oxidation problems. However, it has been found that synthetic antioxidants are carcinogenic and mutagenic agents, so they are being tried to replace them with natural antioxidants (Pourashouri et al., 2009). Several studies have been conducted to extend the shelf life of fresh foods including fish products by using different preservation strategies. Thus, *Rosmarinus officinalis* extracts have been successfully used as natural preservatives; as an antioxidant agent in numerous species of sardines (*Sardine pilchardus*) (Serदारoglu and Felekoglu, 2005) as well as in tilapia (*Oreochromis niloticus*) (Ibrahim and Sherif, 2008). The Mediterranean climate in Libya favors the growth of a great number of plant species, some of which have various medicinal and antioxidant potential properties. Rosemary (*Rosmarinus officinalis* L.) belongs to *Lamiaceae* family is one of the widely spread plant in El-Jabel El-Agadar province (East of Libya) and seems to be a rich source of phenolic acids (Adris et al., 2019), therefore, it is considered to be a promising source of natural antioxidants and antimicrobial agents (Adris et al., 2019). There was an evidence that rosemary is able to prolong the shelf life of food and maintain its quality during storage, and therefore it has already been used as a vital preservative in the food industry. Twenty seven spices have been defined by the US Food and Drug Administration (FDA) as safe, including rosemary (Charalambous, 1994). Because of consumer demand for fresh refrigerated foods with extended shelf life, considerable research has been directed toward using various preservation strategies to preserve or prolong the shelf life, while ensuring the safety, of fresh foods including fishery products (Sallam, 2007). However,

limited data is available regarding the application of rosemary extract to prolong the shelf life of fish. Therefore the objective of this study was to evaluate the effectiveness of rosemary extract on extending the shelflife of fish fillet prepared from Seriola Dumeriri fish.

## II. MATERIALS AND METHODS

### Plant Material

The rosemary plant (*Rosmarinus officinalis* L.) was collected from the Al-Jabal Al-Akhdar region (in the far east of Libya) *Rosmarinus officinalis* L. (Rosemary) in late spring and transferred to the laboratory where the plant leaves were washed, dehydrated (room temperature; ~ 28°C) and powdered with using an electric grinder and the powder was kept in a closed container at -60 degrees until extraction.

### Preparation of Rosemary extract

Because nearly all of the identified components from plants active against microorganisms, are aromatic or saturated organic compounds and they are most often obtained through initial extraction of organic solvents, methanol is used in this study to extract biologically active ingredients (Naili et al., .2010) from rosemary plant. Extraction of rosemary plant was conducted by weighing 50 g of dried rosemary then extraction by adding 500 ml of methanol (80%) with shaking (120 rpm) for 24 hrs. After filtration through gauze, plant debris was re-extracted twice then the collected extract was filtrated by filter paper (Whatman no.1). The filtrates were concentrated under reduced pressure (at 35°C) then lyophilized and saved in closed bottles at 5°C until use.

### Fish Sample preparation and treatment:

Fresh Cerola Dumeriri was caught from the sea of Haniyeh District, Al-Jabal Al-Akhdar Governorate-Libya and immediately cooled by placing in an ice box and transported to the laboratory within three hours of catching. It is a high quality fish and abundant in the shores of Libya in the spring and autumn. It is characterized by a grayish-blue color, a length of about 125 cm, and a streamlined body with a crescent tail. After gutting, the fish muscle was washed and filleted (130 ±10 gm each) then immersed for 10 min in 1% aqueous solution (w/v) of lyophilized methanolic rosemary extract. Fillet samples were left to drain and placed in sterile polyethylene bags and refrigerated at 2 ±1C° for fifteen days. Three samples were withdraw every days for chemical and microbiological analysis.

### pH measurement

pH of treated and untreated fish samples was measured according to the method mentioned by (Abou-Taleb et al.,2007) by adding distilled water to fish fillet sample at a ratio of 1:10 (water: fish meat) and homogenizing the sample using an electric mixer. The pH was measured using a PH meter (Terminal 740 inolab seziess, WTW, Germany).

### Thiobarbituric acid (TBA) Test:

This test was carried out by measuring the malondehyde according to the method described by Siu and Draper (1978), where 10 grams of fish meat were mixed with 50 milliliters of distilled water in an electric mixer for 2 minutes. The homogenate was transferred to a boiling flask and 47.5 ml of distilled water and 2.5 ml of 4N HCl were added (final pH 1.8).The mixture was heated until about 50 ml of distillate was collected. Five ml of distillate was transferred to a test tube then 5 ml of 0.02M thiobarbituric acid (TBA) in H<sub>2</sub>O was added. The tube was placed in a water bath for 35 minutes and cooled for 10 minutes. The absorbance was measured at the wavelength of 538 nm in Aquamate Plus UV/Vis Spectrophotometer (Thermo Scientific, England). The concentration of TBA was expressed as mg Malone Aldehyde/kg of fish meat.

### Total volatile base nitrogen (TVB-N) Test:

Total volatile nitrogen compounds were measured according to the standard method described in (AOAC, 2002). For this 100 g of fish meat were homogenized with a laboratory blender for 2 min after adding 300 ml of Trichloroacetic acid (7.5%). The mixture was filtered to obtain a clear solution ready for analysis. 50 ml of the filter were transferred to a distillation tube and 10 ml of sodium hydroxide (NaOH 20%) were added and the apparatus immediately sealed and the steam distillate was collected in a flask containing 5 mL of 4 % boric acid and a few drops of mixedindicator (methyl red /methylene blue 2:1). The steam distillation procedure was continued for 5 min and the distillate had been collected. The obtained quantity of basic solution was titrated with hydrochloric acid (N 0.05). The TVB-N content was determined after blank correction that had been determined by the steam distillation with 50 mL of distilled water sample. Concentration of volatile nitrogen compounds was expressed as mg nitrogen/100 g of fish meat.

### Microbiological analysis of fish fillet

Microbial loads of rosemary treated and control fillet samples (three samples each) was determined by withdrawing samples periodically during refrigerated storage. Ten gm of each treatment was homogenized into stomacher bag using Stomacher Blender (LB400,VWR,UK) for 2 min. Serial dilutions were prepared and pour plate technique was applied according to the standard methodologies (Gerhardt *et al.*, 1994). The total viable count of bacteria and count of psychrotrophic bacteria. were expressed as log CFU/g.

### Sensory evaluation of treated fish fillet

Sensory analyses of fish fillet samples was conducted using 9-point hedonic scale (Peryam and Girardot, 1952) to determine the consumer acceptability of uncooked rosemary treated and control fish fillet. Testers were received an explanation of the study Fish samples were presented to the testers and asked to score smell, colour and general acceptance from 1 to 9 where 1 = very

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disliked, 5 = disliked and disliked (midpoint), and 9 = very liked. Scores of separate attributes were gathered to give a comprehensive sensory score.

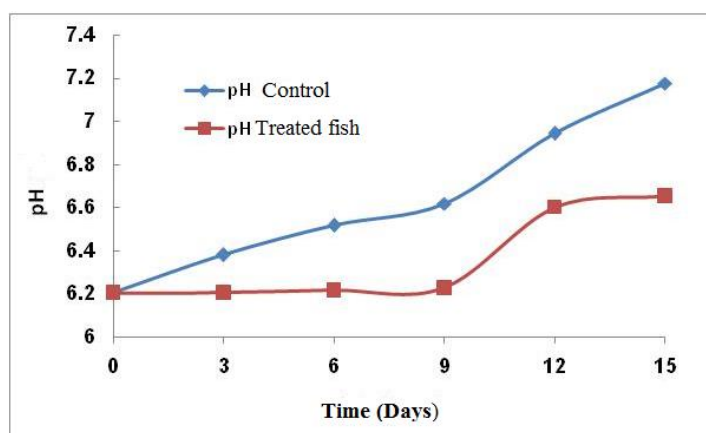
**Statistical analysis**

Statistical analysis was performed using complete randomized design. Two-way ANOVA analysis ( $P \leq 0.05$ ) was conducted to determine the efficacy of the rosemary in keeping quality of refrigerated fish. Means were separated using Duncan’s multiple range test with  $\alpha = 0.05$ . Analyses were performed with SPSS software (Version 14.0; SPSS, Chicago, IL).

**III. RESULTS AND DISCUSSION**

**Change in pH**

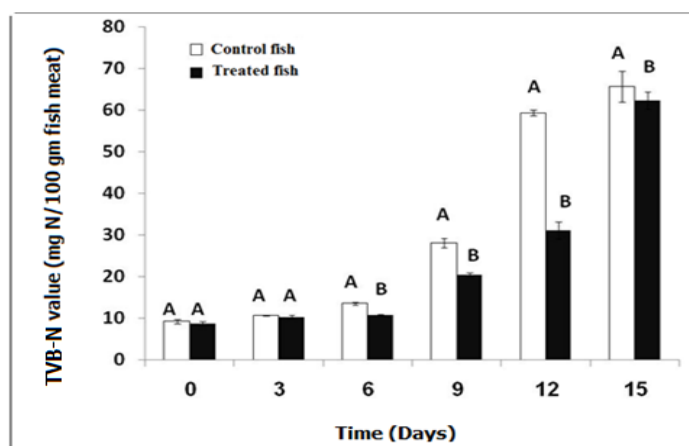
The change in pH of fish fillets during storage at 2°C is shown in Figure (1). Initially, pH was approximately 6.2 at time zero for both not treated (control) and fish treated with rosemary extract. During the storage at 2°C, pH values of control increased rapidly from this sampling day onwards to reach 6.6 on day 9 of cold storage and continued to increase at a faster rate than the treated fish samples to reach a value of 7.2 at the end of the cold storage period (15 days). However, a slight increase (6.5) was reported for treated fish samples at day 15 of storage. The increase in pH values is due to the deterioration of the food substance as a result of microbial activity and basic nitrogenous compounds such as ammonia and other basic nitrogenous compounds, which are produced as a result of the action of microbes (Gram and Dalgaard, 2002).



**Figure (1): Effect of rosemary extract on the pH of fish stored at 20°C for 15 days.**

**Changes in total volatile nitrogen compounds (TVB-N):**

Volatile nitrogen compounds are an important indicator of fish quality during storage. The TVB-N is widely used as a fish spoilage index, as an increase in this parameter has been associated with bacterial spoilage and endogenous enzyme activity, which lead to the loss of quality in fish (Dolea et al., 2018). The acceptability limit of TVB-N varies and depends on the fish species, region, season, age or sex. In addition, this parameter depends on whether the fish is fresh or processed (Dolea et al., 2018). Connel (1990) suggested that 30-40 mg N/100 g and 60 mg N/100 g sample of TVB-N (in terms of wet weight) is an upper limit for freshwater and saline fish respectively. In this study, changes in total volatile basal nitrogen (TVB-N) of fish during storage at 2°C are shown in Figure (2). Nitrogenous compounds value decreased immediately after treatment from 9.24 for untreated fillets to 8.72 mg nitrogen/100 g for treated fish sample.

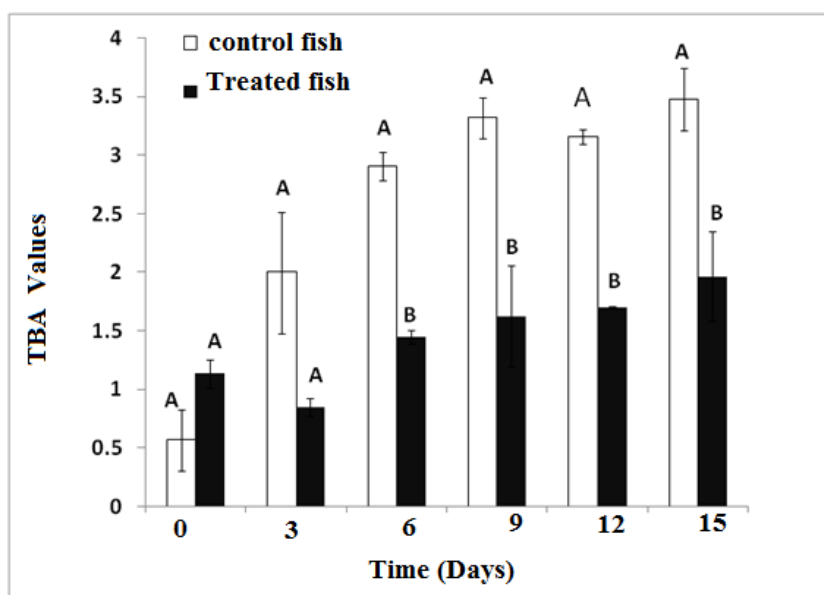


**Figure (2) :Effect of rosemary extract on content of TVB-N (mg nitrogen/100 g of fish meat) of fish stored at 2°C.** In each time period the averages bearing similar letters have no significant differences at ( $p \leq 0.05$ ).

During cold storage, these volatile compounds increased in control samples reaching 28.14 mg nitrogen/100 gm on day 9, although it was still within the acceptable limit for fresh fish. In contrast, the TVB-N levels of samples treated with rosemary extract, kept under the acceptability limit (31.16 mg nitrogen/100 g sample) until day 15. Control samples showing an important increase through to day 15 of study where values were significantly exceeded the upper limit (63 mg nitrogen/100 g sample) of TVB-N (Fig. 2). The increase in shelf life of treated fillets may be due to the inhibitory effect of rosemary on microbial growth, which delayed the hydrolysis of fish protein in treated fish compared to control samples. The ability of this extract to inhibit fat oxidation is related to the total content of phenolic compounds, as phenolic antioxidants prevent the formation of fat free radicals, which interact with the absorbed oxygen in the self-oxidation process, thus delaying the process of self-oxidation of fat (Dolea et al., 2018).

### Changes in TBA

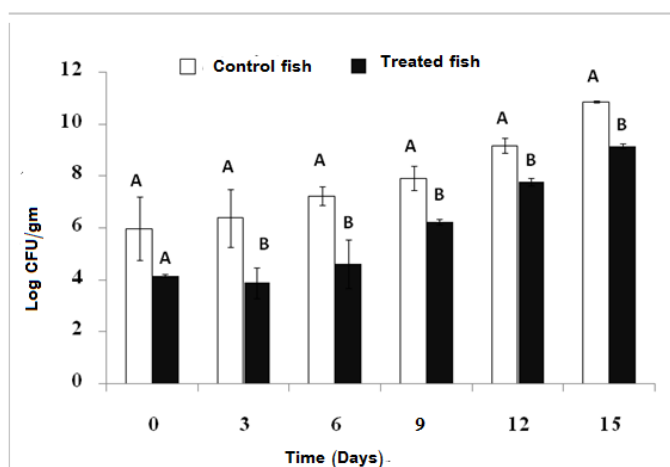
TBA is a reference for measuring secondary lipid oxidation by measuring malonaldehyde, which is a by-product of oxidation resulting from the breakdown of hydroperoxides formed during the oxidation of polyunsaturated fatty acids. The peroxides are compatible with the oxidative deterioration of the unsaturated fatty acids of fish meat and lead to the production of undesirable taste and smell and thus be undesirable. The value of TBA is one of the important methods for the determination of oxidation products in fats and oils (Ramanathan and Das, 1992; Kulas and Ackman, 2011). Bonnl (1994) stated that good quality fish and fish products have TBA values of less than 2 mg malonealdehyde /kg of fish meat and therefore fish with TBA values of more than 2 mg malonealdehyde/kg will give a rancid odor and taste. In current study, changes in TBA of fish during storage are shown in Figure (3): Immediately after treatment and until the third day of cold storage, there were no significant differences ( $p > 0.05$ ) between rosemary treated and untreated fillet samples. During storage, the TBA values of the control increased to reach 2 mg malonealdehyde /kg fish meat on the third day, which is considered within the acceptable limit, while the TBA values for the treated fish did not exceed 1.97 mg malonealdehyde /kg fish meat until the fifteenth day of storage at 2°C. On the contrary, control samples showing a progressive increase through to day 15 of study to exceed (3.4mg malonealdehyde /kg of fish meat) the acceptable limit of this parameter (2 mg malonealdehyde/kg). The obtained results suggested that rosemary extract extended the shelf life of treated fish fillets compared to untreated fillets. The inhibitory effect of rosemary on oxidation in this study is consistent with the results of several other studies. Varelziz *et al.*, (1997) found that using rosemary extract to preserve frozen and chopped mackerel fillets delayed fat oxidation and produced significantly less malonaldehyde compared to untreated samples. Tironi *et al.*, (2010) studied the effect of rosemary extract (200-500ppm) on the changes that could occur in protein and fat in minced salmon frozen at -11°C and found that the high concentration of rosemary delayed the oxidation process up to 50% compared to the untreated samples and there was a decrease in muscle tone loss. Cadun *et al.*, (2008) studied the effect of rosemary extract (300 ppm) on the quality of cryo-stored shrimp (1°C). The study showed that adding rosemary extract significantly decreased TVB-N value during storage and TBA value of the treated group compared with that of the untreated group. A similar study showed that rosemary extract was very effective in reducing lipid oxidation in slices of sea propolis (*Sparus aurata*) stored at 1°C (Gimenez *et al.*, 2004). In another study, rosemary extract (0.2%) was used as a natural preservative to control the chemical properties (TVB-N and TBA values) in vacuum-packed carp (*Carassius auratus*) cooled and stored at 1 m. Rosemary significantly slowed the increase in TVB-N and TBA values in treated samples compared to untreated ones (Li *et al.*, 2012).



**Figure (3): Effect of rosemary extract on fish content stored at 2°C of TBA (mg Malone Aldehyde/kg of fish meat) In each period the averages bearing similar letters have no significant differences at the level of probability ( $p \leq 0.05$ ).**

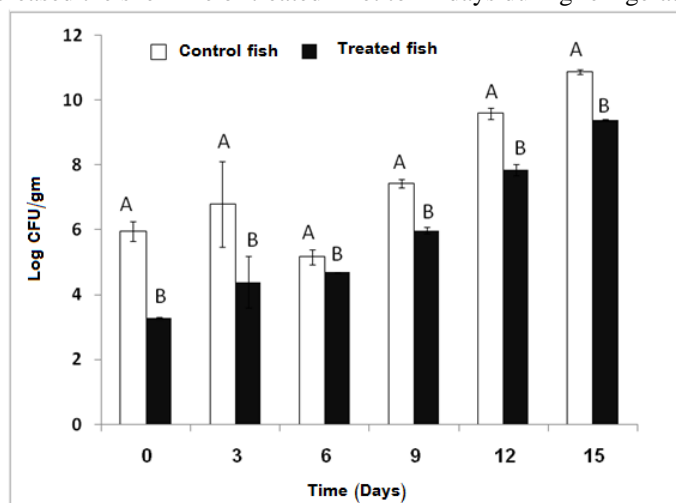
**Microbiological analysis**

Figure (4) represents the total viable counts (TVC) of fish fillet throughout 15-days of cold storage at 2°C ±1. It is clear that dipping fish fillet samples into 1% (w/v) aqueous solution of rosemary extract caused a significant dropping in bacterial count ( $p < 0.05$ ) on fillets samples. The initial count of TVC was around 6.0 log<sub>10</sub> CFU/g and rosemary treatment exhibited an immediate effect on treated fillet as bacterial count decreased significantly to 4.1 log<sub>10</sub> CFU/g. After fish catching, some sources of contamination such as aquatic environment, surface or intestinal microorganisms, equipments, and handling may contribute in quick deterioration of fish muscle. In this study, TVC values of control and treated fillet samples exhibited a gradual increase during storage period starting from day 6 as the control fillet samples reached a value of 7.70 log<sub>10</sub>CFU/g which was beyond the microbiological acceptability limit of fresh fish (7.0 log<sub>10</sub> CFU/g). On the contrary a reduction of up to 2 log cycles between rosemary treated fillet and control samples was reported after 12 days of cold storage suggesting the efficacy of treatment in keeping microbiological acceptable limit up to 12 days compared to only 6 days for controls as total count significantly increased and exceeded the permissible limit to reach a value of 9.0 log<sub>10</sub> CFU/g (Fig. 4).



**Figure 4: Effect of rosemary methanolic extract on total viable count of fish fillets during cold storage at 2°C for 15 days.** Bars: represent means ± SD of three replicates. For each day significant differences ( $P \leq 0.05$ ) indicated by different letters on bars.

Regarding psychrotrophic bacteria (PTC), the same behavior as that of TVC was reported as control again exhibited higher counts throughout the storage period compared to treated samples (Fig. 5). In general, results detected that rosemary extract delayed the rate of microbial spoilage and increased the shelf life of treated fillet to 12 days during refrigerated storage at 2°C.



**Figure 5: Effect of rosemary extract treatment on total psychrophilic bacteria count on fish fillet treated stored at 2°C for 15 days.** Bars: represent means ± SD of three replicates. In each time period the averages bearing similar letters have no significant differences at ( $p \leq 0.05$ ).

The antimicrobial properties of rosemary have been reported in the literature (Kenar *et al.*, 2010; Ucak *et al.*, 2011; Gao *et al.*, 2014). Kenar *et al.*, (2010) reported that shelf life of vacuum packed sardine fillet stored at 3±1°C has extended to seven days after dipping fillet in ethanolic extract of rosemary. Additionally, rosemary extract (at 0.4 and 0.8%) was found effective in controlling biochemical indices and bacterial growth in vacuum packed Atlantic mackerel burgers during cold storage (Ucak *et al.*, 2011). Recently, Gao *et al.*, (2014) displayed a synergistic effect of rosemary extract with nisin in prevention lipid oxidation, protein degradation, nucleotide breakdown and microbial growth in pompano fillet (*Trachinotus ovatus*) during cold storage at 4°C. Turhan *et al.*, (2009) investigated the effect of brining with rosemary extract on the oxidative stability of anchovies stored at 4°C for 28 days. Their results indicated that adding brining with rosemary extract delayed the lipid oxidation of anchovies and the highest antioxidant effect was observed in brined anchovies with rosemary during storage as indicated by peroxide value (POV), thiobarbituric acid reactive substance and oxidative rancidity scores.

### Effect of rosemary extract on sensory characteristics of fish fillet

Sensory assessment is the most popular way of assessing the freshness of fish. It is simple, fast, and provides immediate quality information. The sensory specifications of fish are clearly visible to the consumer and are essential for consumer satisfaction (Sallam *et al.*, 2007). Table (1) displays a summary of the results of sensory evaluation of fillet stored at 2°C. The assessment was performed for all treatments every 3 days up to day15. Rosemary treatment and controls had no significant differences in the acceptability of sensory characteristics during the first three days of storage period. However, from day 6 onwards treated fillet samples were more acceptable since score of all sensory attributes were significantly ( $p < 0.05$ ) higher than those of control samples (Table 1).

**Table 1: Effect of rosemary extract on sensory characteristics of fish fillet stored at 2°C**

Storage time (days) at 2°C	Treatment	Smell	Colour	Overall acceptability
0	Control	8.8 <sup>a</sup>	8.7 <sup>a</sup>	8.6 <sup>a</sup>
	1% rosemary	8.9 <sup>a</sup>	8.8 <sup>a</sup>	8.7 <sup>a</sup>
3	Control	7.5 <sup>a</sup>	8.0 <sup>a</sup>	7.8 <sup>a</sup>
	1% rosemary	8.8 <sup>a</sup>	8.9 <sup>a</sup>	8.6 <sup>a</sup>
6	Control	6.2 <sup>a</sup>	6.9 <sup>a</sup>	6.1 <sup>a</sup>
	1% rosemary	8.0 <sup>b</sup>	8.4 <sup>b</sup>	8.0 <sup>b</sup>
9	Control	4.8 <sup>a</sup>	3.7 <sup>a</sup>	5.5 <sup>a</sup>
	1% rosemary	6.9 <sup>b</sup>	6.9 <sup>b</sup>	6.5 <sup>b</sup>
12	Control	3.0 <sup>a</sup>	2.5 <sup>a</sup>	3.1 <sup>a</sup>
	1% rosemary	6.4 <sup>b</sup>	6.7 <sup>b</sup>	5.2 <sup>b</sup>
15	Control	2.0 <sup>a</sup>	2.1 <sup>a</sup>	2.2 <sup>a</sup>
	1% rosemary	3.2 <sup>b</sup>	3.9 <sup>b</sup>	4.3 <sup>b</sup>

For each sensory attribute; in the same day averages with the same letters indicate no significant differences at ( $P \leq 0.05$ ).

### CONCLUSION

Rosemary extract showed the ability to delay oxidation and microbial spoilage and extend the shelf life of refrigerated fish, which enhances the possibility of using this herb to preserve this type of perishable food. The study showed promising results for the possibility of using extracts derived from these plants as potential alternatives to preservatives in the food industry. However, if plants and extracts are to be used for food preservation, issues of safety and toxicity will always need to be addressed.

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