



RESEARCH ARTICLE

Effect of Garlic (*Allium sativum* L.) on the Microbiological, Chemical and Sensorial Quality of Smoked Atlantic Mackerel (*Scomber scombrus* Linnaeus, 1758) Stored in Vacuumed Packets at Refrigerator Temperature (+4°C)

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ARTICLE INFO

Article History:

Received: 07.02.2019

Accepted: 26.11.2019

Available Online: 26.12.2019

Keywords:

Hot smoking

Atlantic mackerel

Garlic

Quality changes

Shelf life

ABSTRACT

In this study, the effect of garlic (*Allium sativum* L.) on the microbiological, chemical and sensorial quality of smoked Atlantic Mackerel (*Scomber scombrus* Linnaeus, 1758) stored in vacuumed packets at refrigerator temperature (+4°C) were investigated. The results of total volatile basic nitrogen (TVB - N) in control group (without garlic contains 10% salt) showed that the product reached the limit of consumable value on the 43rd day (TVB - N = 36.45±1.133 mg / 100 g) and on the 67th day in the garlic group (with 2% garlic contains 10% salt) (TVB - N = 35.69±1.026 mg / 100 g) ($P < 0.05$). It was observed that garlic group values were higher than control group in terms of sensory analysis (texture, appearance, odour and taste). However, it was determined that the value of taste did not fall below the consumable value in both groups. These results showed that the shelf life of smoked Atlantic Mackerel was 24 days longer with garlic application and the garlic supplement increased both the shelf life of the product and gave sensory appreciation to the product.

Please cite this paper as follows:

Duyar, H. A., Kalaycı, Z. H. and Bilgin, S. (2019). Effect of Garlic (*Allium sativum* L.) on the Microbiological, Chemical and Sensorial Quality of Smoked Atlantic Mackerel (*Scomber scombrus* Linnaeus, 1758) Stored in Vacuumed Packets at Refrigerator Temperature (+4°C). *Alinteri Journal of Agriculture Sciences*, 34(2): 132-141. doi: 10.28955/alinterizbd.665207

Introduction

Atlantic Mackerel (*Scomber scombrus* Linnaeus, 1758) abundant in cold and temperate shelf areas, forms large schools near the surface. They overwinter in deeper waters but move closer to shore in spring when water temperatures range between 11°C and 14°C. Mainly diurnal, it feeds on zooplankton and small fish. The species is traded fresh, frozen, smoked and canned (Froese & Pauly, 2018).

Smoking is a traditional preserving method used for both fish and meat products around the world and also smoked fish products have wide acceptance today due to their accustomed taste and aroma as well as longer shelf life as a result of the

combined effects of dehydration, antimicrobial and antioxidant activities of several smoke constituents mainly: formaldehyde, carboxylic acids and phenols (Doe, 1998). Smoking gives the special color and flavor to the food and extends its shelf life via the effects of dehydration, antimicrobial and antioxidant of the smoke compounds. Smoking also changes the texture of product. Several factors contribute to the quality and safety of such products. These factors are the quality of raw material, salt concentration used water activity of the final product, heat through the smoking process, the quantity of smoke, packaging methods, hygienic circumstances and storage conditions (Koral et al., 2010).

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Atlantic Mackerel used in this study is an important pelagic fish caught in the North Atlantic including the Mediterranean with high economic value (Froese & Pauly, 2018). The fat content is about 6-23%, water content is 56-74% and protein content is 18-20 % throughout the year (NOAA, 2014). It is considered one of the more healthy fish because it is rich in omega-3 fatty acids and an excellent source of selenium, niacin, and vitamins B6 and B12 (NOAA, 2014). Generally, it is sold freshly and frozen, but it is exported from Norway to Turkey as frozen and consumed by Turkish people as frozen.

The use of synthetic antioxidant has been very effective in controlling food rancidity. However, synthetic antioxidants have frequently been associated with certain health problems (Pakawatchai et al., 2009). This has necessitated the use of natural antioxidants, such as ginger (Iheagwara, 2013; Frank et al., 2014), garlic (Duyar et al., 2016), in the prevention of rancidity in smoked fish. It is also important to apply herbal preservatives and sweeteners to extend the fish flavor and shelf life (Balıkcı, 2009; Frank et al., 2014; Duyar et al., 2016).

Garlic is the edible bulb from a plant of the *Allium* (onion) genus, commonly used for flavoring in cooking and for its beneficial effects for human health. Garlic is among the oldest of all cultivated plants. It has been used as a medicinal agent for thousands years. This remarkable plant has multiple beneficial effects such as antimicrobial, antithrombotic, hypolipidemic, antiarthritic, hypoglycemic, antitumor and antioxidant activities. A large number of studies have demonstrated the antioxidant activity of garlic by using. It grows in many parts of the world and is a popular ingredient in cooking due to its strong smell and delicious taste. It is high in a sulfur compound called allicin, which is believed to bring most of the health benefits. However, throughout ancient history, the main use of garlic was for its health and medicinal properties. Garlic not only possesses antibacterial, antifungal and antimicrobial properties, but also its consumption positively influences human circulatory and immune system and hence has a wide range of health benefits (Banerjee & Maulik, 2002, Păcurar & Krejci, 2010). It has been proven effective as a hypolipidemic, antimicrobial, antihypertensive, hepatoprotective, and insecticidal agent in various human and animal therapies (Shakya & Labh, 2014). Nowadays people are very conscious of consuming natural products that no longer contain chemical preservatives. This has led researchers to work on the use of natural materials such as garlic instead of chemicals to produce new flavors and to ensure shelf life for products that are produced.

The aim of this study was to determine the effect of garlic (*Allium sativum* L.) on the microbiological, chemical and sensorial quality of smoked Atlantic Mackerel (*Scomber scombrus* Linnaeus, 1758) stored in vacuumed packets at refrigerator temperature (+4°C).

Materials and Methods

Fish Samples

Atlantic Mackerel (*Scober scombrus*) exported from Norway to Turkey, were bought from Sinop fishermen in boxes as

frozen blocks. The mean length of the *S. scombrus* used in the research is 35 ± 3 cm and the mean weight is 350 ± 20 g ($n = 200$).

Preparation of fish samples

Gutting was carried out manually after beheading and washing in running tap water. Fish were divided into 2 groups as control group (without garlic) and garlic group (with garlic) to be transferred to salt solution. Two salted brines were prepared for salting before smoked. For the control group, a salt solution of 10% (10 g salt to 100 ml fresh water) was prepared with 1/3 (fish / salt). For garlic supplement group; 10% salt solution was prepared and crushed garlic pieces were added so that the brine ratio would be 2% to prepare the fish to stand.

Salting before hot smoke

The first group (control group) prepared for smoked; it was left for one hour on salt water containing 10% salt. The second group of smoked samples (garlic group) was stored for one hour in salt water containing 10% salt and 2% garlic. At the end of the one hour, the fish were removed from the salt water and washed to remove excess salt under the tap water. Then, the fish were hung with hooks, lined up at appropriate intervals in the smoke oven, kept at room temperature for 20 minutes to drain excess water. After this process, smoking process was started

Smoking

The smoking process was carried out in a semi-mechanical smoke oven by hot smoke method. In the production of smoke, sawdust obtained from beech wood is used. The smoking process was carried out in three stages: In the first step, at 30°C for 40 minutes it was expected hardened skin. In the second step, lightly soaked sawdust was placed in a metal tray and the fishes were allowed to cook for 60 minutes at 50°C in sawdust smoke. In the third and last stage, the temperature was raised to 80°C and smoke was made for 45 minutes. Smoked fishes were thawed 30 minutes at room temperature. Later, remove the filets of fishes for packing and stocking.

Packing and stocking

From each sample prepared for packaging, each fillet was packed with a vacuum packed device (Abant brand, MG 42 model table top vacuum machine) by placing a polyethylene vacuum bag. The vacuum packed fillet is stored in the refrigerator ($4 \pm 1^\circ\text{C}$) during the study.

pH Determination

10 grams of smoked fish samples were weighed and 20 ml of purified water was added. These samples were homogenized for 1 minute and then pH measurements were made by immersing the pH-meter probe in the homogenized fish (Vural & Oztan, 1996). Smoked fish are food items that are quickly defeated. The pH values of smoked fish range from 5.4 to 6.9 (Varlık, 2004).

Water Activity (*aw*)

Nova AG, LabSwift-aw that Automatic water activity machine was used for determination of water activity.

Determination of Total Volatile Basic Nitrogen (TVB-N)

The TVB-N assay was performed according to the Lücke-Geidel method modified by Antonacopoulos and the results were given as mg / 100g (Varlık et al., 1993). A 10 g fragmented sample was placed in a balloon. Then add 1 g of magnesium oxide (MgO) and a few drops of silicone oil and some purified water to prevent foaming and also 10 ml of 3% boric acid (H₃BO₃), 8 drops of tashiro indicator mixture and about 100 ml of purified water were added to the 500 ml Erlenmeyer used as a titration vessel. The sample was placed in a balloon, dish and other balloon heater with pure water, and then subjected to distillation for 15-20 minutes by connecting to the coolant tap. The resulting distillate was titrated with 0.1 N hydrochloric acid (HCl) and the total volatile basic nitrogen content was calculated according to the following formula (Inal, 1992, Varlık et al., 1993):

$$\text{TVB-N (mg/100 g)} = \text{Spent (HCl)} \times (0.0014008) \times (100) \times (1000) / \text{Sample amount (g)}$$

There are differences according to TVB-N value in the classification of the quality of fishery products according to various researchers. The TVB-N values obtained in our study were evaluated based on the quality values of Varlık et al. (1993).

Determination of Thiobarbituric Acid (TBA)

The number of thiobarbituric acid was determined according to Erkan & Özden (2008). The homogenized sample was weighed 1.90-2.00 g (\pm 0.01) into the tube and 100 μ l (0.10% = 1 g / l of ethanol prepared BHT) was added. 16-25 ml of TCA (trichloroacetic acid) (5%) was added to it and ultrathoracic was removed and filtered through filter paper. After placing 5 ml of the filtrate on the tube, the same amount of TBA reagent (0.02 mol / l of 10% glacial acetic acid prepared, weighing 0.2883 g for 100 cc) was added. The tubes were held in a water bath at 70-80 °C for 30 minutes and after the tubes had been cooled, they were read against the corpuscles in a spectrophotometer set to a wavelength of 532 nm.

Microbiological Analyzes

For microbiological analyzes, samples were taken from aseptic conditions from two packets of each group. 10 g of fish meat samples were weighed and added with 90 ml of 0.85% sterile saline (8.5 g NaCl, 1000 ml of purified water). The pre-sterilized homogenizer was homogenized and diluted 10⁻¹. Dilutions in the form of dilution solution consisting of 1 ml dilution + 9 ml saline were formed from the homogenized sample. Diluted dilutions of up to 10 were then made, and two parallel runs from each dilution and microbiological analyzes were made using the bulk plate method (Baumgart, 1986, Varlık et al., 1993). Following the incubation, petri dishes were counted and the results were given as log cfu / g.

Total mesophilic aerobic bacteria (TMAB) and total psychrophilic aerobic bacteria (TPAB) count

Plate Count Agar (PCA) medium was used for total mesophilic aerobic bacteria and total psychrophilic aerobic bacteria counts. Samples from smoked fish samples were taken under aseptic conditions and weighed 10 g. The weighed sample was disintegrated and homogenized in sterile disruptor with 90 ml of 0.85% physiological salt water (8.5 g NaCl, 1000 ml distilled water). The first dilution (10⁻¹) was performed with this procedure (Gurgun and Halkman, 1990). Dilutions of 10⁻², 10⁻³ and 10⁻⁴ were then prepared using 0.85% physiological salt water as diluent fluid. Sowing was done by transferring 1 ml of sterilized-end automatic pipettes (eppendorf, 1000 μ L) from each of the dilutions of 10⁻¹, 10⁻², 10⁻³ and 10⁻⁴ ratios. Plate Count Agar (PCA) broth which has been sterilized and cooled to 44-46 °C was added 10-15 ml by pouring method to petri dish boxes and mixed (Roger et al., 1987, Göktaş, 1990). The petri dishes were then incubated for 3 days at 28 °C in an incubator for total mesophilic aerobic bacteria counts and 10 days at 7 °C for total psychrophilic aerobic bacteria counts (Roger et al., 1987, Göktaş, 1990). Then, total mesophilic and psychrophilic aerobic bacteria counts were calculated (Gurgun & Halkman, 1990).

Total yeast and mold count (TYM)

Potato Dextrose Agar (PDA) is used for the cultivation of fungi. Potato Dextrose Agar (PDA) is a general purpose medium for yeasts and molds that can be supplemented with acid or antibiotics to inhibit bacterial growth. Potato Dextrose Agar medium was used for yeast and mold count. Dilutions of 10⁻¹, 10⁻², 10⁻³, and 10⁻⁴ ratios were prepared from homogenized smoked fish samples. Sowing was performed by transferring 1 ml of eppendorf (1000 μ L) into 2 sterile empty petri dishes from each of dilutions of 10⁻¹, 10⁻², 10⁻³, and 10⁻⁴ ratios. Sterilized petri dishes were placed at a temperature of 44-46 °C and poured into the PDA medium by pouring 10-15 ml. After the fermentation, the petri dishes were inverted and the number of yeast and mold was counted after incubation for 3 days at 28 °C (Roger et al., 1987; Göktaş, 1990; Varlık et al., 1993).

Total coliform bacteria count

Dilutions of 10⁻¹, 10⁻², 10⁻³, and 10⁻⁴ ratios were prepared from homogenized smoked fish samples. Sowing was performed by transferring 1 ml of eppendorf (1000 μ L) into 2 sterile empty petri dishes from each of dilutions of 10⁻¹, 10⁻², 10⁻³, and 10⁻⁴ ratios. Sowing petri dishes were placed at a temperature of 44-46 °C with 10-15 ml of Violet Red Agar (VRBA) medium and than the petri dishes were inverted after the supernatant and incubated for 1 day at 35 °C (Roger et al., 1987; Göktaş et al., 1999).

Sensory Analysis

For sensory analysis, smoked fish were placed on labeled plates and presented to panelists. The panelist group of six people evaluated the appearance, smell, taste and texture of the fish. A score of 5 was considered the limit value and below this value was considered not to be consumed (Koral, 2006).

Panelists were asked to make a good assessment from 1 to 10 points in terms of point criteria by the modified method of Koral (2006). Panelists evaluated the sensory analysis using a 0-10 hedonic scale (10-8: very good; 8-6: good; 6-5: consumable and <5: spoilage)

Statistical Analysis

Significant differences between two groups were determined using the t test or/and Mann-Whitney test of PAST software with a significance level of $P < 0.05$.

Results

Chemical Contents

Total volatile basic nitrogen and thiobarbituric acid results during the storage days are shown in Table 1 and Figure 1. The TBA values increased both control and garlic groups depending on storage days and the TBA values did not exceed the consumable limit in both groups during the storage times (Table 1, Figure 1). The TBA values between two groups were not significantly different until the 7th days ($P > 0.05$). From day 7 to day 43, the difference between the two groups was statistically significant ($P < 0.05$).

The TVB - N values was higher in control group than garlic group during the storage times and the TVB - N values increased regularly both groups depending on storage days. The TVB - N values between two groups were not significantly different on days 0th, 16th, 19th and 25th days ($P > 0.05$). On the other days of storage, the difference between the TVB - N

values of two groups was found to be statistically significant ($P < 0.05$). The results of the TVB - N in control group showed that the product reached the limit of consumable value on the 43rd day (TVB - N = 36.45 ± 1.133 mg / 100 g) and on the 67th day in the garlic group (TVB - N = 35.69 ± 1.026 mg / 100 g).

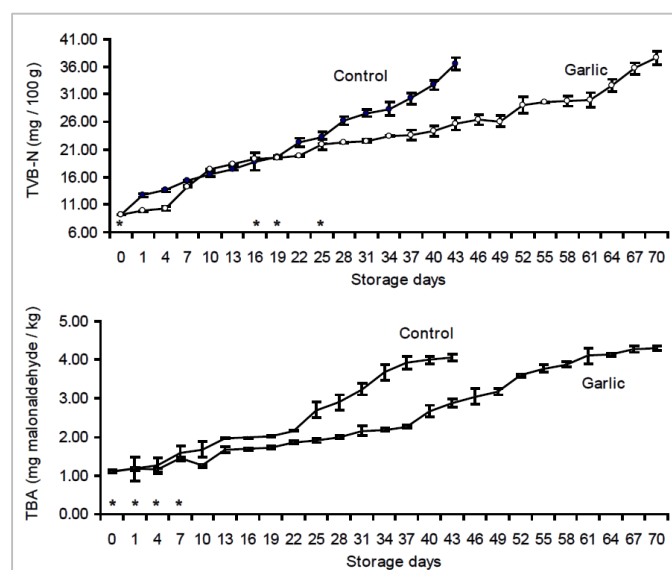


Figure 1. Total volatile basic nitrogen (TVB-N) and thiobarbituric acid (TBA) results during the storage days. The days marked with stars are statistically insignificant between two groups ($P > 0.05$)

Table 1. Total volatile basic nitrogen (TVB - N) and thiobarbituric acid (TBA) results during the storage days

Storage Days	Total volatile basic nitrogen (TVB - N) (mg / 100 g)		Thiobarbituric acid (TBA) (mg malonaldehyde / kg)	
	Control	Garlic	Control	Garlic
0	9.20±0.026 ^a	9.20±0.072 ^a	1.11±0.031 ^a	1.11±0.040 ^a
1	12.65±0.265 ^a	9.89±0.118 ^b	1.17±0.306 ^a	1.18±0.038 ^a
4	13.59±0.361 ^a	10.32±0.427 ^b	1.26±0.200 ^a	1.15±0.041 ^a
7	15.29±0.087 ^a	14.27±0.115 ^b	1.58±0.210 ^a	1.44±0.035 ^a
10	16.47±0.347 ^a	17.34±0.066 ^b	1.68±0.215 ^a	1.26±0.039 ^b
13	17.49±0.262 ^a	18.27±0.111 ^b	1.97±0.020 ^a	1.66±0.083 ^b
16	18.80±1.600 ^a	19.32±0.100 ^a	1.98±0.010 ^a	1.69±0.042 ^b
19	19.64±0.120 ^a	19.55±0.183 ^a	2.01±0.031 ^a	1.73±0.040 ^b
22	22.32±0.763 ^a	19.81±0.092 ^b	2.15±0.012 ^a	1.86±0.046 ^b
25	23.16±1.037 ^a	21.96±0.900 ^a	2.69±0.212 ^a	1.91±0.061 ^b
28	26.20±0.780 ^a	22.23±0.108 ^b	2.90±0.200 ^a	1.99±0.038 ^b
31	27.57±0.626 ^a	22.48±0.274 ^b	3.23±0.153 ^a	2.15±0.122 ^b
34	28.31±1.222 ^a	23.46±0.089 ^b	3.67±0.218 ^a	2.19±0.040 ^b
37	30.28±1.041 ^a	23.58±0.976 ^b	3.93±0.164 ^a	2.26±0.036 ^b
40	32.70±0.872 ^a	24.32±0.930 ^b	4.00±0.092 ^a	2.67±0.142 ^b
43	36.45±1.133 ^a	25.63±1.110 ^b	4.06±0.076 ^a	2.88±0.101 ^b
46		26.40±0.917		3.05±0.200
49		26.06±1.005		3.17±0.083
52		28.97±1.487		3.60±0.040
55		29.55±0.092		3.78±0.101
58		29.82±0.890		3.88±0.061
61		29.98±1.271		4.10±0.200
64		32.58±1.037		4.13±0.040
67		35.69±1.026		4.27±0.092
70		37.59±1.254		4.31±0.061

The different superscript on the same line represent statistical differences between the two groups ($P < 0.05$).

Analyzes were performed in triplicate (n = 3).

aW and pH Values

Water activity (aw) and pH fluctuations during the storage days are shown in Table 2 and Figure 2. Although small statistically significant increases and decreases were detected pH values between two groups during storage ($P < 0.05$), the pH value was found to be within the range of pH = 6 and 6.5 values recommended for freshness (Table 2, Figure 2). The average water activity (aW) values was found between 0.97 ± 0.003 and 0.99 ± 0.003 in control and garlic groups during the storage days (Table 2, Figure 2).

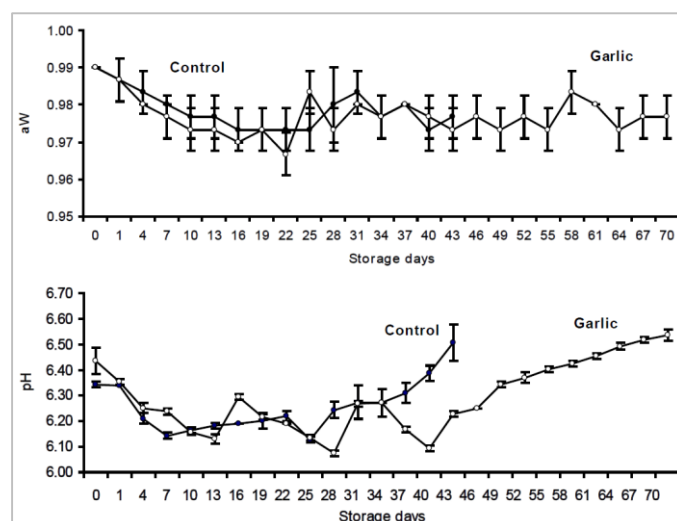


Figure 2. Water activity (aW) and pH fluctuations during the storage days

Table 2. Water activity (aw) and pH results during the storage days

Storage Days	aw		pH	
	Control	Garlic	Control	Garlic
0	0.99±0.000 ^a	0.99±0.000 ^a	6.34±0.003 ^a	6.44±0.102 ^a
1	0.99±0.003 ^a	0.99±0.003 ^a	6.34±0.000 ^a	6.35±0.003 ^a
4	0.98±0.003 ^a	0.98±0.000 ^a	6.21±0.006 ^a	6.25±0.006 ^b
7	0.98±0.000 ^a	0.98±0.003 ^a	6.14±0.003 ^a	6.24±0.003 ^b
10	0.98±0.003 ^a	0.97±0.003 ^a	6.16±0.003 ^a	6.16±0.003 ^a
13	0.98±0.003 ^a	0.97±0.003 ^a	6.18±0.003 ^a	6.13±0.006 ^b
16	0.97±0.003 ^a	0.97±0.000 ^a	6.19±0.000 ^a	6.29±0.003 ^b
19	0.97±0.003 ^a	0.97±0.003 ^a	6.20±0.002 ^a	6.22±0.003 ^a
22	0.97±0.003 ^a	0.97±0.003 ^a	6.22±0.006 ^a	6.19±0.000 ^b
25	0.97±0.003 ^a	0.98±0.003 ^a	6.13±0.032 ^a	6.13±0.003 ^a
28	0.98±0.006 ^a	0.97±0.003 ^a	6.24±0.009 ^a	6.07±0.003 ^b
31	0.98±0.003 ^a	0.98±0.000 ^a	6.27±0.019 ^a	6.27±0.003 ^a
34	0.98±0.003 ^a	0.98±0.003 ^a	6.27±0.015 ^a	6.27±0.000 ^a
37	0.98±0.000 ^a	0.98±0.000 ^a	6.31±0.012 ^a	6.17±0.003 ^b
40	0.97±0.003 ^a	0.98±0.003 ^a	6.39±0.009 ^a	6.09±0.003 ^b
43	0.98±0.003 ^a	0.97±0.003 ^a	6.51±0.020 ^a	6.23±0.003 ^b
46		0.98±0.003		6.25±0.000
49		0.97±0.003		6.34±0.003
52		0.98±0.003		6.37±0.006
55		0.97±0.003		6.40±0.003
58		0.98±0.003		6.42±0.003
61		0.98±0.000		6.45±0.003
64		0.97±0.003		6.49±0.003
67		0.98±0.003		6.52±0.003
70		0.98±0.003		6.54±0.007

The different superscript on the same line represent statistical differences between the two groups ($P < 0.05$). Analyzes were performed in triplicate ($n = 3$).

Microbiological Contents

Total psychrophilic aerobic bacteria (TPAB), total mesophilic aerobic bacteria (TMAB), total coliform bacteria and total yeasts and molds (TYM) results are shown in Table 3 and Figure 3. Total psychrophile aerobics bacteria (TPAB) spillage was not detected in the first 4 days of storage in both groups. No statistical difference could be detected between the two groups on the 7th, 10th, 19th, 22nd, 28th and 31st days of the storage period ($P > 0.05$). However, on the 13th, 16th, 31st, 34th, 37th and 40th days of storage, the total psychrophilic aerobics bacteria count of the control group was found to be statistically higher than the garlic group ($P < 0.05$).

The total number of aerobic mesophilic bacteria (TMAB) showed a steady increase in the storage process in both groups. The TMAB values was found to be 6.63 ± 0.090 log cfu / g in the control group and 4.40 ± 0.062 log cfu / g in the garlic group on the 43rd day of the storage period. This value was found as 6.26 ± 0.029 log cfu / g in the garlic group on the 70th day. The difference in total mesophilic aerobic bacteria count between control and garlic groups during storage was found to be statistically insignificant ($P > 0.05$).

Total yeast and mold (TYM) values were found to be 4.55 ± 0.030 log cfu / g in the control group and 4.09 ± 0.079 log cfu / g in the garlic group on the 43rd day of storage. This value was determined as 5.34 ± 0.075 log cfu / g in the garlic group on the 70th day. The TYM value of the garlic group was

statistically higher than that of the control group until the 13th day of the storage period ($P < 0.05$), whereas the values of the control group after the 13th day were statistically higher than the values of the garlic group ($P < 0.05$).

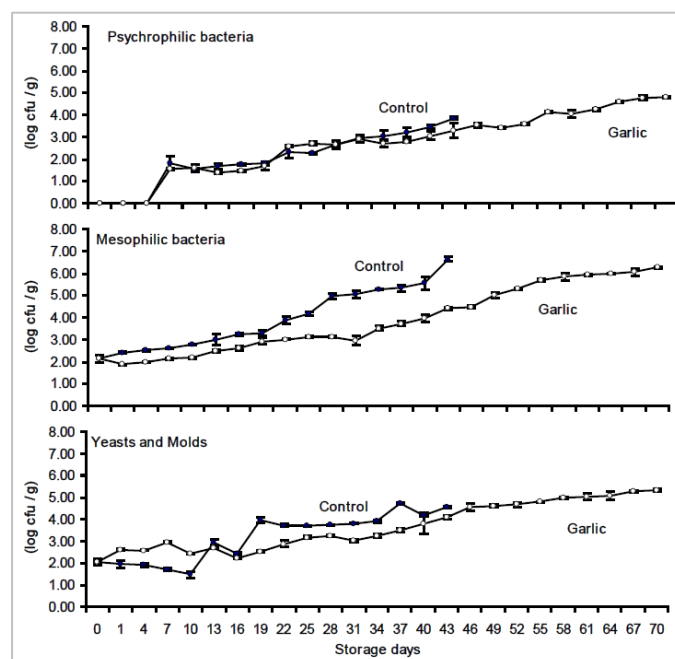


Figure 3. Microbiological fluctuations during the storage days. Vertical lines refer to standard errors (S.E.)

Table 3. Total psychrophilic aerobic bacteria (TPAB), total mesophilic aerobic bacteria (TMAB), total coliform bacteria and total yeasts and molds (TYM) results

Storage Days	Total psychrophilic aerobic bacteria (log cfu/g)		Total mesophilic aerobic bacteria (log cfu/g)		Total coliform bacteria (log cfu/g)		Yeasts and Molds (log cfu/g)	
	Control	Garlic	Control	Garlic	Control	Garlic	Control	Garlic
0	<1.47	<1.47	2.13±0.173 ^a	2.13±0.153 ^a	<1.47	<1.47	2.05±0.050 ^a	2.05±0.150 ^a
1	<1.47	<1.47	2.41±0.029 ^a	1.88±0.035 ^b	<1.47	<1.47	1.95±0.182 ^a	2.59±0.030 ^b
4	<1.47	<1.47	2.51±0.031 ^a	1.99±0.031 ^b	<1.47	<1.47	1.92±0.075 ^a	2.57±0.033 ^b
7	1.83±0.297 ^a	1.57±0.020 ^a	2.60±0.030 ^a	2.15±0.046 ^b	<1.47	<1.47	1.72±0.059 ^a	2.94±0.060 ^b
10	1.54±0.045 ^a	1.60±0.175 ^a	2.76±0.028 ^a	2.19±0.052 ^b	<1.47	<1.47	1.47±0.141 ^a	2.42±0.017 ^b
13	1.68±0.119 ^a	1.39±0.036 ^b	2.99±0.242 ^a	2.48±0.030 ^b	<1.47	<1.47	2.94±0.121 ^a	2.67±0.035 ^b
16	1.78±0.045 ^a	1.48±0.035 ^b	3.22±0.075 ^a	2.61±0.079 ^b	<1.47	<1.47	2.42±0.060 ^a	2.23±0.036 ^b
19	1.80±0.069 ^a	1.70±0.176 ^a	3.28±0.114 ^a	2.92±0.142 ^b	<1.47	<1.47	3.95±0.135 ^a	2.50±0.046 ^b
22	2.30±0.225 ^a	2.57±0.035 ^b	3.87±0.173 ^a	3.00±0.017 ^b	<1.47	<1.47	3.72±0.062 ^a	2.87±0.148 ^b
25	2.28±0.045 ^a	2.69±0.076 ^b	4.17±0.079 ^a	3.11±0.030 ^b	<1.47	<1.47	3.69±0.032 ^a	3.16±0.063 ^b
28	2.67±0.113 ^a	2.65±0.173 ^a	4.97±0.131 ^a	3.13±0.046 ^b	<1.47	<1.47	3.73±0.029 ^a	3.23±0.067 ^b
31	2.95±0.045 ^a	2.90±0.178 ^a	5.06±0.159 ^a	2.95±0.198 ^b	<1.47	<1.47	3.80±0.030 ^a	3.02±0.062 ^b
34	3.05±0.224 ^a	2.70±0.175 ^b	5.28±0.030 ^a	3.51±0.096 ^b	<1.47	<1.47	3.90±0.058 ^a	3.23±0.078 ^b
37	3.20±0.226 ^a	2.80±0.070 ^b	5.33±0.131 ^a	3.71±0.105 ^b	<1.47	<1.47	4.72±0.063 ^a	3.48±0.079 ^b
40	3.45±0.090 ^a	3.04±0.185 ^b	5.55±0.285 ^a	3.94±0.171 ^b	<1.47	<1.47	4.18±0.061 ^a	3.80±0.477 ^b
43	3.85±0.045 ^a	3.28±0.350 ^b	6.63±0.090 ^a	4.40±0.062 ^b	<1.47	<1.47	4.55±0.030 ^a	4.09±0.079 ^b
46		3.53±0.105		4.48±0.060		<1.47		4.56±0.159
49		3.41±0.036		5.02±0.131		<1.47		4.62±0.062
52		3.59±0.035		5.30±0.030		<1.47		4.67±0.096
55		4.13±0.038		5.69±0.063		<1.47		4.80±0.017
58		4.05±0.175		5.85±0.171		<1.47		4.98±0.046
61		4.24±0.070		5.94±0.033		<1.47		5.03±0.156
64		4.59±0.034		5.99±0.030		<1.47		5.07±0.193
67		4.77±0.107		6.04±0.165		<1.47		5.28±0.045
70		4.79±0.035		6.26±0.029		<1.47		5.34±0.075

The different superscript on the same line represent statistical differences between the two groups ($P < 0.05$). Analyzes were performed in triplicate ($n = 3$).

Sensory Analysis

Changes in sensory analysis (texture, appearance, odour and taste) scores during the storage time are shown in Table 4 and Figure 4. It was determined that garlic group values were higher than control group in terms of sensory analysis (texture, appearance, odour and taste) during storage. Texture, appearance and odour values were determined as 10 in the first 4 days in both groups. On the assessment of texture, the control group decreased below the consumable value on the 40th day ($P < 0.05$) and the garlic group on the 61st day. These results were found to be lower than the consumable limit value of the garlic group on the 55th day and the control group on the 43rd day ($P < 0.05$) after evaluation on the odour. However, it was determined that the value of taste did not fall below the consumable value in both groups. According to the results of this study, it was determined that the control group fell below the consumable limit value on the 40th day ($P < 0.05$). According to the results of the sensory evaluation mentioned above, it was determined that appearance values of the control group dropped below the consumption limit value (5) on the 40th day and the garlic group on the 70th day.

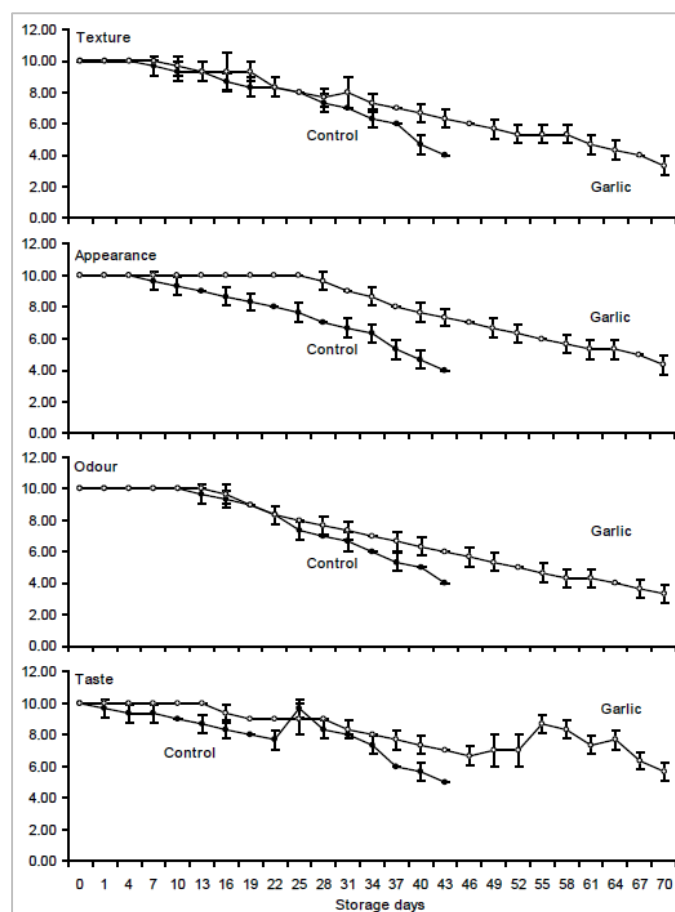


Figure 4. Changes in sensory analysis (texture, appearance, odour and taste) scores during the storage days. Vertical lines refer to standard error

Table 4. Changes in sensory analysis (texture, appearance, odour and taste) scores

Storage Days	Texture		Appearance		Odour		Taste	
	Control	Garlic	Control	Garlic	Control	Garlic	Control	Garlic
0	10.00±0.000 ^a	10.00±0.000 ^a	10.00±0.000 ^a	10.00±0.000 ^a	10.00±0.000 ^a	10.00±0.000 ^a	10.00±0.000 ^a	10.00±0.000 ^a
1	10.00±0.000 ^a	10.00±0.000 ^a	10.00±0.000 ^a	10.00±0.000 ^a	10.00±0.000 ^a	10.00±0.000 ^a	9.67±0.333 ^a	10.00±0.000 ^a
4	10.00±0.000 ^a	10.00±0.000 ^a	10.00±0.000 ^a	10.00±0.000 ^a	10.00±0.000 ^a	10.00±0.000 ^a	9.33±0.333 ^a	10.00±0.000 ^a
7	9.67±0.333 ^a	10.00±0.000 ^a	9.67±0.333 ^a	10.00±0.000 ^a	10.00±0.000 ^a	10.00±0.000 ^a	9.33±0.333 ^a	10.00±0.000 ^a
10	9.33±0.333 ^a	9.67±0.333 ^a	9.33±0.333 ^a	10.00±0.000 ^a	10.00±0.000 ^a	10.00±0.000 ^a	9.00±0.000 ^a	10.00±0.000 ^a
13	9.33±0.333 ^a	9.33±0.333 ^a	9.00±0.000 ^a	10.00±0.000 ^a	9.67±0.333 ^a	10.00±0.000 ^a	8.67±0.333 ^a	10.00±0.000 ^a
16	8.67±0.333 ^a	9.33±0.667 ^a	8.67±0.333 ^a	10.00±0.000 ^a	9.33±0.333 ^a	9.67±0.333 ^a	8.33±0.333 ^a	9.33±0.333 ^a
19	8.33±0.333 ^a	9.33±0.333 ^a	8.33±0.333 ^a	10.00±0.000 ^a	9.00±0.000 ^a	9.00±0.000 ^a	8.00±0.000 ^a	9.00±0.000 ^a
22	8.33±0.333 ^a	8.33±0.333 ^a	8.00±0.000 ^a	10.00±0.000 ^a	8.33±0.333 ^a	8.33±0.333 ^a	7.67±0.333 ^a	9.00±0.000 ^a
25	8.00±0.000 ^a	8.00±0.000 ^a	7.67±0.333 ^a	10.00±0.000 ^a	7.33±0.333 ^a	8.00±0.000 ^a	9.67±0.333 ^a	9.00±0.577 ^a
28	7.33±0.333 ^a	7.67±0.333 ^a	7.00±0.000 ^a	9.67±0.333 ^a	7.00±0.000 ^a	7.67±0.333 ^a	8.33±0.333 ^a	9.00±0.000 ^a
31	7.00±0.000 ^a	8.00±0.577 ^a	6.67±0.333 ^a	9.00±0.000 ^a	6.67±0.333 ^a	7.33±0.333 ^a	8.00±0.000 ^a	8.33±0.333 ^a
34	6.33±0.333 ^a	7.33±0.333 ^a	6.33±0.333 ^a	8.67±0.333 ^b	6.00±0.000 ^a	7.00±0.000 ^b	7.33±0.333 ^a	8.00±0.000 ^a
37	6.00±0.000 ^a	7.00±0.000 ^a	5.33±0.333 ^a	8.00±0.000 ^b	5.33±0.333 ^a	6.67±0.333 ^b	6.00±0.000 ^a	7.67±0.333 ^a
40	4.67±0.333 ^a	6.67±0.333 ^b	4.67±0.333 ^a	7.67±0.333 ^b	5.00±0.000 ^a	6.33±0.333 ^b	5.67±0.333 ^a	7.33±0.333 ^a
43	4.00±0.000 ^a	6.33±0.333 ^a	4.00±0.000 ^a	7.33±0.333 ^b	4.00±0.000 ^a	6.00±0.000 ^b	5.00±0.000 ^a	7.00±0.000 ^b
46		6.00±0.000		7.00±0.000		5.67±0.333		6.67±0.333
49		5.67±0.333		6.67±0.333		5.33±0.333		7.00±0.577
52		5.33±0.333		6.33±0.333		5.00±0.000		7.00±0.577
55		5.33±0.333		6.00±0.000		4.67±0.333		8.67±0.333
58		5.33±0.333		5.67±0.333		4.33±0.333		8.33±0.333
61		4.67±0.333		5.33±0.333		4.33±0.333		7.33±0.333
64		4.33±0.333		5.33±0.333		4.00±0.000		7.67±0.333
67		4.00±0.000		5.00±0.000		3.67±0.333		6.33±0.333
70		3.33±0.333		4.33±0.333		3.33±0.333		5.67±0.333

The different superscript on the same line represent statistical differences between the two groups ($P < 0.05$). Analyzes were performed in triplicate ($n = 3$).

Discussion

TVB - N analysis is a criterion of the products of microbial and enzymatic degradation of protein and non-protein nitrogen compounds in fish meat and it is one of the most important parameters used in determining the quality of fresh and processed fish meat. Quality classification for TVB - N values (Varlık et al., 1993): samples containing 25 mg / 100 g TVB - N are very good, samples containing 30 mg / 100 g TVB - N are good, samples containing 30 - 35 mg / 100 g TVB - N are marketable, more than 35 mg / 100 g TVB - N are considered spoilt. Studies on TVB - N content have reported that fish shelf life is different for different fish species stored by different processing methods. Namely, Rainbow trout (*Oncorhynchus mykiss*) was salted at different rates and smoked hot and stored under refrigerator conditions and the TVB-N value of the 6% salted product reached 35.0 mg / 100 g at the end of the 87th day (Unal, 1995). In a study investigating the shelf life of a hot smoked bonito stored at refrigerator temperature, the TVB-N value reached 41.82±1.20 mg / 100 g on the 13th day of storage (Koral et al., 2010). Dondero et al. (2004) investigated the quality parameters by smoked salmon by the method of cold smoke and packed in vacuum and stored at different temperatures, shelf life has been determined as 46 days at the lowest temperature (0°C) and 19 days at the highest temperature. TVB-N value of garfish meat smoked and stored in refrigerator conditions was determined as 37.47 mg / 100 g on 25th day and also the TVB-N value of garfish meat smoked and stored in room conditions was 38.87 mg / 100 g on the 9th day (Koral et al., 2009). In the present study, the results of TVB - N in control group showed that the product reached the limit of consumable value on the 43rd day and on the 67th day in the garlic group. The TVB - N results obtained in this study showed that the garlic treatment extended the mackerel shelf life by 24 days. When the TVB-N values obtained in this study were compared with other research results, TVB-N values increased similarly to other studies due to storage time (Muratore & Licciardello 2005; Bilgin, 2003; Bilgin et al 2007; Ünal, 1995). Furthermore, the reasons for the differences in the studies may be due to differences in packaging and storage conditions, different fish species, quality of raw material, salt concentration and duration, application of drying process and smoke density etc.

The value of TBA in the meat is a result of oil oxidation and is considered as one of the most important criteria of quality parameters (Varlık et al., 1993). A large part of the changes related to the degradation of fish occurs with the oxidation and disintegration of oils, resulting in a loss of the painful organoleptic value (Varlık et al., 1993). Schormuller (1969) reported that the amount of TBA used in determining the degree of oxidation in aquaculture should not be less than 3 mgMDA / kg in a very good material, not more than 5 in a good material, and a limit value of 7-8 mgMDA / kg in a good material and the aquatic products above this value are evaluated as spoilt. The TBA values obtained in our study were evaluated based on the quality values of Schormuller (1969).

It has been reported that there is no significant difference between the groups in terms of the TBA values after storage of

hot smoked and vacuum packed bonito (*Sarda sarda*) on the refrigerator temperature for 60 days (Duyar et al., 2008). Yanar et al. (2006) examined the TBA value in smoked tilapia (*Oreochromis niloticus*) by hot smoked method using different salt solutions and at the end of the study, it was reported that the TBA values in all groups increased regularly according to the storage period in refrigerator conditions and this increase was directly proportional to the increase in salt concentration. In a study examining the quality parameters of goldfish stored at 4°C with hot smoke, the TBA value was reported to increase to 6.32 mg malonaldehyde / kg on the 28th day of storage (Ünlüsayın et al., 2001). In the present study, the TBA values increased both control and garlic groups depending on storage days and the TBA values did not exceed the consumable limit in both groups. As regards the TBA values reported in previous studies, numerical differences were observed as well as similarities with various research results. The reported differences in TBA between studies may be due to differences in raw materials used, differences in different smoke and storage conditions. In addition, it can be deduced that the garlic used in this study is the positive effect of antioxidant.

It has been reported that the pH value should be between 6.0 and 6.5 for freshness and between 6.8 and 7.0 for consumption limit value (Varlık et al., 1993). In this study, increases and decreases in pH value were determined. However, it has been found at the appropriate intervals of 6.0-6.5 recommended for freshness.

Microbiological parameters are important in determining the shelf life and quality of smoked products (Hansen et al., 1995). Total mesophilic aerobic bacteria (TMAB) and total psychrophile aerobic bacteria (TPAB) are important indicators in determining the microbiological properties of cold stored smoked products. These bacteria play an effective role in the degradation of hot and cold smoked products. In smoked products, the reported microbiological limit of consumption for these bacteria is 6 log cfu / g (Olafsdottir et al., 2005). Generally, when the total bacterial load is being assessed, acceptable microbiological lower limit for fish and fish products is reported as 6 log cfu / g and upper limit 7 log cfu / g (ICMSF, 1986). When the microbiological analysis values obtained in our study were compared with the results of previous studies, coliform bacteria encounter and storage-related bacterial growth were parallel to the results of the previous study (Balıkçı, 2009). When considering the numbers of yeast and mold with TMAB, TPAB, it is generally thought that the differences between the studies may be due to smoke type, sawdust type and raw material.

In this study, sensory evaluation results were regularly decreased in control and garlic groups depending on the storage period. But, the taste values increased on the 25th day in the control group and on the 55th day in the garlic group. This may be due to increasing Inosine monophosphate (IMP). The IMP is responsible for sweetness and characteristics associated with fresh fish muscle (Howgate, 2006). The garlic group showed better results than the control group in all four parameters (texture, appearance, odour and taste). The reason why the value of garlic group in the present study is lower than the control group; it is thought to be caused by the

combination of vitamin C and Organosulfur compounds, which are antioxidant properties found in the content of garlic.

Conclusion

From the above results, it can be concluded that garlic provide antioxidant and antimicrobial benefits to smoked Atlantic Mackerel stored in vacuumed packets at refrigerator temperature (+4°C). Therefore, it is suggested that garlic as a natural herb, could be used to extend the shelf life of meat products, providing the consumer with food containing natural additives, which might be seen more healthful than those of synthetic origin. Further research is required to focus on understanding the mechanisms of action, in particular concentrations of active ingredients of garlic in either powder or fresh form which applied to smoked fish products.

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