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Effects of cold and hot smoking processes and the addition of natural *Dunaliella salina* polyphenol extract on the biochemical quality and shelf life of *Sander lucioperca* fillets after storage for 90 days

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SUMMARY: The effects of cold and hot smoking and the addition of *Dunaliella salina* polyphenol extract on the biochemical quality and shelf-life of *Sander lucioperca* fillets after storage for 90 days at 0–4 °C were examined. The results showed a significant increase in protein, lipid, free fatty acid, and 1,1-diphenyl-2-picrylhydrazyl contents, and a decrease in peroxide and thiobarbituric acid reactive substances, and volatile base nitrogen levels in cold (CSF) and hot (HSF) smoked fillets covered with or without extract and stored for 1, 20, and 90 days compared to fresh fillets (FF). Saturated and monounsaturated fatty acids exhibited a significant increase in FF and CSF and HSF covered with or without extract. The total polyunsaturated fatty acids revealed a significant decrease in FF and CSF and HSF with or without extract. Therefore, cold and hot smoking and polyphenol extract improved the biochemical quality and storage shelf-life of fillets for 90 days at 0–4 °C.

KEYWORDS: *Antioxidants; Cold and hot smoking; Dunaliella salina microalgae; Fatty acids; Freshwater fish; Polyphenols*

RESUMEN: ***Efecto de procesos de ahumado frío y caliente y la adición de extracto polifenólico natural de* Dunaliella salina *sobre la calidad bioquímica y la vida útil de filetes de* Sander lucioperca *almacenados durante 90 días.*** Se examinaron los efectos del ahumado en frío y en caliente y la adición de extracto de polifenoles de *Dunaliella salina* sobre la calidad bioquímica y la vida útil de filetes de *Sander lucioperca* almacenados durante 90 días a 0–4 °C. Los resultados mostraron un aumento significativo en los contenidos de proteínas, lípidos, ácidos grasos libres y 1,1-difenil-2-picrilhidrazilo, y una disminución en las sustancias reactivas de peróxido y ácido tiobarbitúrico, y los niveles de nitrógeno básico volátil en frío (LCR) y caliente (HSF) de filetes ahumados cubiertos con o sin extracto y almacenados durante 1, 20 y 90 días en comparación con los filetes frescos (FF). Los ácidos grasos saturados y monoinsaturados exhibieron un aumento significativo en FF y LCR y HSF cubiertos con o sin extracto. Los ácidos grasos poliinsaturados totales revelaron una disminución significativa en FF y CSF y HSF con o sin extracto. Por lo tanto, el ahumado en frío y en caliente y el extracto de polifenoles mejoraron la calidad bioquímica y la vida útil durante el almacenamiento de los filetes durante 90 días a 0–4 °C.

PALABRAS CLAVE: *Ácidos grasos; Ahumado en frío y en caliente; Antioxidantes; Microalga Dunaliella salina; Pescado de agua dulce; Polifenoles*

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1. INTRODUCTION

Fish is a highly biodegradable food due to its susceptibility to oxidation, which assists in the growth of pathogenic microorganisms (Chaillou *et al*., 2015) and eventually leads to the formation of off-odor and flavor, and finally to rot (De Souza-Franco *et al*., 2010). Therefore, it must be precisely handled and preserved to retard its spoilage and to assure microbial safety and a marketable shelf-life (Amaral *et al*., 2021). Indeed, some chemical quality indices have so far been developed to assess the level and extension of fish spoilage, such as the total volatile basic-nitrogen (TVB-N) and trimethylamine-nitrogen (TMA-N), thiobarbituric acid (TBA) value, and the presence of biogenic amines (histamine, cadaverine, tyramine, and putrescine) produced by the decarboxylation of specific free amino acids by the action of microorganisms (Silbande *et al*., 2018). TVB-N implies the measurement of volatile basic nitrogenous compounds, such as trimethylamine (TMA), dimethylamine (DMA), and ammonia (NH_3), which are produced by bacteria, from the action of enzymes or the deamination of amino acids (Kostaki *et al*., 2009). The proposed value of TVB-N for spoilage initiation is 30–35 mg N/100 g; however, some studies present lower levels depending on the fish species (Kostaki *et al*., 2009). TMA-N is the main constituent of non-protein nitrogen fraction, produced by the bacterial spoilage, enzymatic activity, and decomposition of TMA-N-oxide, and is responsible for the fishy odor. The upper limit of TMA-N values considered for spoilage acceptance is 10–15 mg TMA-N/100 g, but lower limits are also suggested by other authors (Kostaki *et al*., 2009). Regarding lipid oxidation, the TBA value is used to measure the malondialdehyde (MDA) content. The quality values range between 2–4 mg MDA/kg, but this value might not reflect the actual rate of lipid oxidation because MDA can interact with other components (Kostaki *et al*., 2009). In addition, several methods, including vacuum packaging, modified atmosphere packaging, active packaging, and chemical additives, such as organic acids and natural extracts, combined with freezing systems, have been applied to impede its decomposition (Amaral *et al*., 2021).

Extensive fish farming, as in the case of *Sander lucioperca* (pikeperch or zander), offers the opportunity for fishermen to cost-effectively harvest fish. Although the nutritional quality of *S. lucioperca* is high because it is rich in polyunsaturated fatty acids (PUFA), vitamins, and minerals (Bouriga *et al*., 2020), this species is not overly valued by consumers, mainly due to its undesirable taste and flavor compared to marine fish. The smoking process can offer such a marketing alternative to freshwater fish and result in high-quality and acceptable products (Bouriga *et al*., 2012). As far as known, smoking treatments (hot, cold, liquid, and electrostatic) have been documented as useful processes for preserving the quality of seafood and can be achieved through both traditional and innovative techniques (Karsli and Çağlak, 2021). Overall, some constituents of smoking substances are aldehydes, ketones, alcohols, acids, hydrocarbons, esters, phenols, and ethers. These substances are applied to the surface of the wires and then penetrate the muscle, giving the products their final color and taste. However, some reports have shown that smoking processes have a negative impact on the nutritional value of fish fillets (Bouriga *et al*., 2012). Thus, the addition of antioxidants can be a useful technique for preserving the quality of fillets.

Given that consumers are becoming more health conscious, there has been a strong demand for the use of functional foods and the addition of natural ingredients. For this reason, several authors have focused on microalgae as potential sources of compounds with functional, nutritional, antimicrobial, and antioxidant properties (Cakmak *et al*., 2014). Among the compounds that can be obtained from microalgae are antioxidants, which have been widely used as food conserves in the food industry (Madhavi *et al*., 1996**)**. In addition, natural antioxidants, such as polyphenols, are now more extensively used due to their physiological benefits to human health. In this context, herbs have been the most valuable antioxidants used to protect smoked fillets, especially green algae of the genus *Dunaliella*. Of this genus, *D. salina* is a green, halophilic microalga commonly found in sea salt fields. This microalga is famous for its high commercial, economic, and industrial value due to its persistent capacity to produce large amounts of polyphenols and carotenoids, especially β-carotene, which has been widely used as an important natural antimicrobial and antioxidant for nutrient preservation in food, feed, and the pharmaceutical industry due to its high physiological properties, as well as biodiesel because of its high unsaturated fatty acid content (Cakmak *et al*., 2014). In addition, polyphenols and β-carotene function as scavenger compounds to protect the fillets from the generation of free radicals (Burton and Ingold, 1984).

Despite the immense number of works on smoking processes and their potential effects on fish fillets, the use of *Dunaliella salina* as a natural antioxidant has not been well explored. Hence, the current study was conducted to examine the effect of both cold and hot smoking processes and the addition of two graded concentrations (0.5 and 1% v/w) of natural *Dunaliella salina* polyphenol (pp) antioxidant extract on the biochemical quality and shelf-life and consumption of *Sander lucioperca* fillets during storage for 1, 20, and 90 days, respectively, in a refrigerator at 0–4 °C.

2. MATERIAL AND METHODS

2.1. Polyphenol antioxidant extract

Dunaliella salina samples were collected in May 2019 from Chott El Djerid, an endorheic salt lake, situated in southern Tunisia (33°54′42.21″N, 8°31′7.98″E). The antioxidant extract was prepared following the method described by Messina *et al*. (2015), in which 10 g of dried and pulverized microalgae were extracted with 100 mL of distilled water and then incubated in a shaker for 24 h in the dark. Afterwards, the mixture was filtered and lyophilized. The final solution was prepared by dissolving 10 g of the freeze-dried extract in 1000 mL of distilled water (10 $g \cdot L^{-1}$ of distilled water), with a polyphenol content equal to 500 mg of gallic acid equivalents (GAE)/L (Messina *et al*., 2015).

2.2. Sampling and smoking procedure of *Sander lucioperca*

S. lucioperca samples were collected in May 2019 during 10 fishing operations from Sidi El Barrak Reservoir (Beja Governorate, northwest Tunisia, 37°01'N, 09°39'E). A total of 45 fish samples, ranging from 12.5 to 28.6 cm (mean 24.3 ± 3.2 cm) in total length and 3400 to 1900 g (mean 700 ± 2.20 g) in total weight, were collected using a 30 mm mesh trammel net. The samples were preserved in ice and then transported to the laboratory where they were weighed, measured, de-capitated, and cleaned. These samples were longitudinally cut into fillets measuring 13.6 –18.2 cm (mean 15.8 ± 2.6 cm) in length, 3.2–4.4 cm (mean 3.1 ± 0.23 cm) in thickness, 7.4–9.8 cm (mean 6.78 ± 1.12 cm) in width, and 200.4–420 g (mean 264 ± 0.86 g) in weight. The fillets were divided into three groups (100 g each): the first consisted of fresh fillets without any additives, the second comprised fillets covered with a final concentration of 0.5%, i.e., 50 mL pp (v)/100 g fillets (w), of the polyphenol (pp) antioxidant *D. salina* extract using a micropipette, and the third included fillets that were treated with the polyphenol antioxidant extract as the second group, but with a final concentration of 1% pp, i.e., 100 mL pp (v)/100 g fillets (w), extract. Afterwards, the three groups were smoked in cold and hot conditions. The cold-smoked fillets were dried for 2 h in the smoking chamber at a temperature of 30–35 °C, while the hot-smoked fillets were introduced into the industrial smoking chamber using a peripheral smoke generator. The process of smoking the fillets was accomplished as follows: pre-drying the fish surface at 50–60 °C for 150 min, followed by hot smoking at 65–70 °C for 30 min, and finally steaming at a temperature of 68–72 °C. In both smoking processes, oak wood was used (Bouriga *et al*., 2020). Finally, the fillets were cooled in cold air at 10 °C. Subsequently, the smoked fillets were vacuum-sealed and stored in a refrigerator at 0–4 °C for 90 days. For the biochemical analyses, fresh and vacuum-sealed smoked fillet samples were gradually tested after 1, 6, 20, 50, and 90 days of storage at 0–4 °C. However, the results obtained from the storage periods 6, 20, 50, and 90 showed a similar significant gradual increase or decrease in each fillet type, as the case may be, so we reduced and summarized the data by presenting here only the results for three storage periods, namely 1, 20, and 90. All flesh samples were stored at -80 °C until analysis.

2.3. Biochemical analyses related to shelf-life

2.3.1. Protein determination

The total protein content of *S. lucioperca* fillets was determined by estimating their total nitrogen content using the Kjeldahl method 981.10 of the AOAC. Approximately 1 g of raw material was hydrolyzed with 15 mL concentrated sulfuric acid (H_2SO_4) containing two copper catalyst tablets in a heat block (Kjeltec system 2020 digestor, Tecator Inc., Herndon, VA, USA) at 420 °C for 2 h. After cooling, H_2O was added to the hydrolysates before neutralization and titration. The amount of total nitrogen obtained was multiplied with both the traditional conversion factor of 6.25 and species-specific conversion factors (Mariotti *et al*., 2008) in order to determine total protein content. The protein content was expressed as a mean percentage (%) of the wet weight (ww) of three replicates.

2.3.2. Lipid determination

The total lipids were determined according to the protocol described by Bouriga *et al*. (2020). Briefly, 10 grams of fresh or smoked fillet samples were homogenized for 8-10 min at 4 °C in a mixture of chloroform: methanol (1:2) using a Polytron homogenizer (Malaysia). The homogenate was added to 5 mL NaCl saturated solution and 20 mL chloroform with Butylated hydroxytoluene (BHT; 50 ppm), and then homogenized for 7 and 5 min, respectively. Then, 20 mL of distilled water were added and the solution was homogenized again for 1 min. The obtained mixture was incubated in an ultrasound bath for 10 min, and a vacuum cleaner with Buchner funnel and chloroform. The organic fraction was extracted with a separating funnel, dried with sodium sulfate, and evaporated to dryness in the rotary evaporator (Stuart™, UK). The obtained oil was solubilized in a known volume of chloroform with BHT (50 ppm) and stored at -20 °C. The total lipids were expressed as a mean percentage (%) of the wet weight (ww) of six replicates.

2.3.3. Peroxide value (PV)

The peroxide value of the fillet samples was determined according to the IDF standard method, 74A: 1991(9), with the ferric thiocyanate method based on the ability of lipid peroxides to oxidize ferrous ions at a low pH. The resulting ferric ions were reacted to thiocyanate and the concentration of the complex formed was determined by spectrophotometry (Jenway 6315, UK) at 500 nm. The standard sample was determined by the reaction of a series of aliquots of a 10-µg/ml iron (III) chloride standard solution, 10 mM ammonium thiocyanate, and a sufficient amount of chloroform/methanol mixture (7:3). The results were expressed as mequivalent of oxygen per kg of lipid (meq O_2/kg) and the values were presented as a mean percentage (%) of three replicates.

2.3.4. Thiobarbituric acid reactive substances (TBARS)

The production of thiobarbituric acid reactive substances (TBARS) was determined based on the AOAC (1998) method. Oil samples were dissolved in 1-butanol, mixed with 0.2% TBA in 1-butanol, incubated in a water bath for 2 h at 95 °C, then cooled under tap water. The absorbance was determined using a spectrophotometer at 532 nm, and the standard curve was established by the TBARS reaction of a series of aliquots of 0.2 mM TMP (1,1,3,3-tetramethoxypropane) prepared in 1-butanol. The results were expressed in mg MDA (malondialdehyde)/kg of oil and values were presented as a mean percentage (%) of three replicates.

2.3.5. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) antioxidant activity

To measure the antiradical activity, the synthetic radical DPPH (1,1-diphenyl-2-picrylhydrazyl) was used according to the method of Bersuder (Bersuder *et al*., 1998). Briefly, 10 mg of the fillet sample were suspended in 0.5 mL distilled water. Afterwards, 1.2 mL absolute ethanol and 0.2 mL DPPH solution (50 µM in ethanol) were mixed and incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm using a T70 UV-visible spectrophotometer. The results were expressed as a mean percentage (%) of three replicates of inhibition or trapping activity and were calculated by the following formula:

$$\text{DPPH trapping activity} = ((\text{control OD} - \text{sample OD})/\text{control OD}) \times 100.$$

2.3.6. Total fatty acid (FA) determination

Fatty acid methyl esters (FAME) were determined following the AOAC 963.15 methodology (AOAC, 1990), with slight modification. In brief, the analysis was done in a Varian Agilent 6890 N gas chromatograph (Agilent Technologies, Santa Clara, USA), equipped with an auto-sampler and fitted with a split/splitless injector and a flame ionization detector (FID). Separation was performed in an Innowax 30 × 0.25 capillary column (25 m × 0.25 mm i.d., film thickness) (Agilent Technologies, Santa Clara, USA). The temperature was programmed from 180 to 200 °C at 4 °C/min, held for 10 min at 200 °C, heated to 210 °C at 4° C/min, and held at 210 °C for 14.5 min using an injector and FID at 250 °C. The fatty acid contents in the total lipids of the samples were estimated using nonadecanoic acid methyl ester C19:0 Me (Sigma Chemical Co. Ltd), as an internal standard (10 mg/mL) based on the ratio of the peak area. The fatty acid sequences ranged according to their chromatographic retention times, and the values are given as a mean percentage (%) of total fatty acid methyl esters of six replicates.

2.3.7. Free fatty acids (FFA) determination

The samples (50 mg each) were homogenized with cyclohexane and copper acetate-pyridine reagent and stirred by vortex for 2 min, and then centrifuged at 9000 rpm for 20 min, and detected at 710 nm. The quantitative analysis of each fatty acid was performed for six replicates of each sample using nonadecanoic acid (C19:0, Sigma Chemical Co. Ltd), as an internal standard, and values were presented as a mean percentage (%) of three replicates.

2.3.8. Total volatile base nitrogen (TVB-N)

The total volatile basic nitrogen (TVB-N) was measured by direct distillation of the homogenized samples according to the EU Commission Regulation (EC) No 2074/2005 (EEC, 2005). The sample was ground carefully by a meat grinder. Exactly 10 g ± 0.1 g of the ground sample were weighed in a suitable container, mixed with a 90-mL 6% perchloric acid solution, homogenized for two min with a blender, and then filtered. Steam distillation of 50 mL of the extract after sufficient alkalinization with 20% NaOH (6.5 mL) and the addition of several drops of phenolphthalein (1 g/100 mL 95% ethanol) and a few drops of silicone anti-foaming agent, began immediately. The steam distillation was regulated so that around 100 mL of distillate was produced within 10 min. The distillation outflow tube was submerged in a receiver with a 100-mL 3% boric acid solution, to which three to five drops of the indicator solution, Tashiro Mixed Indicator (2 g methyl-red and 1 g methylene-blue, dissolved in 1000 mL 95% ethanol), were added. After exactly 10 min the distillation was ended. The volatile bases contained in the receiver solution were determined by titration with a standard hydrochloric solution 0.01M till the pH reached 5.0 ± 0.1. The TVB-N (mg/100 g sample) = (V1 - V0) × 0.14 × 2 × 100/ M, where V1 = Volume of 0.01 M hydrochloric acid solution in mL for sample; V0 = Volume of 0.01 M HCL solution in mL for blank (50 mL 6% perchloric acid solution was used instead of the extract); M = sample wet weight (ww) in g. The values were expressed as a mean percentage (%) of the wet weight (ww) of three replicates.

2.4. Statistical analysis

The results are presented as means ± standard deviation (SD). All biochemical indicators were statistically analyzed using SPSS software on triplicate samples, except total lipids and fatty acids, and free fatty acids, which were statistically analyzed on six triplicate samples. Normality and homogeneity of the variance of the results were confirmed by Kolmogorov-Smirnov and Levene's tests, respectively. One-way ANOVA followed by the Tukey test was performed to determine the significant differences between fresh, cold-, and hot-smoked fillets with or without antioxidant extract and was set either at $p < 0.05$, $p < 0.01$, $p < 0.001$.

3. RESULTS AND DISCUSSION

3.1. Biochemical composition of fresh and smoked fillets

The biochemical composition of total proteins and lipids (%) in fresh and smoked *S. lucioperca* fillets are presented in Table 1. The percentage of total protein content remained nearly constant (range = 18.36–18.07%) in the fresh fillets (FF) during the 1, 20, and 90 days of storage, and showed a gradual significant increase ($p < 0.001$) in the levels (range = 41.27–51.31%) in both cold- (CSF) and hot-smoked (HSF) fillets covered with or without the two graded concentrations (0.5 and 1%) of *D. salina* polyphenol extract compared to FF during the three storage periods. However, this significant increase remained nearly constant in both CSF (41.27%) *vs* HSF (41.78%) without the covered extracts and CSF (42.57%) *vs* HSF (42.31%) covered with 0.5% of the polyphenol extract; while it increased in HSF (51.31%) covered with 1% of the polyphenol extract compared to CSF (42.31%) covered with 1% of the polyphenol extract during the three storage periods.

In addition, the total percentage of lipids revealed gradually decreased levels (1.86, 1.29, and 0.98%, respectively) in FF during the three storage periods and differentially significantly increased levels ($p < 0.001$) in both CSF and HSF with or without 0.5 or 1% of the polyphenol covering extracts during the three storage periods, with the highest similar levels in CSF and HSF covered with 0.5% of the extract and the highest

TABLE 1. Variations in the biochemical composition of total proteins and lipids (in %) in fresh (FF), cold-smoked (CSF), hot-smoked (HSF) fillets of *Sander lucioperca*, and cold-smoked (CSF) and hot-smoked (HSF) fillets covered with *Dunaliella salina* polyphenol (pp) antioxidant extract at 0.5 and 1% concentrations during three storage periods.

Storage days	FF		CSF		CSF+0.5% pp		CSF+1% pp		HSF		HSF+0.5% pp		HSF+1% pp	
	Proteins	Lipids	Proteins	Lipids	Proteins	Lipids	Proteins	Lipids	Proteins	Lipids	Proteins	Lipids	Proteins	Lipids
1	18.36±1.13^{a}	1.86±0.12^{a}	41.27±2.57^{a}	6.92±1.27^{a}	42.57±1.13^{a}	7.76±2.23^{a}	42.31±2.57^{a}	6.92±1.12^{a}	41.78±3.27^{a}	7.05±1.92^{a}	42.57±1.13^{a}	7.76±2.23^{a}	51.31±3.22^{a}	9.01±2.07^{a}
20	18.20±1.54^{a}	1.29±0.07^{a}	39.94±2.68^{a}	5.87±0.73^{a}	40.20±1.54^{a}	7.19±1.87^{a}	41.98±1.27^{a}	5.87±1.21^{a}	40.36±2.38^{a}	6.36±1.37^{a}	40.20±1.54^{a}	7.19±1.87^{a}	46.07±2.39^{a}	6.22±1.73^{a}
90	18.07±1.17^{a}	0.98±0.09^{a}	39.07±1.31^{a}	2.02±0.15^{a}	39.01±0.22^{a}	5.98±0.09^{b}	41.04±1.72^{a}	4.31±1.62^{a}	38.27±1.43^{a}	5.01±0.18^{b}	39.01±0.22^{a}	5.98±0.09^{b}	44.69±1.86^{a}	4.56±1.57^{a}
ANOVA	*	***	***	***	*	**	*	**	**	**	*	**	**	***

Values are means ± standard deviation (n = 3 for proteins and n = 6 for lipids); Different superscript letters in the same column represent significant differences($p < 0.05$, one-way ANOVA with Tukey test); Significant differences in total proteins and lipids percentages between FF and CSF and HSF with or without antioxidant extract during the three storage periods are presented ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

level, especially after the day 1 of storage, in HSF covered with 1% of the extract (Table 1).

In the present study, the total proteins remained unaltered in FF over the 1, 20, and 90 days of storage. This stability in total protein content may be due to the retention of water in the fillets during these storage periods. However, its significant increase in both CSF and CSF, as well as in HSF and HSF, covered with 0.5 and 1% of the extracts, compared to FF during the three storage periods can be attributed not only to the decrease in moisture and water content, and the increase of ash associated with evaporation during the smoking process, but also to the effect of polyphenols, including phenolic acids and flavonoids, vitamin E, tocopherols, and tocotrienols, which are powerful antioxidants found in the *D. salina* extract and oil (Cakmak *et al*., 2014), which protect the cell membrane from oxidative damage and consequently prevent protein and lipid oxidation (Garavaglia *et al*., 2016). In addition, the significant decrease ($p < 0.05$) in protein levels in both HSF and HSF covered with 0.5% of the extract after the 20 and 90 days of storage may be due to signs of the enzymatic autolytic activity causing the spoilage (Ayeloja *et al*., 2020), which was discontinued in the HSF covered with 1% of the extract after e 90 days of storage.

On the other hand, the significant decrease ($p < 0.001$) in the lipid content in FF during the three storage periods can be attributed to the progression in reducing the antioxidant properties and total phenolic content, which reversibly increased in CSF and HSF with or without 0.5 and 1% of the extracts during the three storage periods. This significant increase can be explained in terms of the dehydration of the fillets during smoking and the incorporation of *D. salina* oil into the fillets covered with 0.5 and 1% of the extracts, which led to stopping the degradation of lipids by eliminating free radicals. Similar results were found with sardine samples canned in grape seed and olive oils (Bouriga *et al*., 2022). Nevertheless, the significant decrease in lipid levels in FF after the 20 and 90 days of storage suggests the hydrolysis of some lipid fractions associated with the progression of microbial spoilage (Burton and Ingold, 1984).

3.2. Lipid oxidation indices in fresh and smoked fillets

The levels of the peroxide value (PV) and TBARS levels in the *S. lucioperca* FF and CSF and HSF covered with or without 0.5 and 1% of *D. salina* polyphenol antioxidant extracts are given in Table 2. The results showed that PV increased gradually during the storage periods from 1, 20 to 90 days, with the highest significant level ($p < 0.001$) found after 90 days of storage (Table 2). However, the PV in both CSF and CSF covered with 0.5 and 1% of the extract revealed a gradual decrease over the three storage periods compared to its level in FF. In HSF, although the PV indicated a significant increase ($p < 0.05$) after day 1 of storage and a significant decrease ($p < 0.05$) after the 20 days, it revealed a highly significant increase after 90 days compared to CSF. In HSF covered with 0.5 and 1% of the extract, compared with HSF, the PV displayed a slight decrease after 1 day of storage, a constant level at 1% concentration of the extract after the 20 days, and a significantly decreased level after 90 days; but this level was reduced significantly at 0.5% concentration

TABLE 2. Variations in the peroxide (PV, in meq active O2/kg lipid), thiobarbituric acid reactive substances (TBARS, in mg MDA/kg lipid), and 1,1-diphenyl-2-picrylhydrazyl (DPPH, in g/100g lipids) values in fresh (FF), cold-smoked (CSF), hot-smoked (HSF) fillets of *Sander lucioperca*, and cold smoked (CSF), and hot smoked (HSF) fillets covered with *Dunaliella salina* polyphenol (pp) antioxidant extract at 0.5 and 1% concentrations during the three storage periods

Parameter	Storage days	FF	CSF	CSF+0.5% pp	CSF+1% pp	HSF	HSF+0.5% pp	HSF+1% pp	ANOVA
PV	1	6.41±0.23^{a}	4.13±0.27^{a}	3.97±0.12^{a}	3.01±0.57^{b}	5.76±0.22^{b}	4.52±0.42^{c}	4.22±0.10^{c}	***
	20	10.06±1.04^{a}	9.13±1.41^{a}	6.08±1.06^{a}	5.27±1.42^{b}	7.27±0.47^{b}	5.58±0.18^{c}	6.73±0.76^{c}	***
	90	47.86±2.53^{a}	26.21±1.43^{a}	16.48±2.57^{b}	15.78±1.27^{c}	31.38±1.67^{b}	17.98±1.79^{b}	14.72±1.56^{c}	***
TBARS	1	0.52±0.12^{a}	0.96±0.17^{b}	0.67±0.08^{a}	0.55±0.03^{a}	1.38±0.31^{b}	1.17±0.38^{c}	1.03±0.15^{c}	***
	20	4.06±0.13^{a}	2.12±0.31^{c}	2.07±0.08^{b}	1.74±0.41^{b}	3.27±0.38^{a}	1.74±0.54^{b}	2.36±0.42^{c}	***
	90	8.41±1.05^{a}	3.68±0.35^{b}	3.31±0.52^{c}	2.11±0.72^{c}	7.12±1.07^{a}	5.03±0.67^{b}	4.78±1.04^{c}	***
DPPH	1	69.13±0.39^{a}	72.0±0.58^{a}	74.08±0.83^{a}	77.48±0.23^{a}	72.75±0.43^{b}	81.94±0.95^{c}	88.57±0.83^{c}	***
	20	33.72±0.57^{a}	37.12±0.53^{c}	44.12±0.43^{c}	53.96±0.32^{c}	39.52±0.26^{b}	48.98±0.46^{a}	57.14±0.35^{a}	***
	90	18.10±0.12^{a}	21.89±0.45^{b}	26.67±0.36^{c}	28.56±0.13^{c}	21.65±0.34^{a}	27.98±0.27^{b}	31.88±0.23^{b}	***

Values are means ± standard deviation (n = 3); Different superscript letters in the same column represent significant differences ($p < 0.05$, one-way ANOVA with Tukey test); Significant differences between FF and CSF and HSF with or without antioxidant extract during the three storage periods are presented ($^{***}p < 0.001$).

of the extract. This significantly increased the PV level in FF after 90 days of storage and it is higher than that found in *Sardinella gibbosa* after 6 and 9 days of storage (Chaijan *et al*., 2006). In addition, the PV levels in both CSF and HSF with or without 0.5 and 1% of the polyphenol extracts during the three storage periods were higher than those reported for *S. lucioperca* by Bouriga *et al*. (2020), although these levels were significantly decreased by the addition of these polyphenols (Table 2). These higher levels of PV are likely due to the hot temperature of the smoking process and the higher content of PUFAs, which are highly sensitive to primary oxidative reactions induced by molecular oxygen, and fall within the acceptable limits (10–20 mEq/kg) declared by Connell (1995).

In *S. lucioperca* FF, Bouriga *et al*. (2020) reported that the TBARS original level was 0.49 mg MDA/kg oil. This level increased here to 0.52 mg MDA/kg after the 1 day of storage, indicating that lipid oxidation had occurred and progressively significantly increased ($p < 0.001$) after 20 and 90 days of storage (Table 2). This significant increase is consistent with that found in *Trachurus trachurus* during frozen storage (Aubourg *et al*., 2002) In CSF, the level was significantly higher than in FF, constant after 1 day of storage, and decreased significantly ($p < 0.05$) after 20 and 90 days in CSF covered with 0.5 and 1% of the extract. In HSF, the level revealed a gradual significant decline in SHF covered with 0.5 and 1% of the extract after the 20 and 90 days of storage. However, compared to its level in FF, it exhibited a significant elevation after 1 day of storage and a reduction in HSF and HSF covered with 0.5 and 1% of the extract after 20 and 90 days of storage. In comparison with both CFS and CSF covered with 0.5 and 1% of the extract, the TBARS level displayed a significant increase ($p < 0.001$), especially in HSF and HSF covered with 0.5% of the extract, and constant in HSF after 1 day of storage. However, its level was significantly higher in both HSF and HSF covered with 1% of the extract than in both CSF and CSF covered with 0.5 and 1% of the extract, constant in both CSF and HSF covered with 1% of the extract after 20 days of storage, and significantly higher in both HSF and HSF covered with 0.5 and 1% of the extract after 90 days of storage. This significant increase in the TBARS level beyond the acceptable limit (8 mg MDA/kg) in FF was significantly decreased in both CSF and HSF with or without the covering polyphenol antioxidant extracts during the three storage periods. Despite this decrease, the levels were significantly higher than those observed by Bouriga *et al*. (2020) and Bouriga *et al*. (2022) and lower than those found by Chaijan *et al*. (2006). This increase in TBARS reflects the increase in the formation of aldehydes, relatively polar secondary reaction products (Kolakowska, 2002) due to the increase in phospholipids. In addition, it is worth not-

ing that the decrease in TBARS levels was concurrent with the gradual increase in PV during the three storage periods, a condition which is inconsistent with that described by Chaijan *et al*. (2006). This was probably due to an increase in hydroperoxides, especially aldehydes, in the later stages of secondary lipid oxidation as a result of the greater release of free iron and other prooxidants from the muscle which were excessively degraded when storage time was increased. However, this increase in hydroperoxides was reduced in both CSF and HSF by the addition of 0.5 and 1% of the covering polyphenol extracts.

3.3. Free radical (DPPH) activity in fresh and smoked fillets

The DPPH activity in *S. lucioperca* FF, as well as in both CSF and HSF covered with or without 0.5 and 1% of *D. salina* polyphenol antioxidant extracts are shown in Table 2. Overall, the DPPH activity exhibited a significant decline in FF over the 1, 20, and 90 days of storage, respectively, while a progressive elevation in the activity was observed in both CSF and CSF covered with the two concentrations of the polyphenol antioxidant extracts during the three storage periods. Conversely, the activity showed a significant decrease ($p < 0.05$) in HSF compared to HSF covered with 0.5 and 1% of the extract during the three periods of storage. However, in comparison with its activity in FF, as well as in CSF and CSF covered with 0.5 and 1% of the extract, the activity level in HSF was significantly higher than in FF and lower than in CSF covered with 0.5 and 1% of the extract during the three periods of storage. On the other hand, the level in HSF covered with 0.5 and 1% of the extract was significantly higher than that in CSF covered with 0.5 and 1% of the extract, respectively, during the three periods of storage. Indeed, the DPPH free radical assay has been used to assess the antioxidant activity based on the electron transfer that produces a violet solution in ethanol (Huang *et al*., 2005). Overall, the DPPH activity showed a gradual significant decrease ($p < 0.05$) in both FF and CSF, as well as in HSF covered with or without 0.5 and 1% of the polyphenol extract, with an increase in the storage periods from 1 to 90 days. However, the levels of activity exhibited a significant increase in both CSF and HSF, compared to FF, with the addition of the polyphenol extract. Moreover, this increased activity was concurrent with the increase in lipid content. Therefore, we can assume that there was a close correlation between the polyphenol content, the DPPH activity, and the increase in lipid peroxidation.

3.4. Total fatty acid (FA) composition in fresh, cold-, and hot-smoked fillets

The total FA composition in FF differed significantly during the three storage periods (1, 20, and 90), with a significant increase ($p < 0.05$) in both total saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs), and a significant decrease ($p < 0.05$) in total polyunsaturated fatty acids (PUFAs) after the three storage periods (Table 3). This significant increase was confirmed by the considerable elevation of all SFAs (C14:0, C16:0, and C18:0) and MUFAs (C16:1n-7, C18:1n-7, and C18:1n-9), while the total decrease in PUFAs was only observed in C20:5n-3, C22:5n-3, C22:6n-3, and PUFA-n-3. Similarly, in both CSF and HSF, all SFAs and MUFAs exhibited a progressively significant increase ($p < 0.05$) in level during the three storage periods (1, 20, and 90). However, the total PUFAs, as well as PUFA–n-3 and –n-6, indicated a significant gradual decrease ($p < 0.05$) during these storage periods. Similarly, when CSF and HSF were covered with both concentrations of 0.5 and 1% of *D. salina* extract, all SFAs and MUFAs showed a remarkable increase ($p < 0.05$) over the 1, 20, and 90 days of storage, while the total PUFAs and PUFA–n-3 and –n-6 displayed a substantial significant decrease ($p < 0.05$) during these storage periods.

Regarding the variation in the FA composition in FF, it was shown that the significant increase in total SAFs was dominated by palmitic acid (C:16), which showed the highest increase (40.49%) after the 90 days of storage. This significant increase was also continued at different rates from day 1 to day 90 of storage in both CSF and HSF covered with or without 0.5 and 1% of the antioxidant extract. In addition, the significant increase in total MUFAs in FF was dominated by oleic acid (C18:1n-9), with the highest increase (17.95%) after 90 days of storage. This increase also persisted in differential proportions from day 1 to day 90 of storage in both CSF and HSF covered with or without 0.5 and 1% of the extract. However, the significant reduction in PUFAs was prevailed by docosahexaenoic acid (C22:6n-3) in FF, with the highest decrease (1.67%) found after

TABLE 3. Total fatty acid (FA) composition (in %) in fresh (FF), cold-smoked (CSF), and hot-smoked (HSF) fillets of *Sander lucioperca*, and CSF and HSF fillets covered with *Dunaliella salina* polyphenol (pp) antioxidant extract at 0.5 and 1% concentrations during the three storage periods

FA	FF			CSF			CSF+0.5% pp			CSF+1% pp		
	1 day	20 days	90 days	1 day	20 days	90 days	1 day	20 days	90 days	1 day	20 days	90 days
C14:0	4.65±0.19a	5.09±1.12a	6.14±1.78b	5.72±0.25a	7.72±1.12b*	8.32±0.67c**	5.89±0.25a	6.41±1.52b	7.92±1.55c	6.72±1.05a*	7.12±1.78b**	8.01±1.23c***
C16:0	16.4±0.89a	20.46±1.52b	22.19±2.5c	17.65±2.14a	18.23±1.95b*	21.29±1.48c	16.5±0.87a	17.57±2.4b**	20.76±2.48c*	18.21±1.56a**	18.31±2.55b	19.7±2.37c
C18:0	2.76 ±0.66a	4.01±1.73b	7.15±3.45c	2.92 ±0.78a	4.57±0.73c	7.89±0.47c	3.19±0.78a	3.73±0.47b	6.37±1.51c*	4.22±0.96a***	4.27±0.36a	6.03±0.58c**
C20:0	0.78 ± 0.03b	0.81 ± 0.05c	1.72 ± 0.18c	0.68 ± 0.04b	0.79 ± 0.07b	1.56 ± 0.11a	0.87 ± 0.02b	0.93 ± 0.01c	1.29 ± 0.06b	0.61 ± 0.03b	0.78 ± 0.02a	1.18 ± 0.21c
C22:0	0.52 ± 0.01b	1.12 ± 0.07b	1.41 ± 0.09b	0.34 ± 0.02c	0.57 ± 0.01b	1.47 ± 0.04b	1.22 ± 0.07b	1.07 ± 0.11b	1.63 ± 0.15b	1.22 ± 0.17c	1.41 ± 0.14b	1.57 ± 0.33b
C24:0	1.33 ± 0.08b	2.48 ± 0.15b	3.29 ± 0.21b	1.69 ± 0.13b	2.54 ± 0.27	3.66 ± 0.31c	1.53 ± 0.26b	1.27 ± 0.17c	2.7 ± 0.31c	1.86 ± 0.47b	2.68 ± 0.48b	2.29 ± 0.53b
Total SFA	26.44±2.03a	33.97±3.12c	40.49±2.30c	29.00±3.41a**	34.42±3.27b	44.19±3.06c***	29.2±2.10a**	30.98±2.92a	40.67±2.94c	32.84±2.10a***	34.57±3.41a	38.78±3.07b
C16:1n-7	6.75 ±1.51a	8.80±1.55a	9.984±2.47c	7.82 ±1.51a*	9.41±1.59b*	12.06±3.02c**	6.40±0.46a	7.37±1.42b	10.12±1.21c	5.92±0.47a*	6.68±1.25b**	9.42±1.36c
C18:1n-7	2.90±0.50a	4.25±0.58b	5.12±2.72c	3.51±0.78a*	4.12±0.68b	6.43 ±0.18c*	3.81±0.27a*	4.21±0.72b	6.87±1.36c*	3.36±0.27a	3.86±0.58a*	4.11±1.27b
C18:1n-9	12.23±2.40a	15.40±2.05a	17.95±3.22b	12.85±2.74a	13.78±2.02b*	16.08±0.87c*	13.53±1.13a	14.31±3.05b	17.31±2.87c	12.68±1.41a	13.57±2.42b*	19.52±2.31c**
C20:1	1.11±0.32a	1.96±0.65b	3.04±0.61c	1.27±0.12a	1.62±0.44a	2.08±0.41b	1.09±0.21a	1.51±0.66b	2.12±0.37a	1.31±0.20b	1.39±0.36a	0.97±0.02b
C22:1	1.02 ± 0.05a	1.68 ± 0.05b	2.74 ± 0.07b	1.08 ± 0.03a	1.31 ± 0.03a	1.55 ± 0.03a	1.01 ± 0.05a	1.32 ± 0.03a	1.83 ± 0.07a	0.98 ± 0.01a	1.09 ± 0.06b	0.65 ± 0.03a
C24:1	0.76 ± 0.01b	1.14 ± 0.06b	1.63 ± 0.04b	1.01 ± 0.02b	1.08 ± 0.06b	1.49 ± 0.02c	0.88 ± 0.03b	0.95 ± 0.04c	1.78 ± 0.06b	0.73 ± 0.01b	1.06 ± 0.02b	0.18 ± 0.01c
Total MUFA	24.77±2.05a	33.23±2.56b	40.47±3.67c	27.54±2.31a**	31.32±3.73b*	39.69±2.73c	26.72±1.58a*	29.70±3.41b*	40.03±3.21c	24.98±1.23a	27.65±2.73b**	34.85±3.14c***
C18:2n-6 (LA)	4.68±0.97a	5.40±1.96b	7.32±1.51b	5.69±0.82a*	3.78±1.25b***	3.02±1.78b***	6.32±2.48a**	4.92±1.63b	3.96±0.69c***	7.01±1.57a***	5.36±0.44b	4.00±0.73c***
C20:2n-6	0.96 ± 0.01a	0.42 ± 0.02b	0.01 ± 0.00a	1.48 ± 0.16a	1.09 ± 0.12b	0.36 ± 0.01c	0.09 ± 0.01b	1.34 ± 0.4c	0.46 ± 0.02b	1.39 ± 0.3c	1.19 ± 0.2b	0.75 ± 0.1c
C20:3n-3	1.06 ± 0.04b	0.51 ± 0.05c	0.03 ± 0.01b	1.29 ± 0.03c	1.12 ± 0.05b	0.49 ± 0.03b	0.03 ± 0.02b	1.2 ± 0.1b	0.57 ± 0.03b	0.99 ± 0.07b	1.2 ± 0.4b	0.46 ± 0.01c
C20:4n-6 (ARA)	11.24±2.35a	12.23±2.65b	13.05±2.29c	12.44±7.78a	11.22±2.42b*	11.23±1.65a*	9.98±1.75a*	7.78±1.57b**	9.52±1.38a***	10.02±1.41a	9.08±1.36b***	7.14±0.68c***
C20:5n-3 (EPA)	7.93 ±1.46a	3.49±0.51b	1.22±0.54c	8.57 ±1.33a*	4.98±1.94b*	1.74±2.31a	5.67±0.36a**	3.98±1.72b	3.21±0.42a***	6.41±0.51a*	5.31±0.87b**	4.12±0.35c***
C22:4n-6	1.00 ± 0.03b	0.59 ± 0.06b	0.01 ± 0.00b	1.25 ± 0.07c	1.04 ± 0.05b	0.5 ± 0.02c	0.21 ± 0.01b	1.43 ± 0.6b	0.13 ± 0.01c	1.69 ± 0.5b	2.08 ± 0.8b	0.88 ± 0.02c
C22:5n-3 (DPA)	1.78±0.23a	1.27±0.72a	0.78±0.06b	2.42±0.27a**	1.63±0.12a	1.02±0,05a	1.31±0.68a	0.97±0.09b	0.98±0.08b	1.76±0.21a	1.36±0,01a	1.02±0.04b
C22:6n-3 (DHA)	13.68±2.78a	8.78±2.86b	1.67±0.57c	12.63±2.56a*	8.46±1.41b	6.98±2.97c***	14.85±2.35a*	10.37±1.52b**	7.21±0.33c***	13.78±2.27a	12.21±1.41b**	9.42±0.84c***
PUFA-n-3	23.39±1.15a	13.54±1.09c	3.67±0.08c	23.62±3.06a	15.07±0.76c**	9.74±0.63c***	21.83±2.58a*	15.32±2.13c*	11.40±1.3c***	21.95±1.61a*	18.88±1.2b***	14.56±2.33c***
PUFA-n-6	15.92±1.22a	17.63±1.11b	20.37±1.98c	18.12±0.97a**	15.00±1.35a*	14.25±1.53b***	16.30±1.00a	12.70±1.18b**	13.48±1.3b***	17.03±1.22a*	14.44±1.37b**	11.14±0.51c***
Total PUFA	42.33±1.32a	32.69±2.78b	24.09±2.09c	45.77±3.02a*	33.32±2.78b	25.34±3.12c	37.8±3.1a***	31.99±3.12b	26.04±3.07c	43.05±4.08a	37.79±2.44b	27.79±3.51b*

the 90 days of storage. This decrease also proceeded to different percentages from day 1 to day 90 of storage in both CSF and HSF covered with or without 0.5 and 1% of the extract. Similar results of the significant reduction in PUFAs at the end of smoking (90 days) due to a decrease in PUFAs of the n-3 series (Tot n-3) and a simultaneous increase in MUFAs have been found in *Argyrosomus regius* fillets cold-smoked in combination with 1% *Halocnemum strobilaceum* antioxidant for 35 days of storage at 4 °C (Messina *et al*., 2021). In comparison with the previous work on *S. lucioperca*, the current increase in total SAFs is consistent with that also found in CSF and HSF by Bouriga *et al*. (2022). However, the increase in total MUFAs recorded here is not in line with that reported by these authors, as total MUFAs showed a significant decrease in CSF and HSF. Nevertheless, there is agreement about the decrease reported here and there in total PUFAs in both CSF and HSF. Therefore, we can assume that the *D. salina* polyphenol antioxidant extract had a significant effect not only on the increase in SAFs and MUFAs but also on their percentages in both CFS and HSF.

3.5. Free fatty acid (FFA) content in fresh, cold-, and hot-smoked fillets

The profile of the FFA content in FF showed a progressive significant increase ($p < 0.05$) over the 1, 20, and 90 days of storage. This significant increase was gradually decreased in CSF covered with or without 0.5 and 1% of *D. salina* polyphenol antioxidant extract and then increased again in HSF during the three periods of storage but with the highest level in HSF (Table 4). Overall, the formation of FFA, as a marker

TABLE 3. Continued

FA	HSF			HSF+0.5% pp			HSF+1% pp		
	1 day	20 days	90 days	1 day	20 days	90 days	1 day	20 days	90 days
C14:0	5.28±0.63a*	6.92±1.51b*	9.01±1.44c***	4.69±0.51a	5.08±1.03b	6.27±1.07c	4.73±0.27a	5.27±1.22b	6.55±1.43b**
C16:0	16.98±3.08a	17.86±2.44b**	22.18±1.55c	15.21±2.45a*	16.12±2.76b***	20.36±1.28c**	15.39±2.64a*	15.68±2.31a***	18.6±1.59b***
C18:0	3.26±0.92a	4.13±0.87b	7.41±0.51c	3.12±0.84a	4.01±0.56a	6.81±0.36c	3.58±0.72a	3.78±0.56a*	5.62±0.36b**
C20:0	0.61 ± 0.01a	0.73 ± 0.03b	1.59 ± 0.03b	0.24 ± 0.00a	0.84 ± 0.02b	1.42 ± 0.16a	0.57 ± 0.02b	0.69 ± 0.03b	1.09 ± 0.07b
C22:0	0.92 ± 0.04b	1.05 ± 0.13b	1.87 ± 0.05c	0.69 ± 0.01b	1.02 ± 0.07c	1.89 ± 0.12b	0.97 ± 0.06b	1.01 ± 0.01a	1.64 ± 0.08b
C24:0	1.61 ± 0.12c	2.21 ± 0.16b	2.55 ± 0.15b	2.1 ± 0.23c	1.9 ± 0.15b	1.91 ± 0.24b	1.7 ± 0.18b	1.86 ± 0.09a	2.08 ± 0.16b
Total SFA	28.66±3.24a**	32.90±3.15b	44.61±4.05c***	26.05±2.54a	28.97±2.69b**	38.66±3.12c**	26.94±3.35a	28.29±2.56b**	35.58±2.86c***
C16:1n-7	8.43±1.26a**	8.78±1.21a	13.76±2.58c**	7.21±0.96a	7.92±1.26a	11.32±2.37c*	7.44±0.82a	7.66±1.41a	9.78±2.57b
C18:1n-9	11.46±2.52a	12.02±2.15b	17.45±2.41c	10.23±1.78a	11.12±2.27b	14.36±2.28c	10.51±1.45a	10.77±2.31b	11.90±2.45b
C18:1n-7	4.36±0.89a***	4.99±0.38b	7.16±0.23c**	3.78±0.27a*	4.97±0.12b	6.46 ±0.47c	3.97±0.68a*	4.37±0.12b	5.86±0.47b
C20:1	1.21±0.57b	1.32±0.61a	1.38±0.72c	1.16±0.21c	1.48±0.22a	1.66±0.63a	1.2±0.18c	1.31±0.09b	1.43±0.12c
C22:1	1.07 ± 0.03a	1.46 ± 0.05a	1.16 ± 0.12b	1.01 ± 0.01b	1.10 ± 0.01b	1.31 ± 0.02b	1.16 ± 0.04b	1.68 ± 0.07b	1.63 ± 0.05b
C24:1	0.96 ± 0.04b	1.16 ± 0.07b	0.84 ± 0.01b	0.95 ± 0.00c	1 ± 0.12b	1.15 ± 0.03b	1.06 ± 0.01b	0.73 ± 0.02c	0.99 ± 0.03b
Total MUFA	27.49±2.72a**	29.73±4.21b	41.75±2.86c	24.34±2.15a	27.59±2.76b**	36.26±3.12c***	25.37±2.68a	26.52±2.39b**	31.59±3.28b***
C18:2n-6 (LA)	6.21±0.74a***	4.21±1.13a	2.27±0.14b***	7.04±0.66a***	6.54±0.76a*	4.31±0.21b***	7.01±0.66a***	6.81±0.15a**	6.01±0.18b**
C20:2n-6	1.22 ± 0.2b	1.51 ± 0.6c	0.89 ± 0.01b	1.96 ± 0.5b	1.79 ± 0.6c	1.44 ± 0.3b	1.65 ± 0.4c	2.48 ± 0.54b	1.22 ± 0.17c
C20:3n-3	1.94 ± 0.5a	1.27 ± 0.1b	0.87 ± 0.01b	1.59 ± 0.2c	1.55 ± 0.36b	0.87 ± 0.01a	1.61 ± 0.7c	1.73 ± 0.31c	1.12 ± 0.09a
C20:4n-6 (ARA)	13.25±2.31a**	12.08±1.31b	8.97±1.13a***	11.78±2.58a	11.12±1.26a	7.33±1.09c***	11.66±2.45a	11.36±1.13a	10.16±1.19c**
C20:5n-3 (EPA)	7.12±1.15a	5.96±1.26b**	1.21±0.27c	7.68±1.08a	6.96±1.37b***	4.95±0.83c***	7.51±1.11a	7.38±1.78b***	6.06±0.27c***
C22:4n-6	2.05 ± 0.02b	1.89 ± 0.03c	0.92 ± 0.02b	2.47 ± 0.54c	2.58 ± 0.33b	2.39 ± 0.12b	3.01 ± 0.8a	3.44 ± 0.92b	1.16 ± 0.53b
C22:5n-3 (DPA)	2.51±0.14a**	2.04±0.17a*	1.02±0.01b	3.07±0.27a**	2.47±0.24a**	1.64±0.03b*	3.05±0.13a**	2.81±0.51a**	2.51±0.15b***
C22:6n-3 (DHA)	12.41±2.22a	11.09±1.28b***	6.14±2.37c***	13.61±2.52a	12.79±1.09b***	9.26±1.99c***	13.87±2.42a	13.16±1.28b***	12.65±1.37c***
PUFA-n-3	22.06±1.14a	19.11±1.67b***	8.37±0.78c***	24.37±2.08a*	22.23±1.53b***	15.86±1.47c***	24.43±2.69a*	23.35±1.31a***	21.23±1.70b
PUFA-n-6	19.46±1.42a**	16.31±1.27b*	11.25±0.99c***	18.83±1.66a**	17.67±2.04a	11.64±1.38c***	18.67±0.76a**	18.17±1.28a*	16.18±1.54b***
Total PUFA	46.71±4.31a**	40.05±3.51b**	22.29±2.05c	49.29±4.57a***	45.80±2.48b***	32.19±2.24c***	49.37±3.34a***	49.17±4.97b***	40.89±2.51c***

Values are means ± standard deviation (n = 6); Different superscript letters in the same column represent significant differences ($p < 0.05$, one-way ANOVA with Tukey test); Significant differences between FF and CSF and HSF with or without antioxidant extract during the three storage periods are presented ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$); NS = non-significant, FAs = fatty acids, SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids, LA = linoleic acid, ARA = arachidonic acid, EPA = eicosapentaenoic acid, DPA = docosapentaenoic acid, DHA = docosahexaenoic acid, PUFA-n-3 = Omega-3 fatty acid, PUFA-n-6 = Omega-6 fatty acid.

of lipolysis in smoked oil fish fillets during storage, has been so far associated with fat content, lipolytic activity, and temperature (Bouriga *et al*., 2020). In *S. lucioperca* FF, FFA was represented by 1.24% of total lipids, a level which is within the range (1–7%) found in crude fish (Bimbo, 1998) and lower than that reported by Aidos *et al*. (2001) in herring oil. This level was significantly increased (7.57%) as the storage periods increased, with the highest increase (7.89%) shown in HSF after 90 days of storage. Similar results have previously been recorded by Bouriga *et al*. (2020). Such an increase, particularly in HSF, can be attributed to the lipolysis generated by lipases or phospholipases (Chaijan *et al*., 2006; Bouriga *et al*., 2020). In addition, the results obtained indicated that both smoking and the addition of 0.5 and 1% of the polyphenol extracts were below the acceptable limit (7 g/100g).

3.6. Total volatile base nitrogen (TVB-N) values in fresh, cold-, and hot-smoked fillets

The TVB-N indicated significantly increased levels ($p < 0.001$) in FF during the three storage periods, with the highest level found after 90 days of storage. In addition, differentially significant decreased levels ($p < 0.05$) were found in CSF and CSF covered with 0.5 and 1% of the extracts, as well as in HSF, and HSF covered with 0.5 and 1% of the extracts, during the three storage periods, with the highest similar level in CSF and HSF, especially after 90 days of storage, compared to CSF and HSF covered with 0.5 and 1% of the extracts (Table 4). This significant decrease ($p < 0.001$) in the level of TVB-N in CSF and HSF covered with or without 0.5 and 1% of the covering extracts, compared to FF during the three storage periods, is inconsistent with the findings of Karsli and Caglak (2021), who report-

TABLE 4. Variations in the free fatty acids (FFAs, in g/100g lipids) and total volatile basic nitrogen (*TVB-N, in* mg MDA/kg lipids) values in fresh (FF), cold-smoked (CSF), hot-smoked (HSF) fillets of *Sander lucioperca*, and cold-smoked (CSF), and hot-smoked (HSF) fillets covered with *Dunaliella salina* polyphenol (pp) antioxidant extract at 0.5 and 1% concentrations during the three storage periods

Parameter	Storage days	FF	CSF	CSF+0.5% pp	CSF+1% pp	HSF	HSF+0.5% pp	HSF+1% pp	ANOVA
FFAs	1	1.24±0.07a	1.78±0.17a	1.52±0.04a	1.38±0.02a	3.42±0.10b	2.14±0.16c	2.04±0.37c	***
	20	3.76±0.49a	2.39±0.71c	2.05±0.13c	1.69±0.39c	4.27±0.38b	3.47±0.44a	3.12±0.37a	***
	90	7.57±1.21a	4.96±0.72b	4.23±0.47c	3.29±0.62c	7.89±1.53a	4.92±0.47b	4.47±1.36b	***
TVB-N	1	5.32±0.47a	3.73±0.06b	3.48±0.10b	2.78±0.10a	2.86±0.12a	2.48±0.09a	2.28±0.17b	***
	20	10.06±0.09a	6.27±0.27b	5.2±0.02b	4.2±0.28a	6.05±1.23a	4,89±0.16a	3.21±1.69b	***
	90	22.21±0.43a	19.42±1.22a	16.8±1.52b	15.7±1.02c	19.42±1.22a	13.62±1.38b	12.7±1.31b	***

Values are means ± standard deviation (n = 3); Different superscript letters in the same column represent significant differences ($p < 0.05$, one-way ANOVA with Tukey test); Significant differences between FF and CSF and HSF with or without antioxidant extract during the three storage periods are presented ($^{***}p < 0.001$).

ed a gradual significant increase in TVB-N during the storage of the smoked fish. In this respect, Arous *et al*. (2014) reported that the increase in TVB-N levels resulted from the production of dimethylamine, trimethylamine, and ammonia associated with the destructive effect of microorganisms on proteins during storage. In addition, Karsli and Caglak (2021) attributed the increase in the TVB-N levels to the loss of water during smoking and the increase in proteolytic activity during salting and smoking. Therefore, in addition to the water loss during smoking, we can assume here that the significant decrease in the TVB-N levels in CSF and HSF with or without 0.5 and 1% of the covering extracts was due to increased deamination of adenosine monophosphate or amino acids, which led to an increase in ammonia release, resulting from the combined effect of smoking and the addition of *D. salina* extract (Bouriga *et al*., 2022).

4. CONCLUSIONS

The results showed a significant increase in proteins, lipids, FFAs, and DDPH contents, and a decrease in PV, TBARS, and TVB-N levels in cold (CSF) and hot (HSF) smoked fillets of *Sander lucioperca* covered with or without 0.5 and 1% of *Dunaliella salina* polyphenol antioxidant extract and stored for 1, 20, and 90 days compared to fresh fillets (FF). Saturated (SFAs) and monounsaturated (MUFAs) fatty acids exhibited a significant increase in FF and CSF and HSF covered with or without polyphenol extract. Total polyunsaturated fatty acids (PUFAs) revealed a significant decrease in FF and CSF and HSF with or without the extract. Therefore, cold and hot smoking processes and the addition of 0.5 and 1% of natural *D. salina* polyphenol antioxidant extract was a valuable and promising method to improve the biochemical quality, shelf-life, and consumption of *S. lucioperca* fillets stored for up to 90 days in a refrigerator at 0-4 °C.

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