

## Assessment of micro-macro elements and fatty acid profiles in Turkish salmon fillets during traditional hot smoking: Implications for human health risks

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### Abstract

Turkish salmon, a variant of rainbow trout farmed in Black Sea cages, is a key species in Türkiye's aquaculture. This study examines the effects of hot smoking on Turkish salmon's fatty acids, elemental content, and safety indicators. Twelve large rainbow trout samples were randomly collected from an international hypermarket in Adana, Türkiye, in February 2024 for analysis post-hot smoking. Hot smoking increased total saturated fatty acids (SFA), particularly C16:0 and C18:0, as well as Zn, K, and Fe ( $p < 0.05$ ), while significantly decreasing C20:2 (n-6) among polyunsaturated fatty acids (PUFAs) ( $p < 0.05$ ). Provisional tolerable weekly intake (PTWI) assessments indicated that the estimated weekly intake (EWI) for Cu, Fe, and Zn remained below the PTWI, suggesting no immediate health risks. Total target hazard quotient ( $\Sigma$ THQ) values for heavy metals were below 1, indicating no significant risk to adults or children, despite a post-smoking increase. Low levels of As, Pb, Cr, Ni, and Cd (below detection limits) prevented reliable target carcinogenic risk (TR) assessment, highlighting limitations in evaluating trace elements.

*Keywords:* Fatty acids, health risk assessment, hot smoking, micro-macro elements, Turkish salmon

### Introduction

Seafood undergoes complex processing techniques before reaching consumers. While traditional methods such as marinating, canning, salting, drying, and smoking are common worldwide, the demand for smoking as a distinct processing technology has increased in recent years because of the unique taste and aroma it imparts to the final product. Smoked products now hold a significant market share among processed seafood offerings in Europe (Durmuş and Kara, 2024). Among these, salmon stands out as a key species, particularly the one marketed under the name "Turkish salmon."

The species known as "Turkish salmon" is, in fact, a large rainbow trout (weighing 1 kg and above) that has gained international recognition as a brand. Initially, the fish reach a weight of 180–220 g in cages located in dam lakes across Türkiye. They are then transferred to aquaculture farms in the cold waters of the Black Sea, where they are grown to a weight of 2–4 kg, depending on consumer preferences. These fish are harvested and marketed under brand name, "Turkish salmon" (Bat *et al.*, 2021; Duran and Çenesiz, 2023; Kocatepe *et al.*, 2022; Öğretmen, 2023). In Türkiye, the export of Turkish salmon has seen a significant upward trend, with a 35% increase from 16,774 tons in the first half of 2022 to 22,623 tons in the

same period of 2023, mainly to the Russian Federation (accounting for an 82% share) and Vietnam, among 20 countries (Duran and Çenesiz, 2023; Öğretmen, 2023).

Seafood, recognized for its significant role in human nutrition, is a rich source of unsaturated fatty acids and minerals (macro- and microelements). Certain macroelements—such as sodium (Na), potassium (K), magnesium (Mg), and calcium (Ca)—along with microelements such as iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn)—are vital components of hormones, enzymes, and enzyme activators. These minerals are involved in numerous biochemical reactions within the human body and must be consumed according to physiological requirements (Artar *et al.*, 2022). However, essential minerals can become toxic when present in excessive concentrations (Lilly *et al.*, 2017). To illustrate the functions of some minerals discussed in this study, K is the primary cation in intracellular fluid, whereas Na predominates in extracellular fluid. Both are critical for maintaining acid–base balance, cell membrane permeability, nerve and muscle function, and osmotic pressure regulation. Mg plays a key role in metabolic processes and regulates the intracellular distribution of Na, K, and Ca. Ca is essential for muscle contraction, nerve signaling, heart function, and the development and maintenance of bones and teeth. Phosphorus (P) is a key component of bones, teeth, nucleic acids, and cell membranes, maintaining pH balance in body fluids and serving as a main nutrient source. Fe and Cu are vital for oxygen transport and cellular respiration. Nickel (Ni) aids in cell maintenance, enzyme activation, and genetic function. Zn supports many metabolic pathways, with its deficiency leading to health issues like stunted growth and immune dysfunction. Chromium (Cr) is crucial for lipid and glucose metabolism. Mn is essential for skeletal health and reproduction. Cadmium (Cd) is highly toxic, affecting mitochondrial function and DNA integrity. Mercury (Hg) is extremely toxic, especially as methylmercury, impacting the central nervous system and kidneys (Karataş, 2021; Lilly *et al.*, 2017; Matloob, 2023; Ustaoglu *et al.*, 2024).

PUFAs are crucial nutrients that help reduce the risk of coronary heart diseases. Fish oils, rich in PUFAs, have significant pharmacological and physiological effects on human health. They offer therapeutic benefits for conditions such as AIDS and diabetes. This has led researchers to study the mineral and fatty acid composition of various fish species worldwide (Artar *et al.*, 2022).

In this research, we sought to address the impact of traditional hot smoking on both the fatty acid profile and elemental concentrations of Turkish salmon. Our findings contribute to the understanding of how smoking affects nutrient profiles in fish, support food safety improvements, and provide insights into the implications for

human health risks. Hot smoking, involving temperatures above 80°C, can significantly alter the chemical composition of fish, affecting both nutritional value and safety. The process may lead to increased concentrations of certain elements and fatty acids because of moisture loss and the effects of high heat (Kiczorowska *et al.*, 2019). Studies have reported elevated levels of potentially toxic elements, such as heavy metals, following hot smoking, posing a potential risk to consumers (Abraha *et al.*, 2018; Igwegbe *et al.*, 2015). Therefore, it is essential to monitor the quality parameters of smoked products, including fatty acid composition and the presence of macro- and microelements, to ensure compliance with food safety regulations (Aremu *et al.*, 2013).

Consequently, this study aims to examine the fatty acid profile before and after the traditional hot smoking process. It also investigates the levels of microelements (Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Pb, Cd, Hg, and Se) and macroelements (Ca, Mg, P, K, and Na) in raw and smoked Turkish salmon fillets. This study evaluates how hot smoking alters nutritional and safety parameters in Turkish salmon, offering critical insights into health implications, product quality, and food safety improvements.

## Materials and Methods

### Raw material

The large rainbow trout, commercially known as Turkish salmon, utilized in this study, is a variant of rainbow trout raised in cages in the Black Sea region. Twelve large rainbow trout samples, each with an average weight of  $2.125 \pm 35$ g, were randomly selected and procured from an international hypermarket in Adana, Türkiye, during February 2024 (Figure 1).

Subsequently, fresh samples were meticulously preserved in polythene bags with crushed ice within an insulated container and transported to the laboratory. Upon arrival, the samples underwent a series of preparatory procedures, including washing with de-ionized distilled water, gutting, cleaning, filleting, and division into two distinct parts: fresh fillets and those designated for smoking. The first part was immediately stored at  $-80^{\circ}\text{C}$  until analyzed, and the second part was used to prepare the smoked product.

### Hot smoking process

The fillet samples were subjected to hot smoking using apricot wood hard sawdust. Prior to smoking, the samples were brined in a 15% solution (salt with de-ionized



**Figure 1.** Turkish salmon used in the study.

distilled water/fish in a 1:1 ratio) at refrigerator temperature for 20 minutes, then partially drained for 10 minutes. The hot smoking process involved different temperatures: initially at 30°C for 1 hour with a slight-intensity smoke, followed by 50°C for 1 hour, and finally at 80°C for 1 hour with a heavy smoke (Figure 2).

Temperature and environmental conditions were observed using a thermometer, and smoking time was tracked with a timer during the operation. Following the process, the hot smoked fish were permitted to cool at ambient temperature for 30 minutes before being packed in polythene bags and stored at -80°C until analysis.

### Micro-macro mineral analyses

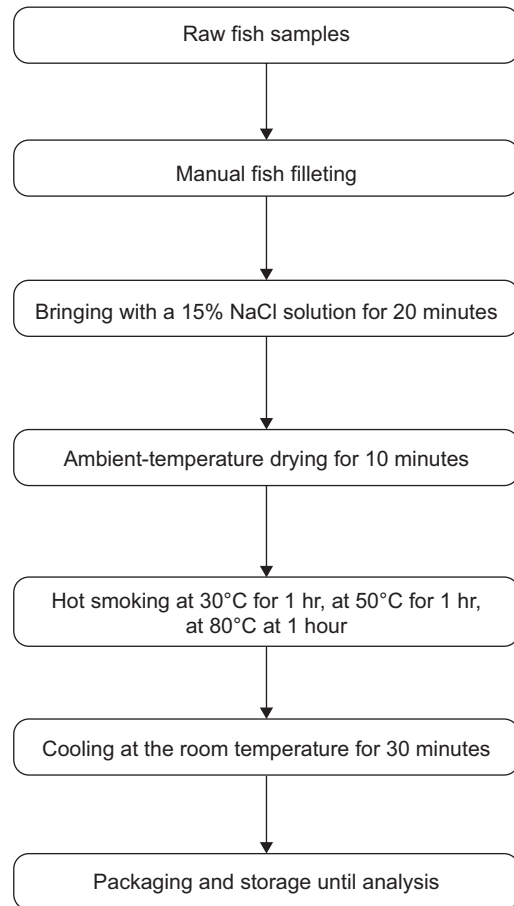
The analysis was conducted on fillets of both fresh and smoked fish. The contents of Al, Cr, Mn, Fe, Ni, Cu, Zn, Ar, Pb, Cd, and Hg in the experimental fish samples (0.25g) were digested in a microwave oven (Berghoff MWS-4) using an acidic mixture of 65% HNO<sub>3</sub>. Subsequently, the concentrations were determined using Inductively Coupled Plasma Optical Emission Spectrometry (Perkin Elmer-NexION 350X).

### Evaluation of health risk

The equation linked with public health risks is as follows:

Estimated daily intake (EDI) of heavy metals (Sadeghi *et al.*, 2020):

$$EDI = \frac{MC \times FDC}{BW}$$



**Figure 2.** Kiln-smoking steps of salmon (Mostafa *et al.*, 2023).

MC (mg/kg) represents the concentration of heavy metals in fish muscle. FDC refers to the average daily food per capita consumption of the Turkish salmon fish species, which is 1.39 g/person/day for consumers in Türkiye. BW denotes body weight, with an average of 70 kg for adults. EDI is expressed as mg/kg bw/day (Sadeghi *et al.*, 2020).

### Calculation of the Average Daily Food Per Capita Consumption (FDC) of Turkish Salmon

While statistics on per capita fish consumption in Türkiye are available, species-specific fish consumption data (e.g., for salmon) are not readily accessible. A reasonable estimate of the average daily food consumption (FDC) of Turkish salmon was calculated using the 2023 production, export, and population data. According to the Turkish Statistical Institute (TURKSTAT, 2023a), total salmon production in 2023 was 66,055 tons, of which 22,623 tons were exported (Duran and Çenesiz, 2023; Öğretmen, 2023). The remaining 43,432 tons were estimated to be available for domestic consumption. Based on Türkiye's 2023 population of 85,372,377

(TURKSTAT, 2023b), the annual per capita consumption was calculated as follows:

*Domestic Consumption*

$$\text{Domestic consumption} = \text{Total production} - \text{Exports}$$

$$\text{Domestic consumption} = 66,055,000 \text{ kg} - 22,623,000 \text{ kg}$$

$$\text{Domestic consumption} = 43,432,000 \text{ kg}$$

*Annual Per Capita Consumption*

$$\text{Annual per capita consumption} = \frac{\text{Domestic consumption}}{\text{Population}}$$

$$\text{Annual per capita consumption} = \frac{43,432,000 \text{ kg}}{85,372,377}$$

$$\text{Annual per capita consumption} = 0.5087 \text{ kg/person/year}$$

*Daily Food Per Capita Consumption (FDC)*

$$\text{Daily Food Per Capita Consumption (FDC)} = \frac{\text{Annual per capita consumption}}{365}$$

$$\text{Daily Food Per Capita Consumption (FDC)} = \frac{0.5087 \text{ kg/year}}{365 \text{ day/year}}$$

$$\text{Daily Food Per Capita Consumption (FDC)} = 0.00139 \text{ kg/person/day}$$

$$\text{Daily Food Per Capita Consumption (FDC)} = 1.39 \text{ g/person/day}$$

Thus, the FDC for Turkish salmon was estimated to be 1.39 g/person/day.

Estimated weekly intake (EWI) of heavy metals (Alipour *et al.*, 2015):

$$\text{EWI} = \text{EDI} \times 7$$

Target hazard quotients (THQ) (Sadeghi *et al.*, 2020):

$$\text{THQ} = \frac{\text{EF} \times \text{ED} \times \text{FDC} \times \text{C}}{\text{RfD} \times \text{BW} \times \text{ATn}} \times 10^{-3}$$

Total THQ ( $\sum \text{THQ}$ ) (Pokorska *et al.*, 2022):

$$\sum \text{THQ} = \text{THQ}(\text{Zn}) + \text{THQ}(\text{Mn}) + \dots \text{THQ}(\text{Hg})$$

A THQ or  $\sum \text{THQ}$  value of  $\leq 1$  is defined as an acceptable risk level, indicating no significant noncarcinogenic health risks. Conversely, values exceeding 1 imply potential health risks for both adult and child consumers (Kilercioglu *et al.*, 2022; Miri *et al.*, 2017).

Factors, units, and values in the THQ formula are given in Table 1.

**Table 1. Parameters and values used in THQ analysis (Çiftçi *et al.*, 2021; Dayananda and Liyanage, 2021; Javed and Usmani, 2016; Li *et al.*, 2020; Pokorska *et al.*, 2022; Sadeghi *et al.*, 2020; Tecimen *et al.*, 2023).**

Statement (Factors; Unit)	Value for Adult	Value for Child
Exposure frequency (EF; days per year)	365	365
Exposure duration (ED; Years)	70	6
Daily Food Per Capita Consumption of Turkish salmon (FDC; g/person/day)	1.39	1.39
Metal concentration (C; mg/kg)	Present study	
Body weight (BW; Kg)	70	30
Average exposure time for noncarcinogens' effects (ATn: days per year × ED)	(365×70)	(365×6)
Oral reference dose (RfDs; mg/kg/day)	Pb=0.0035; Ni=0.02; Cd=0.001; Al=1; Cr=0.003; Cu=0.04; Zn=0.3; Fe=0.7; Mn=0.14; Hg=0.0001; As=0.0003	

*Target carcinogenic risk (TR)*

Among the analyzed heavy metals, such as, Pb, Cr, Ni, and Cd—all classified as carcinogens by the International Agency for Research on Cancer (IARC)—this risk is calculated based on their average concentration in samples, such as fresh and smoked fish fillets. The TR equation, as referenced by Islam *et al.* (2016), Tokatlı and Ustaoglu (2021), and Alam *et al.* (2023), is used to quantify the lifetime cancer risk associated with consuming these contaminants.

$$\text{TR} = \frac{\text{EF} \times \text{ED} \times \text{FDC} \times \text{C} \times \text{CSF}}{\text{BW} \times \text{ATn}} \times 10^{-3}$$

CSF represents the cancer slope factor. The CSF values for As, Pb, Cr, Ni, and Cd are 1.5, 0.0085, 0.5, 0.91, and 15 (mg/kg/day)<sup>-1</sup>, respectively (Alam *et al.*, 2023; Oni *et al.*, 2022).

**Fatty acid analyses**

The methyl esters of fatty acids in the samples were prepared according to the International Union of Pure and Applied Chemistry (IUPAC) Methods II.D.19. The analyses were conducted using a Perkin Elmer Autosystem XL Gas Chromatography equipped with a flame ionization detector (FID) and a Supelco 2330 fused silica capillary column (30m × 0.25 mm × 0.20 μm film thickness) to determine the fatty acid composition. The following working conditions were meticulously maintained:

Sample working conditions on the GC device:

Column temperature program: 2 minutes at 120°C, followed by 10 minutes at 220°C with a 15°C increase, resulting in a total analysis time of 32 minutes.

Injector temperature: 240°C.

Carrier gas: Helium at a flow rate of 0.5 mL/minute.

Detector temperature: 260°C.

Hydrogen (H<sub>2</sub>) flow rate: 45 mL/minute.

Air flow rate: 450 mL/minute.

Sample injection volume: 2 µl.

Maximum column temperature: 280°C.

Split flow: 50 m.

### Lipids' nutritional quality indexes

The nutritional quality of total lipids was evaluated using three indices: the atherogenicity index (AI), the thrombogenicity index (TI), and the hypocholesterolemic/Hypercholesterolemic ratio (h/H). The AI and TI were calculated following the methods of Ulbricht and Southgate (1991) and Santos-Silva *et al.* (2002) with modifications, while the h/H ratio was calculated as described by Cieřlik *et al.* (2018).

$$AI = \frac{C12:0 + (4 \times C14:0) + C16:0}{MUFA + \Sigma(n-6) + \Sigma(n-3)}$$

$$TI = \frac{C14:0 + C16:0 + C18:0}{(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma n-6) + (3 \times \Sigma n-3) + \frac{\Sigma n-3}{\Sigma n-6}}$$

$$H/H = \frac{C18:1n-9 + C18:2n-6 + C18:3n-3 + C20:4n6 + C20:5n3 + C22:5-3 + C22:6n-3}{C14:0 + C16:0}$$

### Statistical analysis

All statistical analyses were conducted using IBM SPSS Statistics, version 21 (SPSS Inc., Chicago, Illinois), ensuring data reliability and statistical accuracy. Results, obtained from analyses conducted in triplicate, are presented as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was used to assess differences in nutrient and element concentrations between fresh and smoked samples, as this parametric test is appropriate for comparing mean values across groups with continuous data assumed to be normally distributed. Post-hoc mean comparisons were conducted using the independent *t*-test with significance levels set at *p* < 0.05. Descriptive statistics, including means and SDs, were generated for each variable group to summarize data trends.

## Results and Discussion

### Changes in micro- and macroelements in the Turkish salmon

Minerals are essential for various physiological processes, including enzymatic reactions, skeletal structure formation, muscle contraction, and the maintenance of colloidal systems such as osmotic pressure, viscosity, and diffusion. They also play a crucial role in regulating the acid–base balance in the body. These minerals are classified into two main groups: macroelements and microelements. Macroelements, including Ca, P, K, Na, Mg, are present in quantities exceeding 5 g in the human body. In contrast, microelements are present in microgram levels per gram of food. Both macroelements and some microelements, such as Zn, Fe, and Cu, are indispensable for maintaining human health (Gokoglu and Yerlikaya, 2015). If they remain within certain concentrations, these essential elements (Zn, Fe, Cu, etc.) do not pose any harm to biota, including fish (Bat *et al.*, 2021). Hence, understanding the concentrations of microelements (also including toxic elements) in fish is crucial for evaluating their impact on human health. For that reason, determining trace element levels in muscle tissue is of paramount importance to ensure food safety (Ouattara *et al.*, 2020). As fish muscle is a commonly consumed dietary component, investigating the composition of muscle tissue in various fish species within their ecosystems holds significant relevance to nutritional science (Rodriguez *et al.*, 2004). The mineral data, categorized as micro- and macroelements, are presented and detailed in Table 2 in this study.

As indicated in Table 2, concentrations of microelements, including Al, As, Hg, Cd, Cr, Pb, Mn, Ni, and Se, were all below detection limits in both fresh and smoked salmon fillets. The low levels of these elements may be associated with the positioning of fish farms in areas devoid of urban, agricultural, and industrial pollution (Bat *et al.*, 2021; Ögretmen 2023). In a study on farmed Turkish salmon by Bat *et al.* (2021), it was noted that the content of Al, Pb, Hg, and Cd between February and June 2019 was below the detectable limit. Similarly, Öztürk (2022) and Dizman *et al.* (2017) found that Cd and Pb levels in rainbow trout remained below the detectable limit. Ögretmen (2023) determined that the levels of Cd, Pb, and Hg in rainbow trout from three different regions in Türkiye were also below the detectable limit. In another study by Öztürk (2022) on the element concentrations of cultured fish in the Black Sea from Türkiye, the levels of As and Cd were reported to be below the detection limit in Turkish salmon, aligning with the findings of the current study. Our findings are in accordance with the prescribed thresholds set forth by the European Commission, FAO, WHO, and Turkish Food Codex

**Table 2.** Concentrations of micro- and macroelements in fresh and hot smoked salmon fillets (mg kg<sup>-1</sup>).

Minerals	Fresh salmon fillets	Hot smoked salmon fillets
<i>Microelements</i>		
Al	<0.5	<0.5
As	<0.05	<0.05
Cu	0.51±0.1	0.53±0.1
Hg	<0.02	<0.02
Zn	4.54±0.86	7.8±1.48*
Fe	2.3±0.46	16.16±5.23*
Cd	<0.02	<0.02
Cr	<0.5	<0.5
Pb	<0.02	<0.02
Mn	<0.5	<0.5
Ni	<0.5	<0.5
Se	<0.5	<0.5
<i>Macroelements</i>		
Ca (190-8810)	134.4±28.2	209.7±44.0
Mg (45-4520)	506.2±91.1	442.3±79.6
P (680-5500)	3785.2±794.9	3651.2±766.8
K (190-5022)	4229.2±888.1	4982.7±1046.3
Na (300-1340)	887.9±186.5	3545.1±716.7*
Na/K	0.21±0.00	0.71±0.04*

The values in parentheses next to each element indicate the concentration range of macroelements in fish muscles as mg kg<sup>-1</sup> (Stoyanova, 2018).

The data are presented as the mean ± SD of triplicate measurements. (\*) indicates the difference between fresh and hot smoked fillets in Turkish salmon.

(Table 3) (Verp and Mutlu, 2022). In the same study by Öztürk (2022), Mn, Cr, Ni, and Se contents were reported as 0.16±0.01 mg/kg, 0.07±0.01 mg/kg, 0.02±0.01 mg/kg, and 0.21±0.01 mg/kg, respectively, which are higher than the values we found. The difference is attributed to variations in the sensitivity of the analytical equipment used in the respective studies. In this research, it was found that Zn was the predominant essential microelement in terms of quantity, followed by Fe and Cu in both fresh and smoked fillets. The concentrations of Zn, Fe, and Cu were initially determined as 4.54±0.86 mg/kg, 2.3±0.46 mg/kg, and 0.51±0.1mg/kg, respectively. The results obtained in this study on fresh salmon fillets are consistent with the report by Gokoglu *et al.* (2004), who noted that Zn is the predominant microelement, with a concentration of 9.68±0.22 mg/kg, followed by Fe with a concentration of 2.10±0.58 mg/kg for rainbow trout (*Oncorhynchus mykiss*). The microelements Zn, Fe, and

Cu are essential for biological systems, playing crucial roles. Consequently, it's reasonable to expect that concentrations of elements such as Zn, Fe, and Cu in fish fillets are higher than those of Al, As, Cd, Mn, Cr, Hg, Ni, Pb, and Se, as suggested by Ouattara *et al.* (2020).

Subsequent to the smoking process, an increase was observed in these concentrations, measuring 7.8±1.48 mg/kg for Zn, 16.16±5.23 mg/kg for Fe, and 0.53±0.1 mg/kg for Cu. In the present study, the smoking process had a significant impact, especially on the content of Fe and Zn, which exhibited a tendency to increase in salmon fillets (p<0.05). Except for Cu (p>0.05), this elevation was found to be statistically significant (p<0.05). In a study conducted by Olgunoğlu (2011) investigating the nutritional components of hot smoked spiny eel (*Mastacembelus mastacembelus*), a similar increase in Fe was reported after the smoking process. Similarly, a minimal increase in the content of Cu in the study by Ajai *et al.* (2019) on the smoking of *Clarias gariepinus* was reported, from 0.40±0.18 mg/100g to 0.42 mg/100g, which was not statistically significant. It has previously been noted that the increase in heavy metal content during smoking of fish could be attributed to the interaction of smoke constituents with the metals present in fresh fish, forming insoluble complexes as suggested by Kourany *et al.* (2021).

In the current study, macroelement parameters were determined within the range reported by FAO (Table 2) for fish muscles (Stoyanova, 2018). It was observed that K was the most abundant macroelement, followed by P in both fresh and smoked fillets. The initial concentrations of K and P were found to be 4229.2±888.1 and 3785.2±794.9 mg/kg, respectively. Following the smoking process, an increase in K content and a slight decrease in P content were noted, resulting in values of 4982.7±1046.3 and 3651.2±766.8 mg/kg, respectively (p>0.05). An increase in Ca content was also observed (from 134.4±28.2 to 209.7±44.0 mg/kg (p>0.05)). The observed increases in these elements may be ascribed to dehydration during the smoking process.

The mean concentrations of macroelements in both fresh and smoked fillets were observed in the following sequence: K> P> Na> Mg> Ca. Similarly, Karataş (2021) reported that the mean concentrations of macroelements in rainbow trout were in the order of K> P> Na> Mg> Ca, measuring 4465.3±3.50, 2807.0±10.3, 1162.6±1.69, 345.4±2.57, and 248.2±5.53 mg/kg, respectively. In a study by Gokoglu and Yerlikaya (2015), it was found that K concentrations in various seafood varieties ranged from 1908 to 4400 mg/kg, exceeding the levels of Na. Luczyńska *et al.* (2009) reported that K and P were the most prevalent macroelements in the muscle tissue of several fish species. Varol and Sünbül (2020) found that K was the most abundant macroelement in the muscle of

**Table 3. Estimated weekly intake (EWI), compared with provisional tolerable weekly intake (PTWI), target hazard quotient (THQ), and total THQ ( $\Sigma$ THQ) of heavy metals in fillets of Turkish salmon.**

	Cu	Zn	Fe	Mn	Cr	Al	Ni	Hg	Cd	Pb	As	Se
PTWI	3.5*	7*	5.6 ++	2.5 **	0.7*	2.86 E-2++	3.5 E-2-	4 E-3+	7 E-3*	2.5 E-2*	3 E-3*	6.6 E-4**
Fresh Fillets												
EWI (F)	7.01 E-2	6.31 E-1	3.20 E-1	-	-	-	-	-	-	-	-	-
Smoked Fillets												
EWI (S)	7.36 E-2	1.084 E0	2.246 E0	-	-	-	-	-	-	-	-	-
Fresh Fillets												
THQ (F)	2.5 E-4 (Ad)	3.0 E-4 (Ad)	6.0 E-5 (Ad)	-	-	-	-	-	-	-	-	-
	5.9 E-4 (Ch)	7.0 E-4 (Ch)	1.5 E-4 (Ch)	-	-	-	-	-	-	-	-	-
$\Sigma$ THQ (F)	6.2 E-4 (Ad)			-	-	-	-	-	-	-	-	-
	1.4 E-3 (Ch)			-	-	-	-	-	-	-	-	-
Smoked Fillets												
THQ (S)	2.6 E-4 (Ad)	5.2 E-4 (Ad)	4.6 E-4 (Ad)	-	-	-	-	-	-	-	-	-
	6.0 E-4 (Ch)	1.2 E-3 (Ch)	1.0 E-3 (Ch)	-	-	-	-	-	-	-	-	-
$\Sigma$ THQ (S)	1.24 E-3 (Ad)			-	-	-	-	-	-	-	-	-
	2.8 E-3 (Ch)			-	-	-	-	-	-	-	-	-
PL <sup>1</sup>	<20	40	100	-	-	-	-	-	-	-	-	-

PTWI: Provisional tolerable weekly intake (mg kg<sup>-1</sup> body weight); EWI: Estimated weekly intake (mg kg<sup>-1</sup> body weight); THQ: Target hazard quotient (PL: Permissible limits (mg kg<sup>-1</sup>) +FAO/WHO, 2011; ++Çiftçi *et al.*, 2021; \*Mohamed *et al.*, 2017; \*\* Mohamed *et al.*, 2019; <sup>1</sup>Verep and Mutlu, 2022; - Kilercioglu *et al.* 2022; (F: Fresh fillets; S: smoked fillets; Ad: Adult; Ch: Child).

fish species from the largest three reservoirs in Türkiye. In addition, Njinkoue *et al.* (2016) noted that the concentrations of K and P in the edible parts of certain fish species (*Pseudotolithus typus* and *P. elongatus*) from the Cameroonian coast were significantly higher than those of Na, Mg, and Ca. The findings in our study regarding fresh salmon fillets align with the reported trends in macroelement concentrations observed across various fish species in previous research.

The level of Na content was reported to range from 270 to 1120 mg/kg in different fish species (Kiczorowska *et al.*, 2019), naturally occurring at low concentrations, rendering it suitable for low-Na diets. However, it has been reported that Na content tends to increase during processing because of the inclusion of salt or compounds containing Na (Gokoglu and Yerlikaya, 2015). In the current investigation, an increase in Na

content was determined after the smoking operation, rising from 887.9±186.5 to 3545.1±716.7 mg/kg (p<0.05). This increase can be attributed to the salting treatment administered prior to the smoking process. Our finding aligns with that reported by Kiczorowska *et al.* (2019), who observed several-fold higher Na amounts in smoked fish compared to fresh fish in their study. A similar increase after smoking in catfish (*Clarias gariepinus*) was reported by Ajai *et al.* (2019) for Na (from 127.56±8.54 to 143.98±33.18 mg/100g), for K (from 239.70±66.91 to 319.44±4.13 mg/100g), and for Ca (from 69.35±3.09 to 110.82±14.27 mg/100g), which is statistically significant. In our study, the smoking operation contributed to diverse changes in the content of Ca, K, and Na, resulting in increases, while Mg and P exhibited decreases.

It was reported that excessive consumption of Na has been linked to hypertension. However, it's worth noting

that the Na/K ratio in foods, rather than the intake of Na or K alone, is more strongly correlated with elevated blood pressure in adults. Therefore, it's recommended that the dietary Na:K ratio (by weight) should be lower than 0.57. This ratio serves as an important indicator for maintaining healthy blood pressure levels (Varol and Sünbül, 2020). In the study, the Na:K ratio of fresh salmon fillets was measured at 0.21, indicating a healthy dietary choice for preventing hypertension. However, after the smoking process, the Na:K ratio significantly increased to 0.71. This substantial increase post-smoking suggests a notable change in the nutritional composition of the salmon fillets, potentially impacting their health implications.

### Health risk assessment

Occasionally, although fish muscle tissue typically contains low levels of metals, the potential risk varies depending on the amount consumed (Kilercioglu *et al.*, 2022). To assess the health risks linked with the common consumption of fish, we computed equivalent weight intake (EWI) and THQ for various heavy metals present in both fresh and smoked salmon fillets.

Table 3 compares estimated EWI with recommended values such as the provisional tolerable weekly intake (PTWI). PTWI denotes the amount of contaminant ingestible by humans throughout their lifetime without significant risk. It is established by the Joint Expert Committee on Food Additives (JECFA), a collaborative body of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) for the United Nations. PTWI standards take into account food consumption volume, duration, and contamination levels (Alipour *et al.*, 2015).

In this study, PTWI values were used as reference standards to evaluate the safety of heavy metal intake from fresh and smoked salmon fillets. The EWI values for Cu, Zn, and Fe were found to be lower than the PTWI, indicating no immediate health risks for either type of fillet. However, these values increased after smoking compared to the initial levels. A similar study conducted by Bat *et al.* (2021) on farmed Turkish salmon compared the EDI (estimated daily intake) values for all chemical elements in fish samples with the toxicologically acceptable levels and oral reference doses (RfD values). The study reported that the intake of all trace elements was below the RfD limits, indicating that trace elements in Turkish salmon do not pose a threat to the regional population. These findings support the results of our current study.

Furthermore, the THQ is recognized as an indicator for assessing potential health risks to consumers. In this

study, the total THQ values for heavy metals in Turkish salmon fillets were below 1, indicating no health hazards for adult and child consumers. However, these values increased after smoking compared to the initial levels. A study by Kilercioglu *et al.* (2022) investigated the  $\Sigma$ THQ of pangasius fillets. Their findings showed that the heavy metal content in the analyzed fillets did not pose a health risk to either adult or child consumers, as both individual THQ and  $\Sigma$ THQ values remained below the safety threshold of 1. In addition, another study by Bat *et al.* (2021) reported that the  $\Sigma$ THQ for farmed Turkish salmon was also  $\leq 1$ . The results of our current study are consistent with these findings.

During the TR assessment, the concentrations of As, Pb, Cr, Ni, and Cd in the fillet samples were determined to be  $<0.05$  mg/kg for As,  $<0.02$  mg/kg for Pb,  $<0.5$  mg/kg for Cr and Ni, and  $<0.02$  mg/kg for Cd (Table 2). These values were below the detection limits required for accurate quantification. Consequently, applying the TR equation would not provide reliable risk estimates. Thus, a definitive TR calculation could not be performed, highlighting the limitations of risk assessment at such low concentration levels. This aligns with a similar study by Bat *et al.* (2021), which reported a negligible carcinogenic risk for farmed Turkish salmon.

### Changes in the fatty acid profile of Turkish salmon

Fish fatty acids, especially PUFAs, play a pivotal role in human health, influencing processes ranging from embryonic development to the prevention and treatment of various diseases. These conditions encompass inflammation, arthritis, type 2 diabetes, autoimmune disorders, hypertension, skin and kidney ailments, as well as cancer, affecting individuals of all ages, from children to adults. Since the human body lacks the ability to synthesize certain essential fatty acids, they must be obtained through diet. Thus, integrating fish into our regular nutrition regimen is essential for promoting overall health and well-being (Taşbozan and Gökçe, 2017).

Twenty-three fatty acids were detected in samples of fresh and smoked Turkish salmon fillets (Table 4).

The analysis revealed that total saturated fatty acid (SFA) levels were higher in smoked fillets ( $p < 0.05$ ) while total PUFA ( $\Sigma$ PUFA) and monounsaturated fatty acids ( $\Sigma$ MUFA) were found to be higher in raw material (in fresh fillets samples) compared to smoked ( $p > 0.05$ ). In addition, the fatty acid composition in fillets of Turkish salmon follows the order MUFA > PUFA > SFA in both fresh and smoked samples. Palmitic acid (C16:0) was identified as the primary SFA in both raw fillet samples, comprising 13.34%, and smoked fillet samples,

**Table 4. Fatty acid profiles in fresh and hot smoked salmon fillets.**

Fatty acids (%)	Salmon fillets	
	Fresh	Hot smoked
C12:0	0.09±0.01	0.08±0.00
C14:0	1.79±0.03	1.79±0.03
C15:0	0.19±0.04	0.18±0.03
C16:0	13.34±0.12	14.22±0.10*
C17:0	0.27±0.03	0.24±0.02
C18:0	4.00±0.10	4.25±0.09 <sup>†</sup>
C22:0	0.1±0.00	0.09±0.01
ΣSFA	19.78±0.09	20.85±0.22 <sup>†</sup>
C14:1	0.05±0.00	0.07±0.02
C16:1	2.95±0.09	2.89±0.10
C17:1	0.35±0.02	0.30±0.03
C18:1 (n-9)	35.68±1.10	35.60±1.00
C20:1 (n-9)	2.19±0.08	2.11±0.09
C22:1 (n-9)	0.30±0.03	0.26±0.03
C24:1 (n-9)	0.22±0.01	0.23±0.00
ΣMUFA	41.74±1.33	41.46±1.17
C18:2 (n-6) (LA)	22.18±1.09	21.67±1.08
C18:3 (n-6)	0.40±0.04	0.36±0.06
C18:3 (n-3) (ALA)	3.66±0.10	3.59±0.11
C20:2 (n-6)	1.5±0.04	1.35±0.05*
C20:3 (n-3)	0.98±0.05	0.99±0.06
C20:3 (n-6)	0.82±0.03	0.81±0.04
C20:5 (n-3) (EPA)	1.67±1.00	1.51±0.8
C22:5 (n-3) (DPA)	0.64±0.06	0.62±0.02
C22:6(n-3) (DHA)	6.60±1.00	6.46±1.15
ΣPUFA	38.45±3.31	37.36±3.33
AI	0.26±0.01	0.24±0.01
TI	0.26±0.03	0.28±0.03
h/H	4.65±0.31	4.57±0.24
PUFA/SFA	1.94±0.16	1.79±0.14
Σn-3	13.55±2.21	13.17±2.10
Σn-6	24.90±1.10	24.19±1.23
n6/n3	1.84±0.22	1.84±0.20
EPA+DHA	7.24±1.06	7.08±1.13
DHA/EPA	10.31±0.60	12.68±2.19

The data are presented as the mean ± SD of triplicate measurements. (\*) indicates the difference between fresh and hot smoked fillets in Turkish salmon.

comprising 14.22%. This was followed by stearic acid (C18:0) within the SFA category, with quantities of 4.00% and 4.25%, respectively ( $p < 0.05$ ). The most abundant fatty acid in the MUFA group was C18:1 (n-9), representing 35.68% and 35.60% in fresh and hot smoked fillets, respectively, while in the PUFA group, C18:2 (n-6) (LA) was the most prevalent, at 22.18% and 21.67% in fresh and hot smoked fillets, respectively

In a study examining Turkish salmon conducted by Keskin *et al.* (2022) and Kocatepe *et al.* (2022), C16:0 emerged as the dominant component among the SFA, with quantities of  $16.23\% \pm 0.00$  and  $17.28\% \pm 0.02$ , respectively. In addition, within the MUFA group, C18:1 (n-9) exhibited significant prevalence, comprising  $28.58\% \pm 0.00$  and  $30.37\% \pm 0.01$  in the respective studies. Furthermore, C18:2 (n-6), categorized within the polyunsaturated fatty acid (PUFA) group, demonstrated notable abundance at  $20.74\% \pm 0.03$  and  $17.21\% \pm 0.01$ , respectively. These findings underscore the substantial contributions of C16:0, C18:1 (n-9), and C18:2 (n-6) to the lipid profile of Turkish salmon, which aligns closely with our study. In another study on commercial Turkish salmon by Durmuş and Kara (2024) to determine the fatty acid composition, the same fatty acid fractions parallel to ours (C16:0, C18:1 (n-9), and C18:2 (n-6)) were reported as dominant, comprising  $15.08\% \pm 0.41$ ,  $32.13 \pm 0.74$ , and  $13.56\% \pm 0.13$ , respectively, for smoked samples. However, in the present study, the percentages of these fatty acids differed from the values reported by researchers. These differences have been interpreted as possibly stemming from the quality of the aquaculture water, its physicochemical composition, or environmental conditions, as well as from the dietary regimen of the fish and season. In addition, it was reported that the nutritional content varies depending on the structure of the fish, region, and the season in which it is harvested (Keskin *et al.*, 2022). In the present study, the EPA+DHA content of fresh fillets was determined to be  $7.24 \pm 1.06\%$ , which is close to the value of 7.57% reported by Keskin *et al.* (2022) for Turkish salmon.

After the smoking process, there was a discernible alteration in the fatty acid composition, characterized by both increases and decreases in some contents. As shown in Table 3, SFA content increased from 19.78% to 20.85% ( $p < 0.05$ ). Conversely, a decrease was observed in the MUFA content from 41.74% to 41.46% ( $p > 0.05$ ) and the PUFA content from 38.45% to 37.36% ( $p > 0.05$ ), respectively. There was also an observed decline in the individual levels of EPA, DHA, and EPA+DHA. The levels of almost all individual acids from the MUFA and PUFA groups were found to be lower than their initial levels after the process. However, the decrease in PUFA and MUFA, including EPA and DHA content, observed in our study aligns with previous reports (Akintola *et al.*, 2013; Bienkiewicz *et al.*, 2019; Bienkiewicz *et al.*, 2022; Bouriga *et al.*, 2020; Colakoglu *et al.*, 2011; Cieřlik *et al.*, 2018; Dhanapal *et al.*, 2012; Kaya *et al.*, 2008; Küçükgülmez *et al.*, 2010; Mostafa *et al.*, 2023; Popelka *et al.*, 2021; Tenyang *et al.*, 2020), where a statistically significant decrease was also found. The reduction in MUFA and PUFA may indicate the degradation of these unsaturated fatty acids during the heat treatment involved in smoking operations, as noted by Olgunođlu (2011) and Bouriga *et al.* (2020).

The increase in SFA is primarily attributed to the rise in fatty acids such as C16:0 and C18:0 following smoking ( $p < 0.05$ ). This increase is consistent with findings from a study on the changing fatty acid profile after the smoking process in common carp and rainbow trout by Cieslik *et al.* (2018), who reported that the smoking process significantly impacted the fatty acid content and profile, showing a statistically significant tendency to increase in SFA ( $p < 0.05$ ).

In this study, the ratio of  $\Sigma$ PUFA/ $\Sigma$ SFA was found to be  $1.94 \pm 0.16\%$  in fresh fillets. However, in smoked fillets, this ratio decreased to  $1.79 \pm 0.14$  ( $p > 0.05$ ). Despite this decline, our study's results indicate that the  $\Sigma$ PUFA/ $\Sigma$ SFA ratio remained above the recommended minimum threshold of 0.45 (Durmuş and Kara, 2024; Kaya *et al.*, 2008; Kocatepe *et al.*, 2022) in both fresh and smoked fillets.

The correlation between n6/n3 unsaturated fatty acids and mortality associated with cancer and cardiovascular diseases is well-established. This ratio is widely recognized as a critical indicator for evaluating the nutritional value of fish oil. It has been proposed that this ratio should not exceed 4 (Durmuş and Kara, 2024). In the present study, the ratio remained the same, at 1.84, before and after the smoking operation, without exceeding the suggested value. Furthermore, nutritional quality is also evaluated using parameters such as the h/H ratio, as well as the AI and TI indexes, which reflect fatty acid fractions and their potential impact on cardiovascular health. While there are no specific optimal thresholds defined for human health, it is generally preferred to have higher h/H ratios and AI and TI indexes below 1 to reduce the risk of cardiovascular disorders (Durmuş, 2019; Gualda *et al.*, 2018). In our study, all these parameters remained within safe ranges in both situations.

## Conclusion

This study highlights the impact of hot smoking on the elemental and fatty acid composition of Turkish salmon. Hot smoking significantly increased levels of SFA, particularly C16:0 and C18:0, as well as essential minerals like Zn, K, and Fe, while reducing certain PUFAs, such as C20:2 (n-6). The results indicate that Turkish salmon remains safe for consumption, as determined by the estimated THQ values, although these values increased after smoking. However, the study also suggests that the smoking process may alter the nutritional profile in ways that warrant careful control, particularly regarding Na content and trace metal concentrations.

To enhance consumer safety and meet regulatory standards, it is recommended that the seafood industry implement regular monitoring and standardized controls

for hot smoking practices. These findings provide valuable insights for improving product quality and safety in the seafood industry.

## Author Contributions

The authors all contributed equally to this article.

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