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Research Article

# The Determination of the Shelf Life and Some Nutritional Components of Gilthead Seabream (*Sparus aurata* L., 1758) after Cold and Hot Smoking

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**Abstract:** This study aimed to determine the shelf life, as well as chemical and microbiological quality of gilthead seabream (*Sparus aurata* L., 1758) prepared by 2 different methods of smoking (hot smoking and cold smoking). The effects of hot and cold smoking on the chemical composition and microbial load of gilthead seabream, as well as organoleptic analysis of the smoked product were investigated. Significant ($P < 0.05$) differences were found in the chemical composition of fresh and smoked seabream. The panelists liked the hot smoked fish more than the cold smoked fish, according to sensory analysis results. Changes in pH, thiobarbituric acid (TBA), and total volatile basic nitrogen (TVB-N) values were significant ($P < 0.05$) during storage at 4 °C. Microbiological analysis results demonstrated that the smoking techniques reduced the microbial content of the fish, whereas microbial content increased during storage. The smoking methods tested had a small effect on the level of vitamin $D_3$ in gilthead seabream.

**Key Words:** Gilthead seabream, hot and cold smoking, shelf life, food components

## Sıcak ve Soğuk Dumanlanmış Çipura Balığı (*Sparus aurata* L., 1758)' nın Bazı Besinsel Bileşenleri ve Raf Ömrünün Belirlenmesi

**Özet:** Araştırmada sıcak ve soğuk dumanlama yöntemine göre dumanlanan çipura (*Sparus aurata* L., 1758) balığının mikrobiyolojik, kimyasal kalitesi ve raf ömrünün belirlenmesi amaçlanmıştır. Her iki şekilde dumanlanan balıkların, kimyasal kompozisyon ve organoleptik analizleri yapılmıştır. Taze, sıcak ve soğuk dumanlanmış çipura balıklarının su, protein, yağ ve kül bileşenlerindeki değişimin farkı önemli ($P < 0,05$) bulunmuştur. Panelistler tarafından gerçekleştirilen organoleptik analiz sonucunda, sıcak dumanlanmış çipura balığı daha çok beğenilmiştir. Dumanlanmış çipura balıklarının depolama süresince pH, tiyobarbutirik asit (TBA) ve toplam uçucu bazik azotu (TVB-N) değerlerindeki değişimin farkının önemli ($P < 0,05$) olduğu saptanmıştır. Mikrobiyolojik analizler sonucunda uygulanan işleme tekniklerinin mikroorganizma sayısını azalttığı tespit edilmiştir. Fakat sıcak ve soğuk dumanlanmış balıkların depolama süresince mikroorganizma sayılarının arttığı belirlenmiştir. Çipura balığının Vitamin $D_3$ düzeyinin sıcak ve soğuk dumanlama işleminden çok az etkilendiği tespit edilmiştir.

**Anahtar Sözcükler:** Çipura, sıcak ve soğuk dumanlama, raf ömrü, besin bileşenleri

## Introduction

Gilthead seabream is one of the main fish species farmed in Turkey. Turkish production of seabream was 16,735 t in 2003 and 27,634 t in 2005. Usually, gilthead seabream is consumed fresh (1,2). The bulk of this species is marketed as whole fish, packed on ice. Production expansion of gilthead seabream in all Mediterranean countries since 1990 has markedly changed the balance of supply and demand, leading, in combination with poor marketing, to a decrease in farm prices. As it is available only as fresh fish, the increasing quantity of this farm species cannot be easily absorbed. A solution to this problem could be the production and promotion of value-added products with high profit

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margins, which could fulfill the present demands of consumers. Such a product would be smoked, ready-to-eat gilthead seabream (3).

Fish oils are the richest known sources of vitamins A and D (4), and fish muscle is a good source of vitamin D (5). Fish muscle also contains remarkable amounts of cholecalciferol (vitamin $D_3$) (6). Vitamin D deficiency in humans has been linked to increased risk of many chronic diseases, including diabetes, cancer, hypertension, and heart disease (7). To the best of our knowledge changes in the cholecalciferol content in fresh and smoked seabream have not been previously studied.

Smoking is probably the oldest known method used for preserving fish. At the present time, the effects of brining and smoking on color and sensory perception are at least as important as the preservative effect due to the use of modern refrigeration systems (8). Smoked meat products exhibit an increased resistance to oxidative changes and whilst oxidation can lead to the destruction of some vitamins it would be expected that certain smoke constituents could help protect such oxidizable vitamins in smoked fish products (9).

Although much research has been conducted on the effect of smoking on the quality parameters of many fish species (10-13), no reference concerning the shelf life and vitamin content of smoked gilthead seabream has been found in the literature. The objective of this work was to investigate the shelf life and changes to the chemical and microbiological parameters of raw gilthead seabream during smoking and storage, as well as the quality characteristics of the hot and cold smoked product.

## Materials and Methods

The study included 80 seabream (300-330 g per fish) (*Sparus aurata*) purchased from a local marine culture farm (Bodrum, Turkey). The fish were kept in boxes with ice flakes. All laboratory analyses was started 24 h after death. Hot smoking and cold smoking techniques were used. An AFOS-type mechanical oven and oak sawdust were used for both smoking procedures. Fish samples were prepared and hot smoked according to a smoldering method previously described (14). The fish were kept in a 20% (w/v) salt solution (fish/brine solution ratio: 1:1) at 16 °C for 45 min. The fish were then removed from the brine solution, hung in a kiln, strained, and kept at 20 °C for about 20 min. For the first 45 min, a temperature of 30 °C was applied and during the next 180 min the temperature was gradually increased to 50, 60, and 70 °C. During the final 45 min the temperature was kept at 80 °C. Other fish samples were prepared and cold smoked using the modified method described by Cardinal et al. (15). The brine salting technique used saturated brine (360 g/l) maintained at 12 ± 1 °C in which the fish were placed (ratio: 50:50 w/v). After 40 min the fish were removed, rapidly rinsed, and stored for 2 h in a cold room at 2 °C. The cold smoking process then began with a drying step in the smoking oven for 30 min at 20 °C, followed by a smoking step at 30 °C and a relative humidity of 50%. Cold smoking was performed for 3 h. Both smoked samples were vacuum packed and stored at 4 °C. All samples was analyzed on the $1^{st}$, $7^{th}$, $14^{th}$, $21^{st}$, $28^{th}$, $35^{th}$, and $60^{th}$ day of storage.

Moisture, crude protein, crude fat, and crude ash content were determined according to standard procedures (16-19). Sodium chloride was determined by the Mohr method (20). pH was measured in the dorsal muscle with a digital electronic pH meter with a glass electrode (WTW Mark 320). TBA number was determined as described by Varlık et al. (21). TVB-N values were estimated using the method described by Lücke-Geidel, as modified by Antonacopoulas and reported by İnal (22). For microbiological analysis, preparation of the samples was carried out according to Refai (23) and Varlık et al. (21). Total mesophilic count (TMC) was determined using plate count agar (Merck 5463) after incubation at 30 °C for 72 h (21,23). Total psychrophilic aerobic (TPA) bacterium was measured using plate count agar (Merck 5463) after incubation for 7-10 days at 5 °C (24). *Staphylococcus* and *Micrococcus* counts were determined using Mannitol salt agar (Merck) after incubation at 37 °C for 48 h (24). Yeast and mold counts were determined using YGC agar (25). Yeast and mold were incubated at 22 °C for 3-5 days. All colonies were counted and the data were reported as colony forming units (log CFU/g).

Immediately after the fish were procured, they were gutted, washed, wrapped in aluminum foil to protect them from light, vacuum packed, and then frozen in an air blast freezer at –80 °C. Vitamin $D_3$ analysis was carried out according to a modified method of Aust et al.

(26) by HPLC with a diode array detector and SCL-10 Avp system controller. Sensory evaluation was carried out according to Stone and Sidel (27), and quantitative descriptive analysis (QDA) was used to evaluate the sensory quality of the different products. Sensory evaluation of the flavor, texture, appearance, and odor of the smoked fish was carried out by 10 selected and trained panelists. A continuous scale from 0 to 9 was used. A value of 0 corresponded to the lowest level of each parameter and a value of 9 to the highest.

In each analysis of both smoking methods 3 fish were filleted and then homogenized (Waring Blender, USA). Every parameter was measured in triplicate, except sensory analysis. Statistical analyses were performed using SPSS v.9.0 for Windows. Analysis of variance (ANOVA) was used and statistical significance was set at $P < 0.05$.

## Results

Table 1 shows the chemical composition of fresh and salted non-smoked samples (SNS), hot smoked gilthead seabream, and cold smoked gilthead seabream.

The results of the sensory evaluation are given in Table 2. pH, thiobarbituric acid (TBA), and total volatile basic nitrogen (TVB-N) values of all samples are given in Table 3. The initial TBA value of raw gilthead seabream was 0.594 ± 0.04 mg MA/kg (Table 3). This value increased to 1.027 ± 0.11 mg MA/kg (1st day of storage of hot smoked samples) and 0.834 ± 0.03 mg MA/kg (1st day of storage of cold smoked samples). Additionally, the TVB-N value increased after the smoking process.

Microbiological findings for smoked gilthead seabream stored at 4 °C are presented in the Table 4. The changes in vitamin $D_3$ of fresh and SNS samples smoked and stored at 4 °C were variable and are given in Table

Table 1. Proximate composition of fresh, salted, and smoked seabream (mean ± SE).

| | Fresh | SNS-H | SNS-C | HS | CS |
|---|---|---|---|---|---|
| Moisture (%) | 72.93 ± 0.28$^{a}$ | 70.35 ± 1.12$^{b}$ | 67.31 ± 0.17$^{c}$ | 60.47 ± 0.71$^{e}$ | 64.53 ± 1.12$^{d}$ |
| Protein (%) | 20.40 ± 0.37$^{d}$ | 20.70 ± 0.53$^{d}$ | 21.86 ± 0.27$^{c}$ | 26.40 ± 0.50$^{a}$ | 22.88 ± 0.33$^{b}$ |
| Fat (%) | 2.98 ± 0.12$^{d}$ | 3.11 ± 0.10$^{cd}$ | 3.52 ± 0.14b$^{c}$ | 4.54 ± 0.40$^{a}$ | 3.85 ± 0.30$^{b}$ |
| Ash (%) | 1.39 ± 0.02$^{d}$ | 2.55 ± 0.41$^{c}$ | 4.13 ± 0.21$^{b}$ | 3.78 ± 0.17$^{a}$ | 4.60 ± 0.26$^{b}$ |
| Sodium chloride (%) | 0.73 ± 0.06$^{e}$ | 2.79 ± 0.16$^{c}$ | 3.15 ± 0.09$^{b}$ | 2.40 ± 0.13$^{b}$ | 4.04 ± 0.10$^{d}$ |

*Different letters in the same line show significant differences among samples ($P < 0.05$).
SNS-H: salted samples before hot smoking; SNS-C: salted samples before cold smoking; HS: hot smoked; CS: cold smoked.

Table 2. Sensory evaluation of smoked gilthead seabream (mean ± SE).

| Parameters | Tech. | Days | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 7 | 14 | 21 | 28 | 35 | 60 |
| Flavor | HS | $^{A}$8.17 ± 0.39$^{a}$ | $^{A}$7.85 ± 0.28$^{a}$ | $^{A}$7.50 ± 0.31$^{ab}$ | $^{A}$6.60 ± 0.40$^{bc}$ | $^{A}$6.00 ± 0.47$^{cd}$ | $^{A}$5.30 ± 0.37$^{d}$ | $^{A}$3.70 ± 0.133$^{e}$ |
| | CS | $^{B}$6.03 ± 0.35$^{a}$ | $^{B}$5.60 ± 0.37$^{a}$ | $^{B}$5.30 ± 0.42$^{a}$ | $^{B}$4.10 ± 0.38$^{b}$ | $^{B}$3.90 ± 0.46$^{b}$ | $^{B}$3.20 ± 0.39$^{b}$ | $^{B}$2.10 ± 0.23$^{c}$ |
| Texture | HS | $^{A}$8.06 ± 0.38$^{a}$ | $^{A}$7.60 ± 0.34$^{a}$ | $^{A}$7.10 ± 0.38$^{ab}$ | $^{A}$6.30 ± 0.30$^{bc}$ | $^{A}$5.60 ± 0.31$^{cd}$ | $^{A}$4.80 ± 0.25$^{de}$ | $^{A}$4.10 ± 0.31$^{e}$ |
| | CS | $^{B}$5.50 ± 0.34$^{a}$ | $^{B}$5.30 ± 0.37$^{a}$ | $^{B}$5.40 ± 0.27$^{a}$ | $^{B}$4.40 ± 0.27$^{b}$ | $^{B}$4.20 ± 0.29$^{b}$ | $^{B}$3.30 ± 0.33$^{c}$ | $^{B}$2.40 ± 0.31$^{d}$ |
| Appearance | HS | $^{A}$7.80 ± 0.44$^{a}$ | $^{A}$7.20 ± 0.25$^{a}$ | $^{A}$6.90 ± 0.31$^{a}$ | $^{A}$6.00 ± 0.26$^{b}$ | $^{A}$5.50 ± 0.27$^{bc}$ | $^{A}$4.90 ± 0.23$^{c}$ | $^{A}$4.00 ± 0.37$^{d}$ |
| | CS | $^{B}$5.30 ± 0.21$^{a}$ | $^{B}$5.20 ± 0.36$^{ab}$ | $^{B}$4.40 ± 0.27$^{bc}$ | $^{B}$4.10 ± 0.31$^{cd}$ | $^{B}$4.30 ± 0.21$^{c}$ | $^{B}$3.40 ± 0.34$^{d}$ | $^{B}$2.20 ± 0.33$^{e}$ |
| Odor | HS | $^{A}$8.35 ± 0.26$^{a}$ | $^{A}$8.40 ± 0.22$^{a}$ | $^{A}$7.10 ± 0.46$^{b}$ | $^{A}$6.50 ± 0.34$^{bc}$ | $^{A}$5.80 ± 0.36$^{c}$ | $^{A}$5.90 ± 0.31$^{c}$ | $^{A}$4.20 ± 0.39$^{d}$ |
| | CS | $^{B}$6.50 ± 0.45$^{a}$ | $^{B}$5.90 ± 0.50$^{a}$ | $^{B}$4.70 ± 0.33$^{b}$ | $^{B}$4.10 ± 0.43$^{bc}$ | $^{B}$3.10 ± 0.23$^{cd}$ | $^{B}$2.60 ± 0.22$^{de}$ | $^{B}$1.60 ± 0.22$^{e}$ |
| General taste | HS | $^{A}$8.10 ± 0.18$^{a}$ | $^{A}$7.76 ± 0.15$^{a}$ | $^{A}$7.15 ± 0.18$^{b}$ | $^{A}$6.35 ± 0.16$^{c}$ | $^{A}$5.73 ± 0.18$^{d}$ | $^{A}$5.23 ± 0.16$^{e}$ | $^{A}$4.00 ± 0.17$^{f}$ |
| | CS | $^{B}$5.83 ± 0.18$^{a}$ | $^{B}$5.50 ± 0.20$^{a}$ | $^{B}$4.95 ± 0.17$^{b}$ | $^{B}$4.18 ± 0.17$^{c}$ | $^{B}$3,88 ± 0.17$^{c}$ | $^{B}$3.13 ± 0.16$^{d}$ | $^{B}$2.08 ± 0.14$^{e}$ |

In same line means ± SE with different lower case letters are significantly different ($P < 0.05$).
In same column means ± SE with different upper case letters are significantly different ($P < 0.05$).
HS: hot smoked; CS: cold smoked.

Table 3. Changes in pH, TBA, and TVB-N values of all samples during storage (at 4 °C) (mean ± SE).

| | pH | | TBA(mg MA/kg) | | TVB-N(mg/100g) | |
|---|---|---|---|---|---|---|
| | HS | CS | HS | CS | HS | CS |
| Fresh | 6.198 ± 0.04$^{d}$ | 6.198 ± 0.04$^{ab}$ | 0.594 ± 0.04$^{d}$ | 0.594 ± 0.04$^{d}$ | 14.280 ± 0.57$^{g}$ | 14.280 ± 0.57$^{h}$ |
| SNS | 6.145 ± 0.01$^{d}$ | 6.123 ± 0.02$^{b}$ | 0.551 ± 0.08$^{d}$ | 0.762 ± 0.04$^{cd}$ | 15.643 ± 0.45$^{f}$ | 16.063 ± 0.47$^{g}$ |
| 1$^{st}$ day | $^{A}$6.394 ± 0.01$^{b}$ | $^{A}$6.349 ± 0.16$^{a}$ | $^{A}$1.027 ± 0.11$^{bcd}$ | $^{B}$0.834 ± 0.03$^{cd}$ | $^{B}$16.307 ± 0.56$^{ef}$ | $^{A}$19.807 ± 0.41$^{f}$ |
| 7$^{th}$ day | $^{A}$6.283 ± 0.02$^{c}$ | $^{B}$6.152 ± 0.01$^{ab}$ | $^{A}$1.386 ± 0.01$^{bc}$ | $^{B}$0.688 ± 0.03$^{d}$ | $^{B}$17.513 ± 0.24$^{de}$ | $^{A}$25.103 ± 0.53$^{e}$ |
| 14$^{th}$ day | $^{A}$6.436 ± 0.01$^{b}$ | $^{B}$6.241 ± 0.01$^{ab}$ | $^{B}$0.612 ± 0.03$^{d}$ | $^{A}$0.879 ± 0.07$^{cd}$ | $^{B}$18.707 ± 0.16$^{d}$ | $^{A}$28.670 ± 0.62$^{d}$ |
| 21$^{st}$ day | $^{A}$6.426 ± 0.01$^{b}$ | $^{B}$6.188 ± 0.01$^{ab}$ | $^{A}$1.189 ± 0.14$^{bc}$ | $^{A}$1.238 ± 0.05$^{c}$ | $^{B}$25.613 ± 0.43$^{c}$ | $^{A}$28.177 ± 0.11$^{d}$ |
| 28$^{th}$ day | $^{A}$6.446 ± 0.01$^{b}$ | $^{B}$6.245 ± 0.07$^{ab}$ | $^{B}$1.490 ± 0.15$^{b}$ | $^{A}$3.057 ± 0.40$^{b}$ | $^{B}$24.870 ± 0.58$^{c}$ | $^{A}$33.30 ± 0.24$^{c}$ |
| 35$^{th}$ day | $^{A}$6.436 ± 0.01$^{b}$ | $^{B}$6.239 ± 0.01$^{ab}$ | $^{B}$0.919 ± 0.02$^{cd}$ | $^{A}$3.627 ± 0.15$^{a}$ | $^{B}$27.113 ± 0.46$^{b}$ | $^{A}$35.01 ± 0.22$^{b}$ |
| 60$^{th}$ day | $^{A}$6.565 ± 0.01$^{a}$ | $^{B}$6.254 ± 0.01$^{ab}$ | $^{B}$2.517 ± 0.44$^{a}$ | $^{A}$3.883 ± 0.08$^{a}$ | $^{B}$33.307 ± 0.47$^{a}$ | $^{A}$40.790 ± 0.51$^{a}$ |

In same column means ± SE with different lower case letters are significantly different (P < 0.05).
In same line means ± SE with different upper case letters are significantly different (P < 0.05).
SNS: Salted non smoked samples (HS: hot smoked, CS: cold smoked)

Table 4. The microbial flora of smoked seabream during storage (at 4 °C) (log cfu/g) (mean ± SE).

| | TMC | | TPA | | Stap -Mic. | | Yeast-Mould | |
|---|---|---|---|---|---|---|---|---|
| | HS | CS | HS | CS | HS | CS | HS | CS |
| Fresh | 3.087 ± 0.02$^{f}$ | 3.087 ± 0.02$^{g}$ | 9.033 ± 0.12$^{a}$ | 9.033 ± 0.12$^{a}$ | 2.580 ± 0.02$^{f}$ | 2.580 ± 0.02$^{f}$ | • | • |
| SNS | $^{A}$2.943 ± 0.02$^{g}$ | $^{B}$2.243 ± 0.01$^{h}$ | $^{A}$2.716 ± 0.01$^{g}$ | $^{B}$2.063 ± 0.02$^{h}$ | $^{A}$2.333 ± 0.02$^{g}$ | $^{A}$2.270 ± 0.02$^{h}$ | • | • |
| 1$^{st}$ day | $^{B}$2.567 ± 0.04$^{h}$ | $^{A}$3.650 ± 0.02$^{e}$ | $^{B}$2.283 ± 0.03$^{h}$ | $^{A}$3.287 ± 0.02$^{f}$ | $^{B}$2.213 ± 0.02$^{h}$ | $^{A}$2.383 ± 0.03$^{g}$ | • | • |
| 7$^{th}$ day | $^{B}$2.933 ± 0.01$^{g}$ | $^{A}$3.333 ± 0.01$^{f}$ | $^{B}$2.827 ± 0.01$^{fg}$ | $^{A}$3.723 ± 0.02$^{e}$ | $^{B}$3.357 ± 0.02$^{e}$ | $^{A}$3.827 ± 0.01$^{c}$ | • | • |
| 14$^{th}$ day | $^{B}$3.643 ± 0.02$^{e}$ | $^{A}$4.957 ± 0.01$^{d}$ | $^{A}$2.927 ± 0.01$^{f}$ | $^{A}$2.970 ± 0.01$^{g}$ | $^{B}$3.790 ± 0.01$^{d}$ | $^{A}$3.973 ± 0.01$^{b}$ | • | • |
| 21$^{st}$ day | $^{B}$4.100 ± 0.01$^{d}$ | $^{A}$5.580 ± 0.02$^{c}$ | $^{A}$3.523 ± 0.02$^{e}$ | $^{A}$3.757 ± 0.01$^{e}$ | $^{A}$4.100 ± 0.03$^{b}$ | $^{B}$2.927 ± 0.01$^{e}$ | • | • |
| 28$^{th}$ day | $^{B}$4.873 ± 0.02$^{c}$ | $^{A}$4.993 ± 0.01$^{d}$ | $^{B}$3.930 ± 0.01$^{d}$ | $^{A}$4.347 ± 0.01$^{d}$ | $^{A}$3.887 ± 0.01$^{c}$ | $^{B}$3.480 ± 0.02$^{d}$ | • | • |
| 35$^{th}$ day | $^{B}$5.387 ± 0.04$^{b}$ | $^{A}$5.767 ± 0.01$^{b}$ | $^{A}$5.413 ± 0.01$^{c}$ | $^{A}$5.553 ± 0.01$^{c}$ | $^{A}$4.137 ± 0.06$^{b}$ | $^{B}$3.967 ± 0.01$^{b}$ | • | • |
| 60$^{th}$ day | $^{B}$6.553 ± 0.03$^{a}$ | $^{A}$7.867 ± 0.01$^{a}$ | $^{B}$6.363 ± 0.07$^{b}$ | $^{A}$6.903 ± 0.02$^{b}$ | $^{A}$4.743 ± 0.01$^{a}$ | $^{B}$4.527 ± 0.02$^{a}$ | $^{A}$2.557 ± 0.01 | $^{B}$2.340 ± 0.01 |

In same column means ± SE with different lower case letters are significantly different (P < 0.05).
In same line means ± SE with different upper case letters are significantly different (P < 0.05).
• Not detected. SNS: salted non-smoked samples; HS: hot smoked; CS: cold smoked.

Table 5. Vitamin $D_3$ content of all samples during storage (at 4 °C) (mean ± SE).

| | HS vitamin $D_3$ (ppm) | CS vitamin $D_3$ (ppm) |
|---|---|---|
| Fresh | 0.019 ± 0.01$^{b}$ | 0.019 ± 0.01$^{b}$ |
| SNS | 0.077 ± 0.02$^{c}$ | 0.025 ± 0.01$^{abc}$ |
| 1$^{st}$ day | $^{A}$0.257 ± 0.08$^{a}$ | $^{B}$0.043 ± 0.01$^{b}$ |
| 7$^{th}$ day | $^{A}$0.066 ± 0.01$^{a}$ | $^{B}$0.032 ± 0.01$^{b}$ |
| 14$^{th}$ day | $^{A}$0.041 ± 0.01$^{a}$ | $^{A}$0,.032 ± 0.01$^{ab}$ |
| 21$^{st}$ day | $^{A}$0.047 ± 0.01$^{a}$ | $^{A}$0.025 ± 0.01$^{a}$ |
| 28$^{th}$ day | $^{A}$0.052 ± 0.01$^{a}$ | $^{B}$0.031 ± 0.01$^{b}$ |
| 35$^{th}$ day | $^{A}$0.13 ± 0.01$^{a}$ | $^{B}$0.039 ± 0.01$^{b}$ |
| 60$^{th}$ day | $^{A}$0.096 ± 0.01$^{a}$ | $^{B}$0.01 ± 0.01$^{b}$ |

In same column means ± SE with different lower case letters are significantly different (P < 0.05).
In same line means ± SE with different upper case letters are significantly different (P < 0.05).
SNS: salted non-smoked samples; HS: hot smoked; CS: cold smoked.

5. The results demonstrated that salting caused a slight increase in vitamin $D_3$.

## Discussion

Proximate composition values as a percentage of fresh sample muscle were in the same range as in previous work on gilthead seabream (28), and protein was within the range reported for a number of *Sparus* spp. (18.1%-22.8%) (29).

Generally, differences in moisture, protein, fat, ash, and sodium chloride content between the samples were significant ($P < 0.05$), but there were no statistically significant differences ($P > 0.05$) in protein content between fresh and salted samples before hot smoking. The salting and smoking process resulted in a significant decrease ($P < 0.05$) in moisture content and a significant increase ($P < 0.05$) in protein and fat content of gilthead seabream samples (Table 1). Similar results for the chemical composition of smoked fish were reported in previous studies (13,14). In another study salting and smoking significantly reduced ($P < 0.05$) the moisture content, and increased the protein and fat content of fish flesh (3). Industrial specifications for smoked finished products generally recommend less than 65% water content in fish flesh (15). This is in agreement with our values of 60.47% ± 0.71 (hot smoked) and 64.53% ± 1.12 (cold smoked) (Table 1). Differences in ash content between fresh and salted smoked gilthead seabream were significant ($P < 0.05$). A similar result was reported by Ünlüsayın et al. (14) for hot smoked fish. Salt content was higher in the salted and smoked samples than in the fresh samples ($P < 0.05$). The increase in the salt content of our smoked samples was partial due to dehydration during the smoking process and subsequent changes in the wet weight of the fillets. The salt content of our cold smoked samples was higher than that of hot smoked fish samples because of saturated brine use.

According to the results of the sensory evaluation, changes in the parameters during storage were significant ($P < 0.05$) (Table 2). Similar results were obtained by Huidobro and Tejada (30), who studied the suitability of freezing cooked gilthead seabream. Vasiliadou et al. (3) studied the suitability of smoking seabream and found the new product was very acceptable. The main changes in the cold smoked fish samples were observed in odor and flavor. Statistically significant differences in all sensory parameters were observed between the hot and cold smoked samples. The results showed that the hot smoked samples received higher scores, especially for flavor and odor, than the cold smoked samples did. These results show that in Turkey this type of product could be accepted by consumers.

No significant differences in pH values were observed during storage, except on the $7^{th}$ and $60^{th}$ days. The pH value of the fish samples increased slightly after smoking (Table 3). The same results were found by Vasiliadou et al. (3). The salting process caused a decrease in pH values in the hot and cold smoked samples (Table 3). Goulas and Kontominas (13) observed that pH values of mackerel (*Scomber japonicus*) decreased after salting. There were no significant differences ($P > 0.05$) in mean pH values (between the $7^{th}$ and $60^{th}$ day of storage) for cold smoked gilthead seabream. Overall, significant differences ($P < 0.05$) were observed between the hot smoked samples and cold smoked samples (Table 3).

The TBA index is widely used as an indicator of the degree of lipid oxidation. The increase in TBA value during the smoking procedure and storage may be attributed to the partial dehydration of fish and to the increased oxidation of unsaturated fatty acids as a result of smoking at relatively high temperatures (up to 70-80 °C) (for hot smoking). During hot smoking, fish are exposed to heating and atmospheric oxygen. These factors can accelerate the oxidation of fish lipids, resulting in an increase in TBA. A statistically significant ($P < 0.05$) but moderate increase was observed in the TBA values of the smoked samples during storage (Table 3). The reason for this moderate increase may have been phenolic constituents' deposition on the smoked fish and salt content of the samples. Among the smoked components, phenols have the highest antioxidant activity (31). Brine is a protective barrier against atmospheric oxygen during brining and, thus, the oxidative process did not proceed as rapidly as was expected. In the cold smoked samples an increase was determined after salting and on the first day of storage, which less than in the hot smoked process. In strongly salted products, the oxidation speed is accelerated because salt increases oxidase enzyme activity (32). Therefore, the TBA value increased as a result of fat oxidation in salted products. This data verified our results related to TBA values of SNS-C and CS gilthead seabream. For the TBA values, generally, significant differences were observed in the cold smoked samples during

storage. Final TBA values of 3.883 ± 0.08 mg MA/kg (for cold smoked fish) and 2.517 ± 0.44 mg MA/kg (for hot smoked fish) exceeded the value of 1-2 mg MA/kg that is usually regarded as the limit beyond which fish will normally develop an objectionable odor and flavor (33).

The TVB-N value increased after the smoking process in this study. According to EEC (34), the TVB-N value of raw fish was much lower than the acceptable upper limits of 25-35 mg/100 g for some fish species. This is in agreement with the initial TVB-N values of untreated fillet samples (14.280 ± 0.57 mg/100 g). As expected, a significant increase ($P < 0.05$) in TVB-N values was observed in both the hot and cold smoked fish. The increased TVB-N in our hot smoked samples was less than that obtained by Goulas and Kontominas (13). They observed that TVB-N values in mackerel (*Scomber japonicus*) almost doubled after hot smoking. In another study a significant increase ($P < 0.05$) in TVB-N was observed after hot smoking seabream (3), but the shelf life of the smoked seabream was not studied. As our results show, the TVB-N level increased gradually with the duration of storage. A statistically significant ($P < 0.05$) but moderate increase was observed in TVB-N values of the hot smoked samples, while a sharp increase was observed in the same values of the cold smoked samples during storage. The changes in TVB-N content of the cold smoked samples were significant ($P < 0.05$) during storage. An increase in TVB-N is expected because it is related to bacterial spoilage (33). The TVB-N limit of 33.307 ± 0.47 mg/100 g of muscle was reached on the 60$^{th}$ day in the hot smoked samples (Table 3). The TVB-N content of the hot smoked samples remained lower than the acceptable limit of 35 mg N/100 g of muscle set by the EEC (34), while the TVB-N value of the cold smoked exceeded it (40.790 ± 0.51 mg/100 g). The increase in TVB-N in cold smoked salmon was detected during storage at 4 °C by Dondero et al. (12) as 44 mg N/100 g of muscle on the 35$^{th}$ day. In our study the effects of the 2 smoking methods were significant ($P < 0.05$) for TVB-N.

As expected, smoking and heating significantly ($P<0.05$) reduced (1$^{st}$ day) the TPA, TMC, *Staphylococcus*, and *Micrococcus* (Table 4) values. Çolakoğlu (11) stated that the hot smoking techniques they used significantly reduced the microbial content of fish compared to fresh fillets of *Rutilus rutilus* and *Coregenus* sp. These results are in agreement with our results (Table 4). In the present study, during storage at 4 °C a statistically significant increase in TMC, TPA, *Staphylococcus*, and *Micrococcus* was observed in the hot and cold smoked gilthead seabream stored at 4 °C from day 1 to 60. A similar result for microbiological values of smoked fish was reported in a previous study (11). Vasiliadou et al. (3) stated that smoking and heating significantly ($P < 0.05$) reduced the total aerobic count. No yeast or mold was detected until the 60$^{th}$ day of storage in any of our smoked samples.

The vitamin $D_3$ (cholecalciferol) content of the smoked fish samples changed to varying degrees during storage (Table 5). Statistically, no significant ($P > 0.05$) changes in vitamin content of the hot smoked samples were seen between the 1$^{st}$ and 60$^{th}$ days. As for cold smoked samples, no significant changes were found between fresh and cold smoked samples during storage, except in the salted samples on the 14$^{th}$ and 21$^{st}$ days. Burt (9) reported that vitamin D is only slightly reduced during cooking. This knowledge is in agreement with our results for the cold smoked samples (fresh samples: 0.019 ± 0.01; 60$^{th}$ day samples: 0.01 ± 0.01). Aminullah Bhuiyan et al. (35) reported that during smoking vitamins A and D decreased slightly, but not significantly, while vitamin E remained unchanged in smoked Atlantic mackerel (*Scomber scomber*).

In conclusion, hot smoking and cold smoking can be used for processing farmed gilthead seabream in Turkey. The hot smoking and cold smoking technology that has was used led to the production of a high-quality delicatessen food item, which could be an alternative to cooked fresh fish in Turkey. Currently, only one study was located about the effects of hot smoking on the quality parameters of gilthead seabream, but no references to the shelf life of hot and cold smoked gilthead seabream were found. To the best of our knowledge this is the first study of the shelf life of hot and cold smoked gilthead seabream. Based on the presented data (pH, TBA, TVB-N values, and microbial flora counts) (Tables 3 and 4) the optimal shelf life of hot smoked gilthead seabream is approximately 35 days versus 21 days for cold smoked gilthead seabream.

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