



BASIC QUALITY CRITERIA AND SHELF LIFE OF HOT SMOKED ANTALYA BARB (*CAPOETA ANTALYENSIS*)

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ABSTRACT

In this study, the hot smoking process and storage time of *C. antalyensis* was examined. The moisture, protein, ash, and fat content of fresh raw fish was $76.52 \pm 0.43\%$, $17.29 \pm 0.05\%$, $1.90 \pm 0.29\%$, and $1.73 \pm 0.13\%$, respectively. The changes in the findings of the smoked samples compared to the raw fresh sample were found to be significant ($p < 0.5$). During the study, TVB-N, TBA and pH findings changed. The TVB-N value was $35.04 \pm 0.77 \text{ mg/100g}$ on the day 91st, and the TBA value was $9.11 \pm 0.65 \mu\text{g MDA/g}$ on the day 70th. The pH values obtained as 6.8 on the day 49th, and 7.0 on the day 91st. An average score of 1.90 ± 0.46 was obtained for the odor criterion on the day 56th. On the day 35th, the Total Plate Count (TPC) was $6.30 \pm 0.00 \log \text{ cfu/g}$, and the Total Psychrophilic Bacteria count was $6.43 \pm 0.15 \log \text{ cfu/g}$. The total number of yeast-mold was determined as $7.15 \pm 0.15 \log \text{ cfu/g}$ on the day 42nd. In the analysis of *C. antalyensis*, 27 different fatty acids were determined. Of the saturated fatty acids (SFA), C16:0 had the highest value. It was determined that the species can be processed by the hot smoking method, by nutrient content, and by the sensory taste appreciated.

1. Introduction

Fish, which has rich protein, amino acid, unsaturated fatty acid, and mineral content, is of great importance to healthy and balanced nutrition. In 2013, the world's average fish consumption was 19.8 kg per capita. Per capita consumption was 6.1 kg in the same period in Türkiye. This corresponds to 1.7 kg of seafood-originated protein (FAO, 2018). So, increasing the amount of consumption per capita in our country is a necessity for raising healthy generations.

To make up for the animal-originated protein need, it will be necessary to either make more use of existing sources or find alternative sources. Although known to be found in inland

waters, there are many fish species not consumed as human food. These species are of great importance to fulfilling the animal-originated protein deficit.

All fish species die in their natural populations by several effects including predators, natural mortality, and fishing mortality. When they are not caught, fish populations occurring in inland water, complete their life without being used for human use. In addition, recent studies have shown that the number of fish species in those freshwater resources is increasing day by day. So, these fish species should be caught from lakes, dams, and rivers with suitable fishing tools and methods

and should be processed with different processing techniques and presented for consumption. Thus, the per capita fish consumption could be increased.

Capoeta antalyensis (Antalya barb) has not been previously processed by any processing method. After the above explanations, the aim of this study was to investigate the effects of hot smoking processing technology on the quality of *C. antalyensis* and on the storage time. For this purpose, sensory, chemical, and microbiological analyses investigated changes in proximate composition and shelf life.

2. Materials and methods

2.1. Materials

C. antalyensis caught by using gill nets (16x16, 20x20, 25x25, 30x30, 35x35, 40x40 mm mesh) (Özekinci *et al.*, 2003) from Karacaören I Dam (Burdur-Isparta-Turkiye). The fish were iced and carried to the laboratory in an ice-insulated box within 1 h of catching. Approximately 12 kg of fish, which the mean weight of fish was 208.28 ± 39.54 g, were processed with hot smoking technology.

2.2. Methods

2.2.1. Smoking process

The gills and viscera of the fish were removed and cleaned out. Blood, mucus, etc., washed with plenty of icy cold water. The cleaned fish were immersed in an 18% saline solution for 45 minutes. The fish taken out from this solution were mounted to the hanging hooks to be hung in the hot smoking cabinet. It was left hung at room temperature for 20 minutes to remove the water on the fish's surface. The temperatures and times given in Table 1 were applied to the fish under oak shavings in a temperature-controlled mechanical hot smoking cabinet (Bilgin, 2003; İzci, 2004). At the end of the procedure, the fish were allowed to cool to room temperature in the cabinet. The smoked fish was fillet under aseptic conditions. Sufficient samples were reserved for analysis. The remainder fillets were vacuumed packed and placed in the refrigerator ($3 \pm 1^\circ\text{C}$).

Table 1. Time and temperature values applied in the smoked process

Time (minutes)	Temp. ($^\circ\text{C}$)
45	30
60	50
60	60
60	70
45	80

2.3. Analysis

Total protein, fat, moisture, ash, TVB-N, TBARS analysis, pH measurement, microbiological analysis, and sensory evaluation were performed to determine the initial values of raw and smoked fish. Sufficient samples were saved for fatty acids analysis (stored at -80°C). TVB-N, TBARS analysis, pH measurement, microbiological analysis, and sensory evaluation were applied to the smoked fish over a weekly period.

2.3.1. Proximate analysis

Moisture (%) was measured with Kern DBS automatic moisture analyser (Günlü, 2007). Ash (%) and total lipid content (%) were determined according to Lovell (1981). Total protein (%) was determined according to the Kjeldahl method ($\text{Nx}6.25$) AOAC (2000) (modified). The carbohydrate (%) value was obtained by calculation according to the following formula.

$$\text{Carbohydrate (\%)} = 100 - (\text{Moisture} + \text{Fat} + \text{Protein} + \text{Ash})$$

(1)

(Varlık *et al.*, 2007).

2.3.2. Chemical analysis

2.3.2.1 The pH

The pH was measured using a digital pH meter. The previously homogenized fish meat was mixed with distilled water to 1/10 (w/v). The pH was determined by using the pH meter newly calibrated considering the ambient temperature (Erkan and Özden, 2007).

2.3.2.2. TVB-N analysis

TVB-N analysis was conducted by modifying the method of Botta *et al.*, (1984) (Nicholas, 2003). 50 ml 7.5% TCA (Trichloroacetic acid) was added to 25g of

homogenized fish meat. It was homogenized for 30 seconds in the mixer. The mixture was filtered through coarse filter paper under vacuum using a Buchner funnel. The homogenate obtained was put in the refrigerator until analysis. For analysis, 15 ml homogenate was placed in a distillation tube. Distillation was performed by adding 4 ml of 10% NaOH and 10 ml of distilled water. 15 ml of 4% boric acid and 7-8 drops of Tashiro's Indicator (2:1 0.2% alcohol methyl red: 0.2% alcohol methylene blue) were added to the Erlenmeyer where the distillate would be collected. The >50 ml distillate obtained by the distillation process (3 min) was titrated with 0.25 N HCl. By saving the amount of HCl consumption;

$TVB-N (mg / 100 g) = [(HCl \text{ consumption in titration } ml \times \text{Normality of HCl} \times 14,007(\text{Mass Number of Nitrogen}) \times (67.5 \text{ mL} / 15 \text{ mL}))] \times (100 \text{ g} / 25 \text{ g})$ calculated with the formula.

2.3.2.3. TBA analysis

TBA analysis was conducted according to Erkan and Özden (2007). The fish meat was put into 50ml centrifuge tubes by weighing 1.9-2.0 g (with a precision of 0.01 g). 100µl of BHT (Butylated hydroxytoluene) solution prepared in ethanol to be 1 g / l was added. Then 25 ml of TCA (Trichloroacetic acid) solution prepared as 50g / l was added. The mixture was mixed with the homogenizer at medium speed and disintegrated. The resulting mixture was filtered through Whatman No: 1 filter paper. 2 ml was taken from the filtrate and transferred to a 20 ml tube. The mouth is tightly closed by adding 2 ml of newly prepared TBA reagent (0.2883g TBA Reagent + 90 ml of Glacial acetic acid + 10 ml of pure water). It was kept in a water bath at 70-80°C (up to 100°C to complete color formation) for 40 minutes for the reaction to occur. At this stage, the same process was applied to the blind and standards. After the tubes had cooled down, they were read on the spectrophotometer at 532 nm.

Preparation of the Standard: 50µl of TEP (tetra ethoxy propane) was completed with 50 ml of 0.1 N HCl and heated at 100°C for 10 minutes. The hydrolysed acetal obtained was taken 2.4 ml and completed to the mark with 100

ml of pure water. This stock standard has 0.1mM MDA (malondialdehyde). Here, the following standards were prepared in order.

Standard 1: 1 ml was taken from the stock standard and completed to 50 ml with pure water. This standard has 0.002mM MDA (0.14406µg MDA / g).

Standard 2: 3 ml was taken from the stock standard and completed to 50 ml with pure water. This standard has 0.006mM MDA (0.43218µg MDA/g).

Standard 3: 5 ml was taken from the stock standard and completed to 50 ml with pure water. This standard has 0.01mM MDA (0.7203µg MDA/g).

Standard 4: 7 ml was taken from the stock standard and completed to 50 ml with pure water. This standard has 0.014mM MDA (1.00842µg MDA/g).

5 ml (or 2 ml, same the sample amount) were taken from these prepared standards. The same amount of TBA solution was added to it. The tubes were then kept in a water bath at 70-80°C (up to 100°C to complete color formation) for 40 minutes. After the tubes were cooled, by spectrophotometer was read against the blank at 532nm. The sample results read in the spectrophotometer were calculated using the regression curve equation of the standards, and the TBARS concentrations (µg MDA / ml) were found. These densities were put into the formula below.

$$TBARS (\mu g \text{ MDA/g}) = MDA (\mu g \text{ MDA/ml}) \times 25 \text{ ml/ Sample Weight (g)} \quad (2)$$

2.3.3. Microbiological analysis

The first dilution was prepared with fish meat and peptone water (1/10 w/v). Other dilutions were prepared from this first dilution, respectively. Sowing was performed in Petri dishes using the cast plate method. Total plate count (TPC) was incubated at 30±1 °C for 72 hours by using Plate Count Agar (PCA). Total Number of Psychrophilic Microorganisms (TPA) was incubated at 4±1 °C for 10 days by using PCA. Total *Enterobacteriaceae* was incubated at 30±1 °C for 24 hours by using Violet Red Bile Agar (VRB). The yeast-mold

count was incubated at $22 \pm ^\circ \text{C}$ for 4 days by using Potato Dextrose Agar (PDA) (ICMSF 1978; Anonim, 1979; Refai, 1979; Varlık *et al.*, 1993; Anonim, 1994; Arslan *et al.*, 1997). Colonies formed as a result of incubation were counted. 6 log CFU/g limit accepted.

2.3.4. Sensorial analysis

The sensorial analysis was performed completely by using human sensory organs referenced with a hedonic scale. (Huss, 1995; Altuğ and Elmacı, 2005). 10 panellists were informed about the evaluation criteria for evaluation (this was a mixed group, and 7 of the panellists were trained, and 3 were untrained. One of the trained panellists disliked eating freshwater fish). When the products heated in the microwave reached the ambient temperature, they were asked to test in terms of odor, flavor, texture and structure, color and general taste. 10 points were the highest and 0 points were the lowest. The average score of 2 points and below was determined as the limit of deterioration.

2.3.5. Fatty acids analysis

5g of previously homogenized fish meat was weighed and extracted by chloroform/methanol method. Then the chloroform was evaporated to give the oil. 0.01 g of oil was weighed and 2 ml of hexane and 4 ml of 2M KOH were added. The mixture was shaken in the vortex for 2 min. Centrifugation was performed for 10 minutes at 4000 rpm. The upper clear hexane phase was taken into vials and read in the GC (Özoğul *et al.*, 2007).

GC conditions: The device Shimadzu TQ 8040 GC / MS / MS analyzer, and MS detector were used as a detector. The column: TRCN100 (100 m x 0.25 mm x 0.20 μm). Column temperature: 140°C , Injection temperature: 240°C , Column temperature program: 6 minutes at 140°C , 240°C with an increase of $4^\circ\text{C}/\text{min}$, and 10 minutes hold time, Split ratio: 1/100, Carrier gas: He, Flow rate 1.18 ml/min., Pressure 274.6kPa, Injection Volume: 1 μL . Restek Fame mixture was used. Library: FAME and NIST. MS conditions: Ion source temperature: 200°C , Interface temperature: 240°C , Solvent cut time: 7.35 minutes, Scan speed: 1428, Mass range: 41-450 m/z, ϵ : 70 V.

2.3.6. Statistical analysis

The T-test was used to compare proximate composition data, one-way ANOVA was used to determine variance differences, and multiple comparison test DUNCAN was used at a 0.05 significance level to compare groups (Özdamar, 2001).

3. Results and discussions

3.1. Proximate composition

The results obtained from the proximate composition analysis of raw fish and smoked fish are given in Table 2. Moisture content was determined as $76.52 \pm 0.43\%$ in raw fish and $66.58 \pm 0.08\%$ after smoking. Protein was determined as $17.29 \pm 0.05\%$ in raw fish, and $24.46 \pm 1.04\%$ after smoking. Ash content in raw fish was $1.90\% \pm 0.29\%$, and lipid content was found as $1.73 \pm 0.13\%$. The change of the data obtained after the smoking process with the raw sample in moisture, protein, ash, lipid, and carbohydrate values was found statistically significant ($p \leq 0.05$). After the hot smoking process, a significant decrease was observed in the moisture content with the effect of the applied heat, while an increase was observed in the protein, ash, and lipid content.

Table 2. Chemical composition of raw and smoked samples ($\pm\text{SE}$)

	Moisture (%)	Protein (%)	Ash (%)	Lipid (%)	CH (%)**
Raw	76.52 ± 0.43^a	17.29 ± 0.05^a	1.90 ± 0.29^a	1.73 ± 0.13^a	2.56
Smoked	66.58 ± 0.08^b	24.46 ± 1.04^b	5.10 ± 0.61^b	2.91 ± 0.19^b	0.94

* Different lower case letters show significant differences ($p \leq 0.05$)

** Obtained by calculation (CH = 100- all others)

3.2. Chemical analysis

TVB-N value, which was determined as $11.01 \pm 0.92 \text{ mg} / 100\text{g}$ in the raw sample, and $18.94 \pm 0.15 \text{ mg} / 100\text{g}$ in the smoked fish, showed a fluctuating change depending on the storage time (Table 3). According to Connell (1980), 15-20 mg N / 100 g TVB-N value in

marine fish shows good quality, and 50 mg N / 100 g of bad quality (Cadun et al., 2005). According to TVB-N content, Kietzmann et al. (1969) fish products are very good up to 25 mg in 100 grams of fish, good up to 30 mg, can be marketed up to 35 mg, over 35 mg has degraded; Ludorf and Meyer (1973) have considered the product containing 35 mg / 100 g TVB-N as marketable and 40 mg / 100 g TVB-N value as degraded (Dokuzlu, 1997). The European Union requests TVB-N analysis in a case of doubt because of the sensory evaluation to determine fish freshness and reports 25, 30, and 35 mg-TV B-N / 100 g as the critical limit for different fish species (Çakli et al., 2006). Findings obtained during storage showed a discontinuous change. The value of 35mg / 100 g for TVB-N was exceeded on the 91st day of storage. It was observed that the findings changed discontinuously of TVB-N obtained in the study where the hot smoking technology was applied in freshwater fish species, in which the samples were obtained by catching from nature (Yanar, 2007), as in our study (Table 7). In some studies given in Table 7, where the fish processed using the smoking technology was obtained by breeding, it was found that this change was continuously and increased tendency from the beginning to the end of storage, but the findings obtained by Bolat et al. (2009) showed a discontinuously change. In none of the studies in Table 7, it was seen that the deterioration limit value was not exceeded in terms of TVB-N, or the study was not continued until the deterioration occurred.

TBA was determined as $1.57 \pm 0.27 \mu\text{g MDA} / \text{g}$ in raw fish and $1.83 \pm 0.03 \mu\text{g MDA} / \text{g}$ in smoked fish (Table 3). Classifications were made by various researchers according to the amount of TBA contained in seafood. Schormüller (1968, 1969) reported TBA as less than 3 mg malonaldehyde/kg in excellent quality products, less than 5 mg malonaldehyde/kg in good quality fish, and consumption limit as 7-8 mg malonaldehyde/kg (Cadun et al., 2005). According to Curran et al. (1980), when the TBA value exceeds 4 mg malonaldehyde/kg in fish meat, it starts the rancidity, and the

consumption limit value is 8 mg malonaldehyde/kg (Erdem et al., 2005). Kundakçı (1989) reports the maximum limit of consumption for the products containing TBA as 4 mg malonaldehyde/kg. Sinhuber and Yu (1958) stated that TBA value should be 3 mg malonaldehyde/kg and below in good quality products, however products with values between 4-27 mg malonaldehyde/kg can be considered as bad quality (Yapar, 1998).

Table 3. Change of TVB-N, TBA and pH values of raw and smoked samples by the time (\pm SE)

Days	TVB-N (mg /100 g)	TBA ($\mu\text{g MDA} / \text{g}$)	pH
Raw	11.01 \pm 0.92	1.57 \pm 0.27	6.84 \pm 0.07
0.	18.94 \pm 0.15 ^b	1.83 \pm 0.03 ^a	6.83 \pm 0.08 ^d
7.	23.11 \pm 0.34 ^d	3.32 \pm 0.24 ^b	6.84 \pm 0.04 ^d
14.	21.60 \pm 0.42 ^c	4.61 \pm 0.19 ^{bc}	6.77 \pm 0.00 ^{cd}
21.	22.10 \pm 0.51 ^c	3.97 \pm 0.43 ^b	6.62 \pm 0.01 ^b
28.	16.89 \pm 0.14 ^a	5.62 \pm 0.53 ^{cd}	6.61 \pm 0.01 ^b
35.	25.05 \pm 0.80 ^e	6.41 \pm 0.70 ^d	6.62 \pm 0.00 ^b
42.	21.10 \pm 0.08 ^c	6.68 \pm 0.31 ^d	6.51 \pm 0.00 ^a
49.	21.93 \pm 0.64 ^c	3.82 \pm 0.22 ^b	6.98 \pm 0.00 ^c
56.	30.09 \pm 0.80 ^f	3.75 \pm 0.84 ^b	6.79 \pm 0.01 ^{cd}
63.	26.05 \pm 0.08 ^c	4.26 \pm 0.28 ^b	6.75 \pm 0.00 ^c
70.	32.19 \pm 0.22 ^g	9.11 \pm 0.65 ^e	6.58 \pm 0.00 ^{ab}
77.	30.51 \pm 0.14 ^f		6.58 \pm 0.01 ^{ab}
84.	33.45 \pm 0.22 ^g		6.55 \pm 0.01 ^{ab}
91.	35.04 \pm 0.77 ^h		7.31 \pm 0.01 ^f

* The difference between the values shown in different letters is significant ($p \leq 0.05$)

It is seen that the findings we obtained in fresh and smoked samples of *C. antalyensis* species showed a discontinuously change. TBA analyses were continued until the samples deteriorated. With the $9.11 \pm 0.65 \mu\text{g MDA} / \text{g}$ obtained on the day 70th of storage, it was decided that the samples deteriorated in terms of TBA. As in our study, in some studies where smoking technology was applied to freshwater fish, the TBA value showed a discontinuous change (Özkütük, 2002; Çakli et al., 2006; Bolat et al. 2009), while in other studies a continuous and deteriorative tendency change (İzci, 2004; Yanar, 2007; Salama and Ibrahim, 2012; Fıccılar and Gencecep, 2017) (Table 8).

For raw fresh samples of *C. antalyensis* species, the pH value was determined as 6.84 ± 0.07 (Table 3). The pH value (6.83 ± 0.08) determined after the hot smoking process is close to this value and there is no statistically significant difference between them ($p > 0.05$). With the progress of time depending on the changes in the fish structure during the storage process, the pH value showed a change in the tendency of decreasing until the day 35th. As of this day, it showed a change in the increase's tendency. The pH, which is frequently used in determining the product freshness, is not sufficient alone to determine the product quality, and it is reported that the results obtained from this should be evaluated together with other analysis methods (Erkan and Özden, 2007; Bilen, 2009). The product is considered being degraded when the pH value is 7 and above in processed seafood (Gülyavuz and Ünlüsayın, 1999), and the consumption limit value is reported to be 6.8-7.0 (Bilgin, 2003). Diaz et al. (2011) report that a pH-related change could not be determined in the deterioration of cooked trout. Garcia-Linares et al. (2004) determined that the fresh salmon and trout pH values were 6.33 and 6.56 respectively. Also, the highest pH value was 6.61 in the sous-vide cooked trout and reported that the changes in pH value obtained are not effective in microorganism behaviors during the storage period.

Although the limit of 6.8 was exceeded with the pH value of 6.98 ± 0.00 obtained on the day

49th measurement, the subsequent measurements showed a discontinuous change. The 7.0 limit value was exceeded with the pH value of 7.31 ± 0.01 on the day 91st measurement. These results show that the pH value alone cannot determine product quality, as expressed by Erkan and Özden (2007), and Bilen (2009). As in our study, the raw fresh fish and smoked sample initial pH values obtained in some studies where the hot smoking process was applied to freshwater fish are high (Diler et al. 2002; İzci 2004; Yanar 2007; Bolat et al. 2009), by an advance of storage time, the pH value has a decreasing tendency first, but then it has an increasing tendency. Similar changes were not seen in other studies examined (Özkütük 2002; Çakli et al. 2006; Salama and Ibrahim 2012; Fıccılar and Genccelep 2017).

3.3. Sensorial analysis

Sensorial analysis points at the beginning of the study of smoked fish were 7.90 ± 0.43 for color, 8.00 ± 0.39 for flavor, 8.00 ± 0.45 for odor, 7.90 ± 0.43 for texture structure, 7.90 ± 0.41 for appearance, and 8.40 ± 0.31 for general acceptance (Table 4). The samples stored in the refrigerator scored below the limit value (2.00) on the day 56th in terms of the odor parameter. The scores of other parameters were also very close to the limit value.

Table 4. Sensorial analysis score (\pm SE)

Days	Color	Flavor	Odor	Texture Structure	Appearance	General Rating
0.	7.90 ± 0.43^d	8.00 ± 0.39	8.00 ± 0.45^{ef}	7.90 ± 0.43^{ef}	7.90 ± 0.41^e	8.40 ± 0.31^d
7.	8.10 ± 0.30^d	8.20 ± 0.25	8.65 ± 0.23^f	8.85 ± 0.17^g	8.45 ± 0.29^e	8.60 ± 0.18^d
14.	6.40 ± 0.31^c		7.30 ± 0.40^{efg}	7.10 ± 0.31^{de}	6.60 ± 0.40^d	6.80 ± 0.33^c
21.	5.80 ± 0.39^{bc}		5.50 ± 0.58^{cd}	6.40 ± 0.52^{cd}	6.00 ± 0.37^{cd}	6.10 ± 0.46^{bc}
28.	4.60 ± 0.37^b		4.60 ± 0.50^{bc}	5.00 ± 0.37^b	5.00 ± 0.37^c	5.10 ± 0.41^b
35.	6.10 ± 0.38^c		6.70 ± 0.50^{def}	6.50 ± 0.78^{cd}	6.40 ± 0.43^d	6.40 ± 0.43^c
42.	6.00 ± 0.50^c		6.50 ± 0.76^{de}	5.40 ± 0.64^{bc}	6.00 ± 0.45^{cd}	6.00 ± 0.45^{bc}
49.	4.60 ± 0.60^b		4.00 ± 0.60^b	4.10 ± 0.55^b	3.50 ± 0.58^b	3.80 ± 0.57^a
56.	2.40 ± 0.34^a		1.90 ± 0.46^a	2.20 ± 0.36^a	2.30 ± 0.37^a	2.90 ± 0.50^a

*The difference between the values shown in different letters is significant ($p \leq 0.05$)

Although smoking technology is traditionally used in the preservation of fish in many regions of the world, the acceptability of the product depends on its sensory properties (Yanar, 2007; Fıccılar and Genccelep, 2017). It is out of the question that a product whose sensory results are not suitable can be offered for consumption and thus marketed (Dokuzlu, 1997). Because if a product acceptable in terms of quality parameters is unacceptable in terms of sensory properties, this product cannot be consumed (Özden et al., 2001). In our study, the sensory scores obtained on the day 7th were higher than the initial values. This case shows that the structural changes that occur in the fish after the hot smoking process have turned into a stable form. For example, the smoke aroma fully integrates with fish meat during storage in the refrigerator. This was seen in all the criteria. In some examined studies, there was a similar change (Bolat et al., 2009; Alçiçek, 2010; Kaba et al., 2013). But there were some studies where this change was not observed (Özkütük, 2002; Çakli et al., 2006; Yanar, 2007). By the progression of the storage period, a relative decrease in sensory scores was detected. These changes depending on time were found statistically significant ($p \leq 0.05$). On the day 56th, the odor criterion was effective in making the sensory deterioration decision for the samples with an average score of 1.90 ± 0.46 . On the other hand, the scores obtained for other criteria (color, texture structure, appearance, general rating) were determined above the deterioration limit.

3.4. Microbiological analysis

Total *Enterobacteriaceae* was not observed in the samples during storage in the refrigerator. On the day 35th, the number of TPC was determined as 6.93 ± 0.03 log cfu /g, and the number of TPA was 6.43 ± 0.15 log cfu /g. The total of yeast mold was determined as 6.00 ± 0.05 log cfu /g on the day 28th. In the analysis performed on the 49th storage day, TPC was determined as 7.02 ± 0.02 log cfu /g, TPA 6.84 ± 0.06 log cfu /g, and yeast-mold 6.89 ± 0.01 log cfu /g (Table 5).

Seafood is generally considered as being degraded when it reaches 6-7 log cfu/g microorganisms (ICMSF, 1986; Huss, 1988; Çakli, 2007; Sallam, 2007; Kiliç et al., 2018). In the analysis made on the day 35th, by the obtained a value of 6.30 ± 0.00 log cfu / g the limit value of 6.00 log cfu / g was clearly exceeded. In the analysis made on the day 42nd, the total number of yeast-mold was determined as 7.15 ± 0.15 log cfu / g. It is seen that the product can be consumed up to day 35th in terms of the TPC and the TPA. When tested in terms of yeast-mold count, we can say that the product can be consumed safely until the day 28th.

It is reported that there is no yeast-mold growth in studies that examined smoked trout (Çakli et al., 2006; Bolat et al., 2009). As in our study, in the studies where the fish are supplied by catching from nature, canal catfish (Efiuvwevwere and Ajiboye, 1996), Tench (İzci, 2004), Vimba bream (Diler et al., 2002), yeast-mold development were observed.

Table 5. Microbiological analysis results (\pm SE)

Days	TPC (log kob/g)	TPA (log kob/g)	Enterobacteriaceae (log kob/g)	Mould-Yeast (log kob/g)
Raw	0.54 ± 0.06^b	0.65 ± 0.05^a	$<10^1$	1.04 ± 0.04^a
0.	$<10^1$ ^a	2.36 ± 0.25^b	$<10^1$	2.72 ± 0.10^b
7.	2.85 ± 0.00^c	2.71 ± 0.03^c	$<10^1$	2.73 ± 0.03^b
14.	4.79 ± 0.03^d	4.00 ± 0.00^d	$<10^1$	4.04 ± 0.03^c
21.	$4.95 \pm$	$5.10 \pm$	$<10^1$	$5.03 \pm$

	0.00 ^e	0.02 ^e		0.01 ^d
28.	5.85± 0.01 ^f	5.91± 0.03 ^f	<10 ¹	6.00± 0.05 ^e
35.	6.93± 0.03 ^g	6.43± 0.15 ^g	<10 ¹	6.30± 0.00 ^f
42.	8.03± 0.00 ⁱ	6.77± 0.07 ^h	<10 ¹	7.15± 0.15 ^g
49.	7.02± 0.02 ^h	6.84± 0.06 ^h	<10 ¹	6.89± 0.01 ^h

* The difference between the values shown in different letters is significant ($p \leq 0.05$)

It is grown in trout-controlled conditions and in cold waters. The used fish in studies in which the yeast-mold development was observed, is cyprinid and their habitat is relatively warmer waters. This temperature is also suitable for the survival and development of yeast-mold. In studies that examined the smoked rainbow trout (Arashisar et al., 2004; Bolat et al., 2009; Mutlu 2016) while the development of bacteria, psychrophilic bacteria, and *Enterobacteriaceae* were observed, but it was reported there was no *Enterobacteriaceae* in a study (Çakli et al., 2006).

3.5. Fatty acids analysis

In the analysis of *C. antalyensis* raw sample, 27 different fatty acids were determined (Figure 6). The number of fatty acids without double bonds (SFA) is 8, the number of fatty acids containing one double bond (MUFA) is 7, and the number of fatty acids containing two or more double bonds (PUFA) is 12. Of the double-bonded, 5 fatty acids were determined in the n-3 group, 6 in the n-6 group, 2 in the n-9 group, 1 in the n-11 group, and 1 in the n-12 group (Table 6).

Among fatty acids without double bonds (SFA), C16: 0 had the highest value. C16: 1 value, which was determined as $10.95 \pm 0.17\%$ in raw fish, decreased to $6.41 \pm 2.52\%$ after the hot smoking process. Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) values were higher in smoked fish than in raw fish. Low amounts of C19:0 fatty acids were detected in

raw and smoked samples. While the total SFA value determined in the raw sample was 24.62%, this value increased to 25.38% in the smoked samples. On the other hand, in the raw sample, the total value of fatty acids containing one double bond was determined as 31.44%, while it fell to 26.86% in the smoked samples.

While the total value of the n-3 group fatty acids of the raw sample increased in the smoked sample, the opposite result was obtained for the n-6 group fatty acids.

Humans cannot synthesize fatty acids double-bonded containing the 9 C atom and more when counted from the end of the carboxyl molecule. For example, linoleic acid (C18: 2n-6), which is an essential fatty acid, cannot be synthesized from the human body, so it should be taken from the outside (Leaf and Weber, 1988).

In studies conducted, it has been reported that Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), which are among the n-3 group of fatty acids, are found in seafood oils intensely and are a natural source of these fatty acids (Eseceli et al., 2006; Nollet and Toldrá, 2010).

The fat content in fish and the fatty acid composition of these oils may vary depending on the various factors, such as species, individuals, body areas, feeding, fishing season, gender, etc., (Özden et al., 2001; Kaya et al., 2004).

Table 6. Fatty acids detected in the raw and smoked sample (%) (\pm SE)

	Raw	Smoked
C4:0	0.17 \pm 0.05 ^a	0.56 \pm 0.05 ^a
C14:0	1.94 \pm 0.07 ^a	1.88 \pm 0.97 ^a
C15:0	2.10 \pm 0.05 ^c	1.50 \pm 0.16 ^{bc}
C16:0	7.70 \pm 0.04 ^a	15.84 \pm 1.2 ^b
C16:1	10.95 \pm 0.1 ^b	6.41 \pm 2.52 ^{ab}
C17:0	7.92 \pm 0.46 ^{ab}	0.87 \pm 0.03 ^a
C17:1	0.53 \pm 0.05 ^a	0.88 \pm 0.49 ^a
C18:0	2.45 \pm 0.01 ^a	4.01 \pm 0.95 ^a
C18:1	3.56 \pm 0.37 ^b	2.09 \pm 0.63 ^{ab}
C18:1n-9(c)	12.29 \pm 4.13 ^a	11.33 \pm 1.85 ^a
C18:2n-6(t)	3.10 \pm 0.03 ^b	0.15 \pm 0.10 ^a
C18:2n-6(c)	2.84 \pm 1.72 ^a	3.66 \pm 1.34 ^a
C18:3n-6	0.74 \pm 0.07 ^a	0.81 \pm 0.23 ^a
C18:1n-12	1.13 \pm 0.10 ^{ab}	2.72 \pm 2.28 ^b
C18:1n-11	1.15 \pm 0.07 ^a	2.51 \pm 2.43 ^a
C19:0	2.18 \pm 0.17 ^b	0.50 \pm 0.00 ^a
C22:5n-3	3.42 \pm 0.31 ^a	6.43 \pm 0.40 ^c
C20:0	0.16 \pm 0.02 ^a	0.22 \pm 0.06 ^a
C18:3n-3	11.06 \pm 4.18 ^b	6.77 \pm 2.67 ^{ab}
C20:1n-9	1.83 \pm 0.23 ^{ab}	0.92 \pm 0.12 ^a
C20:2n-6	0.35 \pm 0.02 ^{ab}	0.25 \pm 0.08 ^a
C20:3n-6	0.52 \pm 0.34 ^a	0.52 \pm 0.07 ^a
C20:3n-3	0.37 \pm 0.14 ^a	0.40 \pm 0.20 ^a
C20:4n-6	3.31 \pm 0.19 ^a	4.06 \pm 1.44 ^a
C22:2	0.20 \pm 0.11 ^a	0.28 \pm 0.00 ^a
C20:5n-3	4.81 \pm 0.38 ^a	7.24 \pm 0.24 ^{ab}
C22:6n-3	5.91 \pm 0.35 ^a	11.36 \pm 3.57 ^{ab}
Σ SFA	24.62	25.38
Σ MUFA	31.44	26.86
Σ PUFA n-3	25.77	32.48
Σ PUFA n-6	10.86	9.45

*The difference between the values shown in different letters in the same line is significant ($p \leq 0.05$)

Table 7. TVB-N values and variation obtained in some studies where smoked technology is applied to freshwater fish

Study	Species	Supply Area	Initial TVB-N (mgN/100 g)	After Smoking TVB-N (mgN/100 g)	End of Storage TVB-N (mgN/100g)	Storage period (day)	Tendency
Yanar 2007	<i>Clarias gariepinus</i>	Fisheries	15.47±0.22	17.67±0.81	29.16±1.68	24	Discontinuous
Özkütük 2002	<i>Oreochromis niloticus</i>	Breeding		15.02±0.66	22.63±0.23	75	Continuous Increase
Bolat et al. 2009	<i>Oncorhynchus mykiss</i>	Breeding	14.56±0.70	18.72±0.24	18.53±0.24	30	Discontinuous
Çaklı et al. 2006	<i>Oncorhynchus mykiss</i>	Breeding		9.3±0.60	27.90±0.10	40	Continuous Increase
Fıcıcılar and Gencelep 2017	<i>Oncorhynchus mykiss</i>	Breeding		16.56 ± 0.34	19.74 ± 0.38	21	Continuous Increase
İzci 2004	<i>Tinca tinca</i>	Fisheries	13.53±0.47	16.33±0.47	26.60±0.81	28	Continuous Increase
Salama and Ibrahim 2012	<i>Ctenopharyngo don idella</i>	Breeding		9.20± 0.03	22.9± 0.5	30	Continuous Increase

Table 8. TBA values and changes obtained in some studies where smoked technology is applied to freshwater fish

Study	Species	Supply Area	Initial TBA (mg mda/kg)	After Smoking TBA (mg mda/kg)	End of Storage TBA (mg mda/kg)	Storage period (day)	Tendency
Yanar, 2007	<i>Clarias gariepinus</i>	Fisheries	0.45±0.04	0.84±0.03	2.67±0.62	24	Continuous Increase
Özkütük 2002	<i>Oreochromis niloticus</i>	Breeding		0.16±0.01	0.11±0.00	75	Discontinuous
Bolat et al. 2009	<i>Oncorhynchus mykiss</i>	Breeding		0.85±0.03	0.48±0.07	30	Discontinuous
Çaklı et al. 2006	<i>Oncorhynchus mykiss</i>	Breeding		1.4	2.1	40	Discontinuous
Fıcıcılar and Gencelep 2017	<i>Oncorhynchus mykiss</i>	Breeding		0.48±0.07	3.65 ± 0.24	21	Continuous Increase
İzci 2004	<i>Tinca tinca</i>	Fisheries	0.15±0.00	0.32±0.01	3.08±0.01	28	Continuous Increase
Salama and Ibrahim 2012	<i>Ctenopharyngo don idella</i>	Breeding		0.13± 0.01	1.72± 0.1	30	Continuous Increase

4. Conclusions

In this study, applying the hot smoking technology to *C. antalyensis* species and the storage time of the obtained product under refrigerator conditions were studied. It was determined that the species can be processed by hot smoking technology owing especially to the obtained rather high sensory taste score.

Preserving the smoked product in frozen storage instead of a refrigerator and conducting a study to measure the consumer response to a product stored in this way can give up useful results.

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