

## ROSEMARY OIL WITH DECONTAMINATION OF HORSE MACKEREL (*TRACHURUS TRACHURUS* L. 1758) AND DETECTION OF SHELF LIFE

O. P. CAN<sup>1</sup>, S. SAHIN<sup>2</sup> and H. YALCIN<sup>3</sup>

<sup>1</sup>University of Cumhuriyet, Department of Food Engineering, Faculty of Engineering, 58140 Sivas, Turkey

<sup>2</sup>University of Cumhuriyet, Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, 58140 Sivas, Turkey

<sup>3</sup>University of Mehmet Akif Ersoy, Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, 15030 Burdur, Turkey

### Abstract

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This study was performed to examine the effect of decontamination with rosemary oil on the shelf life changes of the horse mackerel (*Trachurus trachurus* L. 1758). The samples were divided into three groups as control (C), application with 0.5% rosemary oil (A) and application with 1% rosemary oil (B) and storage at +4°C, day 21. Regarding with microbiological, chemical and sensory quality changes, the samples were examined on 0, 3<sup>rd</sup>, 6<sup>th</sup>, 9<sup>th</sup>, 12<sup>th</sup>, 15<sup>th</sup>, 18<sup>th</sup> and 21<sup>st</sup> days. Total mesophilic aerobic bacteria count reached 6.1 log<sub>10</sub> cfu/g at samples of group A on 15<sup>th</sup> day, while 4.4 log<sub>10</sub> cfu/g samples of group B on 21<sup>st</sup> day. Rosemary oil groups (0.5%-1%) had more shelf life than control, microbiologically. According to chemical analyses results, the application of 0.5% and 1% of rosemary oil has an effect on control of lipid oxidation. Panelists preferred 0.5% rosemary oil (Group A) with regard to sensory quality changes. As a result; rosemary oil (1%) has significant effect on the protection from deterioration of horse mackerels stored at +4°C until 21 days.

**Key words:** Horse Mackerel, rosemary, quality changes, shelf life

**Note:** This study was a poster presentation at the 11<sup>th</sup> Food Congress, October 10-12, 2012 in Hatay, Turkey.

### Introduction

With the rapid increase of world population, one of the most important resources that provide the animal protein needs of humans is aquaculture products. Aquaculture products are important food products due to their rich nutrient compositions. Their high protein content, essential amino acids and vitamin rich structure along with high digestibility and right amounts of unsaturated fats that they contain make aquaculture products valuable (Turan et al., 2006).

Aquaculture products and fish are important food sources and even though they are kept under suitable conditions, they suffer high quality losses due to their sensitive and rapidly spoiling structure. The fundamental reasons for quality loss in aquaculture products and fish are autolysis, oxidation and microbial spoilage. Hence, starting from the time that they are caught they should be rapidly cooled, processed and presented to the consumer healthily (Unluturk and Turantas, 1999).

The shelf life of fish products with their easily spoiling structure is limited with enzymatic and microbiologic spoilage. There are many quality control methods to determine spoilage in fish. Measurement of post mortem changes has been taken as basis in the determination of sensory, microbiologic and chemical quality control changes (Celik et al., 2002; Metin et al., 2002).

Fish oil is richer in unsaturated fatty acids in comparison with the fats of other animals thereby causing fish products to be more sensitive to oxidative spoilage. It is important to prevent lipid oxidation that might arise during the storage time of aquaculture products and fish in order to preserve their freshness and quality. The most effective materials used to prevent lipid oxidation are antioxidants (Karpinska et al., 2001). Many synthetic and natural antioxidants are used to prevent lipid oxidation in foods. With the determination of the toxic effects of synthetic antioxidants, the demand for natural antioxidants has increased. Even though plants are a

good source of natural antioxidants, their antioxidant activities are due to the presence of polar phenolic compounds and essential oils (Ozogul et al., 2010).

It has been stated that natural phenolic antioxidants isolated from various plants such as rosemary, thyme, sage, black pepper and turmeric have strong effects in the preservation of the freshness of processed foods (Coban and Patir, 2010). Rosemary (*Rosmarinus officinalis* L.) has become one of the most preferred plant types due to its rich antioxidant content and less colour and odour in comparison with other plants. The antioxidant property of rosemary extract is due to the carnosol, carnosic acid and rosmarinic acid content (Riznar et al., 2008).

Studies regarding the increase of the shelf life of fish products via various technological processes have been the topic of different studies. Guran et al. (2011) have stated that fish patties made using bonito treated with rosemary oil have shelf lives of up to 14 days at  $4\pm 1^\circ\text{C}$ . Dikici et al. (2011) indicate that the shelf life of rainbow trouts prepared with different concentrations by adding rosemary extract is about 12 days. Ozogul et al. (2010) have stated that the shelf life of rosemary extract added vacuumed sardine fillet at  $+4^\circ\text{C}$  is 13 days. In a study carried out by Bozkurt et al. (2006), it has been stated that shelf life of raw fish fillets prepared by adding euganol have increased up to 42 days at  $4\pm 1^\circ\text{C}$ . Patir et al. (2011) the consumability of rainbow trouts in vacuumed packages prepared by adding euganol has been preserved at least for 70 days.

This study has been carried out to examine the effects of applying various concentrations of (0.5%, 1%) rosemary (*Rosmarinus officinalis* L.) oil to eviscerated horse mackerel (*Trachurus trachurus* L. 1758) stored at  $+4^\circ\text{C}$  on the microbiologic, chemical and sensory quality changes.

## Material and Methods

In the study, horse mackerel fish with an average weight of 50-55 g obtained from the Sivas Fish Market have been used. Rosemary (Herbalox® Seasoning) essential oil used in the preparation of experimental samples was obtained from Kalsec® (Kalsec®, Inc, Kalamazoo, MI USA). The rosemary essential oil used is soluble in both water and oil.

### Preparation of the decontamination solution

In the study, decontamination solution containing rosemary has been prepared. Mixing was applied for a period of 10 minutes using a magnetic mixer while preparing the decontamination solution in order to ensure the homogeneous distribution of the essential oil in water and this process was carried out at room temperature. 500 ml of distilled water was added to a 1 liter volumetric flask during the preparation of 0.5% rosemary solution after which 5 ml rosemary oil

was added and mixing was started following the addition of 2 ml Tween 80 to ensure the homogeneity of the mixture and after 5 minutes of mixing the solution was completed up to 1 liter and mixing was continued. 10 ml rosemary oil was used during the preparation of 1% rosemary solution and the steps described above were repeated.

### Preparation of the fish samples

The fish brought to the laboratory were washed thoroughly with water after which evisceration was carried out with a cross section from the abdominal region. They were rewashed after this process and left to drain for a few minutes. The fish were divided into three groups. The first group was the control (C), the second group (A) was treated with 0.5% rosemary containing decontamination solution while the third group (B) was treated with 1% rosemary containing decontamination solution. During decontamination process, fish from two groups (eviscerated but as a whole) were placed in 5 liter capacity bags which were sealed tightly following the addition of the solution that was prepared previously and the bags were shaken by hand for a period of 1 minute. Following this process, the samples were first drained for a short time and afterwards they were placed in plastic boxes to be stored at  $+4^\circ\text{C}$ . The stored bags from all 3 groups were randomly sampled on the 0, 3<sup>rd</sup>, 6<sup>th</sup>, 9<sup>th</sup>, 12<sup>th</sup>, 15<sup>th</sup>, 18<sup>th</sup> and 21<sup>st</sup> days which were subject to microbiological, chemical and sensory analyses. The study was repeated three times.

### Microbiological analysis

Ten grams of samples were aseptically weighed and homogenized in a Stomacher (Lab Blender 400) for 2 min with 90 ml of sterile peptone water (0.1% peptone, Merck). Further decimal dilutions were made with the same diluents (Harrigan, 1998). For total mesophilic aerobic bacteria (TMAB), Plate Count Agar (Merck, 1.05463) was used. Plates were incubated at  $30^\circ\text{C}$  for 48 hours. Psychrotrophic bacteria (PB) count was determined on Plate Count Agar (Merck, 1.105463) after incubation at  $7\pm 1^\circ\text{C}$  for 240 hours. For *Staphylococcus-Micrococcus* spp. (SM) were used Mannitol Salt Agar (Merck 1.05404), was used after incubation  $37\pm 1^\circ\text{C}$  for 48 hours. For the *Enterobacter* Violet Red Bile Agar (Oxoid CM 485), after incubation  $37\pm 1^\circ\text{C}$  for 24 hours (Halkman, 2005).

### Chemical analysis

Total volatile basic nitrogen (TVBN) content was determined according the method of Varlik et al. (2004). The pH value was determined according the method of AOAC (AOAC, 1990). The TBA value was determined according the methods Tarladgis et al. (1960) and milligrams of malondialdehyde (MDA)/kg flesh.

### Sensory evaluation

For sensory analysis, fish samples were cooked individually in a pan fried for 10 min and immediately presented to the panelists. Sensory evaluation was conducted in individual booths under controlled conditions of light, temperature, and humidity. The samples were tested by eight panelists in small aluminum trays. The panelists were selected and trained according to ISO standards (ISO 8586-1; 1993). The quality of each sample was classified using characteristics to describe the texture, taste, colour, odour, appearance, and overall acceptance. A hedonic scale from 1 to 5 was used to evaluate fish samples: 1 - very bad, 2 - bad, 3 - normal, 4 - good and 5 - very good (Kurtcan and Gonul, 1987).

### Statistical analysis

Analysis of the data was conducted using Statistical Analysis System (SAS) package programmed. Values between groups and within group-between days were compared. Data were subjected to variance analysis in accordance with 3x1x3x1 factorial design and in terms of fix effects and inter-variable interactions so that "repetition number x sampling time x test groups x number of samples examined at one instance from each test group". According to General Linear Model procedure, Fisher's smallest squares average (LSD) test was used. Standard deviation figures of all averages were calculated (SAS, 1996). \* $p < 0.05$  was considered as statistically significant.

### Results

The total number of mesophilic aerobic bacteria (TMAB), psychrophilic bacteria, *Staphylococcus-Micrococcus* spp. and *Enterobacter* in the beginning for horse mackerel was determined respectively as 3.7 log<sub>10</sub> cfu/g, 2.8 log<sub>10</sub> cfu/g, 3.9 log<sub>10</sub> cfu/g and 2.6 log<sub>10</sub> cfu/g (Table 1).

On the 15<sup>th</sup> day of storage, TMAB, psychrophilic bacteria, *Staphylococcus-Micrococcus* spp. and *Enterobacter* reached respectively 7.6 log<sub>10</sub> cfu/g, 6.3 log<sub>10</sub> cfu/g, 5.2 log<sub>10</sub> cfu/g and 6.1 log<sub>10</sub> cfu/g. Whereas for the A and B groups decontaminated with rosemary oil, the number of TMAB, psychrophilic bacteria, *Staphylococcus-Micrococcus* spp. and *Enterobacter* were determined respectively as 6.1-3.6 log<sub>10</sub> cfu/g, 2.5-1.6 log<sub>10</sub> cfu/g, 3.8-2.4 log<sub>10</sub> cfu/g and 4.1-2.6 log<sub>10</sub> cfu/g (\* $P < 0.05$ ).

In horse mackerel samples stored at +4°C, the pH, TVB-N, TBA values have been given in Table 2. Data obtained from sensory analyses have been given in Table 3. The samples of control group were analyzed for colour, odour, taste, texture and general appeal level on the 0, 3<sup>rd</sup> and 6<sup>th</sup> days of storage. Whereas A (0.5%) and B (%1) groups decontaminated with rosemary oil were analyzed on the 0<sup>th</sup>, 3<sup>rd</sup>, 6<sup>th</sup>, 9<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> days of storage.

**Table 1**

**The results of microbiological analyses of horse mackerel during storage period at +4°C (log<sub>10</sub> cfu/g)**

Microorganisms	G	Storage time (day) ( $\bar{x} \pm S$ )							
		0	3	6	9	12	15	18	21
TMAB	C	3.7±0.1 <sup>c.Z</sup>	4.1±0.3 <sup>bc.Z</sup>	4.6±0.1 <sup>b.Z</sup>	5.3±0.1 <sup>b.Z</sup>	6.1±0.3 <sup>b.Z</sup>	7.6±0.2 <sup>a.Z</sup>	-	-
	A	3.0±0.1 <sup>c.ZY</sup>	3.6±0.3 <sup>c.Z</sup>	3.9±0.2 <sup>c.Z</sup>	4.9±0.2 <sup>b.Z</sup>	5.7±0.1 <sup>b.Z</sup>	6.1±0.1 <sup>a.Z</sup>	6.8±0.1 <sup>a.Z</sup>	7.3±0.2 <sup>a.Z</sup>
	B	2.1±0.1 <sup>b.Y</sup>	2.3±0.2 <sup>b.Y</sup>	2.8±0.1 <sup>b.Y</sup>	2.4±0.1 <sup>b.Y</sup>	3.1±0.2 <sup>a.Y</sup>	3.6±0.3 <sup>a.Y</sup>	4.1±0.1 <sup>a.Y</sup>	4.4±0.1 <sup>a.Y</sup>
Psychrophilic bacteria	C	2.8±0.1 <sup>c.Z</sup>	3.6±0.1 <sup>bc.Z</sup>	4.2±0.2 <sup>b.Y</sup>	4.4±0.1 <sup>b.Z</sup>	5.6±0.1 <sup>a.Z</sup>	6.3±0.2 <sup>a.Z</sup>	-	-
	A	1.8±0.1 <sup>b.Y</sup>	2.1±0.1 <sup>ab.Y</sup>	2.0±0.2 <sup>ab.Y</sup>	2.4±0.3 <sup>a.ZY</sup>	2.7±0.1 <sup>a.Y</sup>	2.5±0.1 <sup>a.Y</sup>	3.0±0.1 <sup>a.Z</sup>	3.4±0.1 <sup>a.Z</sup>
	B	1.1±0.2 <sup>a.Y</sup>	1.3±0.3 <sup>a.X</sup>	1.8±0.1 <sup>a.Y</sup>	1.6±0.3 <sup>a.Y</sup>	1.8±0.1 <sup>a.Y</sup>	1.6±0.1 <sup>a.Y</sup>	1.3±0.1 <sup>a.Y</sup>	2.1±0.2 <sup>a.Z</sup>
<i>Staphylococcus-Micrococcus</i> spp.	C	3.9±0.1 <sup>b.Z</sup>	4.3±0.1 <sup>ab.Z</sup>	4.6±0.2 <sup>a.Z</sup>	4.8±0.2 <sup>a.Z</sup>	4.6±0.3 <sup>a.Z</sup>	5.2±0.2 <sup>a.Z</sup>	-	-
	A	2.6±0.1 <sup>b.Z</sup>	2.8±0.1 <sup>b.Y</sup>	3.1±0.1 <sup>b.Y</sup>	3.6±0.2 <sup>a.Z</sup>	3.2±0.1 <sup>a.Z</sup>	3.8±0.1 <sup>a.Y</sup>	4.1±0.1 <sup>a.Y</sup>	4.0±0.1 <sup>a.Z</sup>
	B	1.3±0.1 <sup>b.Y</sup>	1.8±0.2 <sup>b.X</sup>	2.1±0.2 <sup>ab.X</sup>	2.3±0.2 <sup>a.Y</sup>	2.6±0.1 <sup>a.Y</sup>	2.4±0.1 <sup>a.X</sup>	2.8±0.1 <sup>a.Y</sup>	3.2±0.1 <sup>a.Y</sup>
<i>Enterobacter</i>	C	2.6±0.1 <sup>b.Z</sup>	2.4±0.1 <sup>b.Z</sup>	3.2±0.2 <sup>b.Z</sup>	4.8±0.2 <sup>a.Z</sup>	5.3±0.2 <sup>a.Z</sup>	6.1±0.2 <sup>a.Z</sup>	-	-
	A	1.8±0.1 <sup>b.Z</sup>	2.3±0.1 <sup>b.Z</sup>	2.7±0.2 <sup>b.Z</sup>	3.3±0.1 <sup>a.Z</sup>	3.8±0.1 <sup>a.Y</sup>	4.1±0.2 <sup>a.Y</sup>	4.3±0.2 <sup>a.Z</sup>	4.6±0.1 <sup>a.Z</sup>
	B	1.4±0.1 <sup>b.Y</sup>	1.6±0.1 <sup>b.Y</sup>	1.8±0.1 <sup>b.Y</sup>	2.0±0.1 <sup>ab.Y</sup>	2.0±0.1 <sup>ab.X</sup>	2.6±0.2 <sup>a.X</sup>	2.4±0.1 <sup>a.Y</sup>	2.8±0.1 <sup>a.Y</sup>

G: Groups, a,b,c: Means within a column lacking a common superscript letter are different ( $P < 0.05$ ). x,y,z: Means within a row lacking a common superscript letter are different ( $P < 0.05$ ). -: Not analyzed. Values are means for three trials at each groups (n=3x2).

**Table 2**  
The results of chemical analyses of horse mackerel during storage period at +4°C

Analysis	G	Storage time (day) ( $\bar{x} \pm S$ )							
		0	3	6	9	12	15	18	21
pH	C	6.02±0.1 <sup>a,Z</sup>	6.28±0.3 <sup>a,Z</sup>	6.44±0.3 <sup>a,Z</sup>	6.56±0.1 <sup>a,Z</sup>	6.62±0.1 <sup>a,Z</sup>	6.73±0.1 <sup>a,Z</sup>	-	-
	A	6.03±0.1 <sup>a,Z</sup>	6.10±0.2 <sup>a,Z</sup>	6.02±0.1 <sup>a,Z</sup>	6.08±0.1 <sup>a,Z</sup>	6.12±0.1 <sup>a,Z</sup>	6.16±0.2 <sup>a,Z</sup>	6.18±0.2 <sup>a,Z</sup>	6.09±0.2 <sup>a,Z</sup>
	B	6.00±0.1 <sup>a,Z</sup>	6.01±0.1 <sup>a,Z</sup>	6.04±0.1 <sup>a,Z</sup>	6.00±0.1 <sup>a,Z</sup>	6.00±0.1 <sup>a,Z</sup>	6.01±0.1 <sup>a,Z</sup>	6.04±0.1 <sup>a,Z</sup>	6.00±0.1 <sup>a,Z</sup>
TVB-N, mg/100 g	C	15.8±0.3 <sup>c,Z</sup>	18.8±0.2 <sup>c,Z</sup>	23.7±0.4 <sup>c,Z</sup>	33.7±0.3 <sup>b,Z</sup>	35.9±0.4 <sup>b,Z</sup>	49.8±0.3 <sup>a,Z</sup>	-	-
	A	12.4±0.2 <sup>b,Z</sup>	12.8±0.2 <sup>b,Z</sup>	12.0±0.3 <sup>b,Y</sup>	14.8±0.2 <sup>b,Y</sup>	22.8±0.2 <sup>a,Y</sup>	24.0±0.1 <sup>a,Y</sup>	28.0±0.1 <sup>a,Z</sup>	35.0±0.1 <sup>a,Z</sup>
	B	10.6±0.2 <sup>a,Z</sup>	10.2±0.3 <sup>a,Z</sup>	10.1±0.2 <sup>a,Y</sup>	10.4±0.2 <sup>a,Y</sup>	12.2±0.2 <sup>a,X</sup>	14.8±0.2 <sup>a,X</sup>	14.0±0.1 <sup>a,Y</sup>	16.8±0.2 <sup>a,Y</sup>
TBA, mg MDA/kg	C	0.54±0.1 <sup>c,Z</sup>	0.98±0.2 <sup>c,Z</sup>	1.70±0.2 <sup>b,c,Z</sup>	3.80±0.2 <sup>b,Z</sup>	6.10±0.3 <sup>a,Z</sup>	7.30±0.3 <sup>a,Z</sup>	-	-
	A	0.32±0.2 <sup>b,Z</sup>	0.44±0.2 <sup>b,Z</sup>	0.62±0.3 <sup>b,Y</sup>	0.89±0.3 <sup>b,Y</sup>	1.20±0.2 <sup>a,Y</sup>	1.56±0.2 <sup>a,Y</sup>	1.24±0.2 <sup>a,Z</sup>	2.40±0.2 <sup>a,Z</sup>
	B	0.38±0.2 <sup>a,Z</sup>	0.56±0.2 <sup>a,Z</sup>	0.42±0.2 <sup>a,Y</sup>	0.58±0.2 <sup>a,Y</sup>	0.72±0.2 <sup>a,Y</sup>	0.68±0.2 <sup>a,X</sup>	0.92±0.3 <sup>a,Y</sup>	1.01±0.3 <sup>a,Y</sup>

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**Table 3**  
Results of sensory analyses of horse mackerel during storage period at +4°C

Analysis	G	Storage time (day) ( $\bar{x} \pm S$ )					
		0	3	6	9	12	15
Color	C	4.0±0.1 <sup>a,Z</sup>	3.8±0.1 <sup>a,Z</sup>	3.0±0.1 <sup>a,Z</sup>	-	-	-
	A	4.0±0.1 <sup>a,Z</sup>	4.2±0.1 <sup>a,Z</sup>	3.8±0.2 <sup>a,Z</sup>	3.6±0.2 <sup>a,Z</sup>	3.0±0.1 <sup>a,Z</sup>	3.0±0.1 <sup>a,Z</sup>
	B	4.0±0.1 <sup>a,Z</sup>	4.0±0.1 <sup>a,Z</sup>	4.0±0.1 <sup>a,Z</sup>	3.8±0.2 <sup>a,Z</sup>	3.2±0.2 <sup>a,Z</sup>	3.0±0.1 <sup>a,Z</sup>
Odor	C	4.0±0.1 <sup>a,Z</sup>	3.2±0.2 <sup>b,Z</sup>	3.0±0.1 <sup>b,Z</sup>	-	-	-
	A	4.2±0.2 <sup>a,Z</sup>	4.0±0.1 <sup>a,Z</sup>	3.8±0.2 <sup>a,Z</sup>	3.6±0.2 <sup>a,Z</sup>	3.8±0.2 <sup>a,Z</sup>	3.8±0.2 <sup>a,Z</sup>
	B	3.0±0.1 <sup>a,Z</sup>	3.6±0.3 <sup>a,Z</sup>	3.0±0.1 <sup>a,Z</sup>	2.8±0.2 <sup>a,Z</sup>	3.0±0.1 <sup>a,Y</sup>	2.8±0.3 <sup>a,Y</sup>
Taste	C	4.0±0.1 <sup>a,Z</sup>	3.8±0.2 <sup>a,Z</sup>	3.0±0.1 <sup>b,Z</sup>	-	-	-
	A	3.8±0.2 <sup>a,Z</sup>	4.0±0.1 <sup>a,Z</sup>	3.8±0.2 <sup>a,Z</sup>	3.6±0.2 <sup>a,Z</sup>	3.4±0.2 <sup>ab,Z</sup>	3.0±0.1 <sup>b,Z</sup>
	B	3.0±0.1 <sup>a,Z</sup>	3.2±0.2 <sup>a,Z</sup>	2.0±0.1 <sup>b,Y</sup>	2.0±0.1 <sup>b,Y</sup>	2.0±0.1 <sup>b,Y</sup>	2.2±0.2 <sup>b,Z</sup>
Texture	C	3.0±0.1 <sup>b,Y</sup>	3.4±0.2 <sup>a,Z</sup>	3.0±0.1 <sup>b,Y</sup>	-	-	-
	A	4.0±0.1 <sup>a,Z</sup>	3.8±0.2 <sup>a,Z</sup>	4.0±0.1 <sup>a,Z</sup>	3.8±0.1 <sup>a,Z</sup>	3.6±0.2 <sup>a,Z</sup>	4.0±0.1 <sup>a,Z</sup>
	B	4.2±0.2 <sup>a,Z</sup>	3.6±0.2 <sup>a,Z</sup>	4.2±0.3 <sup>a,Z</sup>	3.6±0.2 <sup>a,Z</sup>	3.4±0.2 <sup>a,Z</sup>	3.8±0.2 <sup>a,Z</sup>
General acceptance	C	4.2±0.1 <sup>a,Z</sup>	3.0±0.1 <sup>b,Z</sup>	2.8±0.2 <sup>b,Y</sup>	-	-	-
	A	4.4±0.2 <sup>a,Z</sup>	4.6±0.1 <sup>a,Z</sup>	4.6±0.1 <sup>a,Z</sup>	4.4±0.2 <sup>a,Z</sup>	4.0±0.1 <sup>a,Z</sup>	4.0±0.1 <sup>a,Z</sup>
	B	4.2±0.2 <sup>a,Z</sup>	3.0±0.1 <sup>b,Z</sup>	3.6±0.2 <sup>b,Y</sup>	3.0±0.1 <sup>b,Y</sup>	2.0±0.1 <sup>b,Y</sup>	2.0±0.1 <sup>b,Y</sup>

G: Groups, a,b,c: Means within a column lacking a common superscript letter are different ( $P < 0.05$ ). x,y,z: Means within a row lacking a common superscript letter are different ( $P < 0.05$ ). -: Not analyzed. Values are means for three trials at each groups (n=3x2).

## Discussion

### Microbiological evaluation

Whereas the number of TMAB for control group samples on the initial day was 3.7 log<sub>10</sub> cfu/g, it was determined to be smaller for 0.5% rosemary oil applied A group and 1% B group. It has been observed that the microbial development during storage was faster for the control group in

comparison with the rosemary oil applied A and B groups. Whereas the acceptable limit value for TMAB (10<sup>6</sup> cfu/g) was exceeded in the control group during the 12<sup>th</sup> day of storage (6.1 log<sub>10</sub> cfu/g), it was exceeded on the 15<sup>th</sup> day for the rosemary oil applied A group (6.1 log<sub>10</sub> cfu/g) and for the B group it remained within acceptable limit values throughout the storage period. When these results are evaluated, it was determined that the control group was spoiled starting

from the 15<sup>th</sup> day of storage whereas rosemary oil applied A group spoiled starting from the 21<sup>st</sup> day (Table 1). The difference between the rosemary oil applied groups was determined to be statistically significant (\*P<0.05). Dikici et al. (2011) found that in whole rainbow trout prepared by addition of 0.5% and 1% rosemary oil, the TMAB number was 3.01-2.58 log<sub>10</sub> cfu/ml and respectively as 8.03-7.5 log<sub>10</sub> cfu/ml on the 12<sup>th</sup> day after storage. Güran et al. (2011) stated that bonito meatballs prepared by the addition of rosemary (8.5 g/kg) reached 7.50 log<sub>10</sub> cfu/g levels at 4±1°C on the 14<sup>th</sup> day. Ozogul et al. (2010) state the shelf life of sardine fillet in vacuumed packages with 1% rosemary extract added and stored at 4±1°C as 13 days. Whereas in our study, the TMAB number on the 21<sup>st</sup> day of storage for 1 % rosemary oil added B group was determined to be 4.4 log<sub>10</sub> cfu/g. The obtained results are smaller than those obtained by Guran et al. (2011), Dikici et al. (2011) and Ozogul et al. (2010). It is thought that this difference arises from the types of fish used in the study, the initial microbial load, the location from where the fish was obtained along with the processing and storage methods.

The psychrophilic bacteria number which is accepted as an indicator for the spoiling of food stored in the cold was initially determined as 2.8 log<sub>10</sub> cfu/g for raw fish, whereas for the control group it started to increase starting from the 3<sup>rd</sup> day of storage. Whereas the psychrophilic bacteria number in the control group reached its highest value of 6.3 log<sub>10</sub> cfu/g on the 15<sup>th</sup> day, a decrease of about 3 log<sub>10</sub> cfu/g (\*P<0.05) was determined for the 0.5% and 1% rosemary applied group. This can be stated to be due to the antibacterial property of rosemary oil.

The number of *Staphylococcus-Micrococcus* spp. which was initially determined to be 3.9 log<sub>10</sub> cfu/g for raw fish reached a maximum value of 5.2 log<sub>10</sub> cfu/g in the control group on the 15<sup>th</sup> day. Even though there was initially a decrease in the rosemary applied groups, this value reached 4.1 log<sub>10</sub> cfu/g in the A group on the 18<sup>th</sup> day of storage and 3.2 log<sub>10</sub> cfu/g in the B group on the 21<sup>st</sup> day. The number of *Staphylococcus-Micrococcus* spp. in the 1% rosemary oil applied B group was determined to be 2 log<sub>10</sub> cfu/g smaller in comparison with the control group (Table 1) (\*P<0.05). Güran et al. (2011) have stated that the bonito meatballs prepared by adding rosemary (8.5 g/kg) reached the maximum value of 3.21 log<sub>10</sub> cfu/g at 4±1°C on the 14<sup>th</sup> day. It is thought that this difference arises due to technological process.

*Enterobacter* which is an indicator of the hygienic quality of the product was initially determined to be 2.6 log<sub>10</sub> cfu/g in this study. An increase was observed in the control group starting from the 6<sup>th</sup> day of storage reaching the

maximum value of 6.1 log<sub>10</sub> cfu/g on the 15<sup>th</sup> day. A slow development in terms of *Enterobacter* was observed in 0.5% and 1% rosemary oil applied groups and on the 15<sup>th</sup> day of storage, a decrease of about 2 log<sub>10</sub> cfu/g was determined in the 0.5% rosemary oil applied group (Table 1). A statistically significant difference was determined between groups (\*P<0.05).

### Chemical evaluation

The initial pH value for horse-mackerel was determined as 6.02. Whereas no significant change was observed in rosemary oil applied A and B groups during storage. The highest pH value was determined as 6.73 on the 15<sup>th</sup> day of storage and this value were obtained for the control group. Sengor et al. (2000) have stated the average pH value of horse mackerel stored under refrigerator conditions as 6.73 on the 14<sup>th</sup> day. This result is in accordance with our findings regarding pH. Dikici et al. (2011) measured the pH values for 0.5% and 1% rosemary extract added rainbow trouts on the initial day as 6.51-6.53 respectively and as 6.59-6.65 on the 15<sup>th</sup> day. Application of different rosemary oil concentrations had no significant effects on raw fish in terms of pH value throughout the storage time (Table 2) (P>0.05).

Total volatile basic nitrogen value (TVB-N) is used to determine the level of spoilage in aquaculture products and the fish meat quality during storage. This value can increase due to the activities of endogenous enzymes and bacteria that cause spoilage (Kyrana et al., 1997). When the TVB-N value exceeds 35-40 mg/100 g, the product is accepted to be spoiled (Connell, 1990). The TVB-N value which was determined to be 15.8 mg/100 g initially for raw meat exceeded 35 mg/100 g for the control group on the 12<sup>th</sup> day, reached 35.9 mg/100 g for the A group on the 21<sup>st</sup> day of storage and 16.8 mg/100 g for the B group. The lowest TVB-N value throughout storage was determined in the 1% rosemary oil applied B group followed by the 0.5% rosemary oil applied A group and the control group (Table 2) (\*P<0.05). Ozogul et al. (2010) have stated that they have determined the TVB-N value of 1% rosemary oil extract applied sardine fillet in vacuumed packages stored at 4±1°C as 20.59 mg/100 g initially and as 33.64 mg/100 g on the 17<sup>th</sup> day.

Another indication of lipid oxidation which is one of the reasons for the spoiling of food is the thiobarbituric acid (TBA) number. TBA value for the rosemary oil applied horse mackerel and control group samples have been given in Table 2. It has been stated that the TBA value should be less than 3 for a very good material and should not exceed 5 for a good material and that its consumability limit value is between 7-8 (Varlik et al., 1993). In this study, the TBA number which was initially determined to be 0.54 mg

MDA/kg was smaller for the control group in comparison with those for the rosemary oil applied groups (Table 2) (\* $P < 0.05$ ). The lowest TBA values throughout the storage time were observed for the B group treated with 1% rosemary oil. The TBA value for the control group on the 15<sup>th</sup> day of storage was determined to be 7.30 mg MDA/kg. For rosemary oil applied A and B groups, this value was determined to be 2.40 mg MDA/kg and 1.01 mg MDA/kg on the last day of storage (\* $P < 0.05$ ). According to the TBA analysis results, it was observed that during the 21<sup>th</sup> day storage period of horse mackerel fish, the value of malondialdehyde which is the lipid oxidation product for two groups except the control group was observed to be below the limit values. According to the obtained data, the lowest oxidation occurred in the fatty acids of horse mackerel fish treated with rosemary oil. A similar situation was indicated by Ozogul et al. (2010) and Ucak et al. (2011).

### Sensory evaluation

Foods can be stored for long time due to the antioxidant and antimicrobial properties of rosemary oil. Spoiling in aquaculture products is generally observed with rancid taste and putrid smell. Even though in this study the 1% rosemary oil applied B group samples were better than the 0.5% rosemary oil applied A group samples in terms of microbiological and chemical properties, the A group samples were enjoyed more by the panelists (Table 3) (\* $P < 0.05$ ). Because the taste and odour of the A group was preferred more in comparison with those of the B group which had a more concentrated rosemary oil effect. Differences in terms of colour between the A and B groups were determined to be insignificant during the storage period ( $P > 0.05$ ). In this study, it was determined that the control group samples were spoiled in terms of sensory properties starting from the 9<sup>th</sup> day whereas the 1% rosemary oil applied B group samples spoiled in terms of sensory properties starting from the 12<sup>th</sup> day of storage.

### Conclusion

It was observed that the groups with rosemary oil application (0.5%-1%) had longer shelf life microbiologically in comparison with the control group. According to the chemical analysis results, it has been determined that addition of 0.5% and 1% rosemary oil was effective in the lipid oxidation control for both groups. It was determined that the A group with 0.5% rosemary oil applied had better sensory preference results. It has been determined that 1% rosemary oil is effective in the preservation of the consumability of horse mackerel fish for up to 21 days.

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