Combination effect of smoking conditions and *Dunaliella salina* as natural antioxidant on biochemical characters of stored *Sander lucioperca* fillets

N. Bouriga
University Tunis el Manar
S. Mili
University of Carthage, Higher Institute of Fisheries and Aquaculture of Bizerte
D. Troudi (dhaker.troudiest@laposte.net)
University of Carthage, Higher Institute of Fisheries and Aquaculture of Bizerte, Exploitation of Aquatic Environments
A. Ben Atitallah
University of Sfax
W. Rjiba Bahri
University Tunis el Manar
S. Bejaoui
University of Carthage, Higher Institute of Fisheries and Aquaculture of Bizerte
J.-P. Quignard
University of Montpellier II, Sciences et Techniques Languedoc
M trabelsi
University of Carthage, Higher Institute of Fisheries and Aquaculture of Bizerte, Exploitation of Aquatic Environments

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Abstract

Sander lucioperca is a fresh water fish characterized with its remarkable nutritional value. Thus, smoking improves sensory and microbiological characteristics. Therefore smoking processes is associated to alterations in some physicochemical fish properties. The object of this study is to evaluate the combined effects of two different processes of smoking (cold and hot) and natural antioxidants obtained from the microalgea *Dunaliella salina* administrated at two graded concentrations (+0.5pp and +1pp) on the biochemical quality and shelf life of *Sander lucioperca* fillets were investigated during 1, 20 and 90 days of storage.

Smoking *Sander lucioperca* using *Dunaliella salina* extract increases polyphenols content in the fillets smoked fish. This natural antioxidant leads to a better preservation for this smoked fish. *Dunaliella salina* increases of the amounts of lipids and decreases TVBN, PV, TBARS and FFA in cold and hot smoked fillets.

Therefore, combined effects of two different processes of smoking (cold and hot) and natural antioxidants could be a promising technique to preserve the *Sander lucioperca* fillets quality.

Introduction

Extensive fish farming offers to fishermen the opportunity to produce profitably fish like *Sander lucioperca* (zander, pikeperch or sander) at low prices. Despite the high nutritional quality of *S. lucioperca* (polyunsaturated fatty acids (PUFA), vitamins and minerals) [1] this specie is not well appreciated by consumers because of its undesirable taste and flavour compared to marine fishes.

The application of smoking process can offer such a marketing option for fresh water fish and to obtain high quality products with good acceptance [2]. It is well reported that smoking treatments (hot, cold, liquid and electrostatic) are useful processes for preserving the quality of seafood and can be performed using both traditional and innovative techniques [3]. Among the components of smoke, aldehydes, ketones, alcohols, acids, hydrocarbons, esters, phenols and ethers which settle on the surface of the threads and later enter the muscle, are responsible for the final color and taste of the products. However, some reports have demonstrated that smoking processes have a harmful impact on the nutritive value of fish fillets [2], thus, antioxidant addition could be useful to preserve fillets quality.

Since consumers are more health conscious, functional foods and the addition of natural ingredients are in great demand. For this, several authors focused on microalgae as potential sources of compounds with functional nutritional properties. Among these compounds that can be obtained from microalgae, antioxidants are highly used as food preservatives for the food industry [4]. Also, natural antioxidants like polyphenols are currently more widely employed because of their physiological benefits in human health [5].

In line with this, plants were the most valuable antioxidants used to protect smoked fillets especially the green algal genus Dunaliella. Among them, *Dunaliella salina* (*D. salina*) is known as an important natural antioxidant for fillets protection because of their high physiological characters. Additionally, *D. salina* is a commercial source of natural polyphenols and β-carotene that operates as a scavenger compound to protect fillets against free radicals generation [6].

Despite the wide investigations on smoking processes and its possible effects on fish fillets characters the use of *Dunaliella salina* as natural antioxidant have not been well investigated. For this reason, this study aimed to examine the combined effects of two smoking processes (cold and hot) and two graded concentrations (+ 0.5 and + 1pp) of *D. salina* polyphenols extract as natural antioxidants on the biochemical qualities of zander fillets (*Sander lucioperca*). Our study was maintained during long-term periods when zander fillets were stored at 4°C during 1, 20 and 90 days. In this study we analysed the proximate composition (proteins, lipids, total basic volatile nitrogen) and fatty acid profiles of fresh and smoked fillets. To prove the protective role of *D. salina*, we examined the peroxide value (PV), free fatty acids (FFA) and thiobarbituric acid (TBARS) as lipid peroxidation indices.

Materiels And Methods

Polyphenols extract

*Dunaliella salina*is an halophile green micro-algae especially found in sea salt fields, known for its antioxidant activity because of its ability to create large amount of carotenoids. Microalgea samples were caught in May 2019 from a continental salt lake: Chott El Jerid located in the south of Tunisia (33°54'42.21"N and 8°31'7.98"E).

The antioxidant solution was prepared according to the method described by [7] briefly; 10 g of dried and powdered microalgea was extracted with 100 ml of distilled water for 24 h. The sample was then filtered and lyophilized. The final solution of *Dunaliella salina* was prepared by dissolving 10 g of lyophilized extract in 1,000 ml of distilled water, having a polyphenols content equal to 500 mg Gallic acid equivalents (GAE)/L [7].

Smoking procedure

S. lucioperca samples were caught in May 2019 during 10 fishing operations from the dam of Sidi El Barrak (Governorate of Beja, Northwest Tunisia, 37°01’N, 09°39’E). Sampling surveys were conducted during the daytime with the fishermen operating in the dams. A trammel net with a mesh size of 30 mm was used for fish samplings with a total of 45 individual *S. lucioperca* of various sizes. The fish samples were preserved in ice and transferred to the laboratory where they were weighed, measured de-headed and cleaned. The average weight and length of sampled fish were 3022±16.00 g and 52.00±4.22 cm, respectively. The fillets were separated into three groups:(i) fresh fillets without any additive; (ii) on which polyphenols extracted from *Dunaliella salina* were spread on each side of each fillet using a micropipette to achieve a final concentration 0.5% PP (v/w); and lot (3) as for (2) but with a final concentration of 1% PP (v/w). All three lots of fillets were then cold and hot smoked. Cold smoked fillets are dried for 2 hours in the smoking chamber with temperature fluctuating from 30 to

35°C, and the hot smoked fillets are introduced in industrial smoking chamber with a peripheral smoke generator. This process of thermal treatment of fillets was conducted according to the following sequence: pre-drying of fish surface at 50–60 °C for 150 min, then hot smoking at 65–70 °C for 30 min and finally, steaming to obtain a temperature of 68–72°C. Oak wood was used for both smoking processes [1]. Finally, the fillets were chilled by cold air at 10°C. Smoked fish fillets were vacuum-packed and stored at 0-4°C for 90 days. For biochemical and sensorial analysis, flesh samplings were made on the following 1, 20 and 90 days of refrigerated storage of the smoked vacuum-packed fillets. All muscle samples were stored at -80°C until analysis.

Biochemical analysis

Protein determination: The total protein content in *S. lucioperca* fillets was determined according to Kjeldahl method. Our results were expressed as a percentage of the wet weight (n = 3).

Total volatile basic nitrogen (TVBN) amounts. The total volatile base (TVBN) was determined by flow injection analysis according to method by [8].

Determination of lipids. The lipids were determined according to the Folch protocol. Ten grams of fresh or smoked fillet sample (n=6 for each fillet) were homogenized with a chloroform/methanol (1:2) mixture for 8–10 min at 4 °C 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 min and 5 min, respectively. Then, 20 ml of distilled water were added and homogenized again for 1 mn. The obtained mixture was incubated in an ultrasound bath for 10 min, and vacuum cleaner with Buchner funnel and chloroform. The organic fraction was extracted with a separating funnel, dried with sodium sulphate and evaporated to dryness in the rotary evaporator (Stuart™, UK). When evaluating, the obtained oil was solubilised in a known volume of chloroform with BHT (50 ppm) and stored at -20 °C (n = 6 for each sample).

Determination of fatty acids. The analysis of fatty acid methyl esters (FAMEs) was performed in a Varian Agilent 6890N gas chromatograph, equipped with an auto-sampler and fitted with a split/splitless injector and a flame ionization detector (FID). The separation was carried out in an Innowax 30x0.25 capillary column (25 m × 0.25 mm i.d., film thickness). The temperature was programmed from 180 to 200 °C at 4 °C/min, held for 10 min at 200 °C and heated to 210 °C at 4 °C/min, held at 210 °C for 14.5 min with injector and FID at 250 °C. The fatty acid compositions of the polar and neutral fractions were expressed as the percentage of total fatty acids (n = 6). The fatty acid contents in the total lipids of the fillets were calculated by using C21:0 as the internal standard (10 mg/ml) based on the peak area ratio (n = 6).

Free fatty acids (FFA). 50 mg of each sample were homogenized with cyclohexane and cupric acetate-pyridine reagent and stirred by a vortex for 2 min and then centrifuged for 20 min at 9,000 rpm. The activity was detected at 710 nm. The analysis was developed with oleic acid, which used as a standard (n = 3).

Peroxide value (PV). The peroxide value of the fillet samples was determined by the ferric thiocyanate method with slight modifications based on the ability of lipid peroxides to oxidize ferrous ions at a low pH. The resulting ferric ions reacted with 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 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 in mequivalent of oxygen per kg of oil (meq O2/kg) (n = 3).

Thiobarbituric acid reactive substances (TBARS). The TBARS were assessed based on the AOAC method. An oil sample was solubilised in 1-butanol, mixed with 0.2% TBA in 1-butanol, incubated in a water bath for 2 hours at 95 °C and then cooled under tap water. The absorbance was determined using a spectrophotometer at 532 nm. 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 as mg malonedialdehyde /kg of oil (n = 3).

The antioxidant properties (DPPH). In order to measure the antiradical activity, we used the synthetic radical DPPH (1,1-diphenyl-2-picrylhydrazyl) following the method of Bersuder [9]. Briefly, 10 mg of the sample was suspended in 0.5 ml of distilled water. After that, 1.2 ml of absolute ethanol and 0.2 ml of 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 the T70 UV–visible spectrophotometer. The results were expressed as % of inhibition or % of scavenging activity and calculated using the following formula:

DPPH scavenging activity = ((OD control – OD sample) / OD control) * 100

Statistical analysis. The results are presented as means ± standard deviation (SD). The statistical analysis was determined using SPSS software version. The results were checked for normality and homogeneity of variance by Kolmogorov–Smirnov and Levene's tests, respectively. A one-way analysis (ANOVA) was performed to establish the significant difference between the fresh and smoked fillets and was set at $p < 0.05$.

Results And Discussion

Proximate composition in fresh and smoked S. lucioperca **fillets**

The amounts of proximate composition in fresh and smoked *S. lucioperca* fillets are reported in Fig. 1.

The amounts of proteins in fresh and smoked S. lucioperca fillets. Our data showed that proteins amounts remained stable in fresh fillets during the storage period (Fig. 2.A). Similar trends were observed for cold smoked fillets with and without *D. salina* (+ 0.5pp and + 1pp) that showing related amounts of proteins. Concerning hot smoking fillets, the amounts of proteins showed significant decreases *S. lucioperca* fillets especially after 20 and 90 days of storages ($p < 0.01$). Among the studied period, our results demonstrated significant increases of proteins amounts in all smoked fillets processes as compared to the fresh ones ($p < 0.001$).

The amounts of lipids in fresh and smoked S. lucioperca fillets. The amounts of lipids in fresh and smoked *S. lucioperca* fillets showed similar variations during the storage days (Fig. 2.B). Our results revealed significant decreases of lipids in fresh and smoked fillets after 20 and 90 days of storages ($p < 0.01$). On the other hand, significant increases of the amounts of lipids were recorded in cold and hot smoked fillets containing *D. salina* as natural antioxidant when compared to the fresh fillets ($p < 0.001$).

The amounts of total volatile basic nitrogen (TVBN) in fresh and smoked S. lucioperca fillets. The total volatile basic nitrogen in smoked *S. lucioperca* fillets changed significantly than the fresh ones (Fig. 2.C). Our results demonstrated that the TVBN increases progressively and significantly in fresh and smoked fillets after 20 days ($p < 0.05$) and remaining highly after 90 days of storage ($p < 0.001$). When comparing fresh with cold smoked fillets, our data showed that this process significantly decreased the amounts of TVBN especially when *S. lucioperca* fillets were mixed with + 1pp of *D. salina* ($p < 0.01$). Concerning the hot smoking process, similar variations were reported between the fresh and the hot smoked fillets while significant decreases were noted for TVBN amounts in *S. lucioperca* fillets mixed with + 0.5pp and + 1pp of *D. salina* ($p < 0.001$).

Fatty acid composition in fresh and smoked S. lucioperca **fillets**

According to Table 1, fatty acid composition of fresh fillets showed significant differences during the storage days as evidence by important increases of SFA, MUFA and decreases of PUFA after 20 and 90 days ($p < 0.05$). Data presented in Table 1 clearly revealed that cold smoking process changed significantly the fatty acids composition of *S. lucioperca* during the storage days. These changes were noticed by significant increases of SFA, MUFA and PUFA mainly at the first day of storage ($p < 0.05$), confirming by significant enhancement of their main groups such as C16:0, C14:0, C16:1n-7, C18:1n-7, PUFA n-6, *eicosapentaenoic* acid (EPA), arachidonic acid (ARA) and C18:2n-6 ($p < 0.05$). When zander fillets were mixed with *D. salina* at + 0.5pp, the amounts of SFA, MUFA increased significantly after 1 and 20 days while PUFA showed significant decrease only after 90 days of storage ($p < 0.05$). These depletions were appeared principally PUFA n-3, PUFA n-6, EPA, DHA and ARA acids ($p < 0.001$). Different trends were reported for zander fillets mixed with + 1pp of *D. salina*(Table 1). Our results showed significant increase of SFA after 1 day and PUFA after 20 and 90 days of storage ($p < 0.05$). However, MUFA and their principal compound C18:1n-7 decreases significantly with 1.5-fold after long term storage (20 and 90 days).

Table 1
Fatty acid composition of fresh (FF) and cold smoked (CSF) *Sander lucioperca* with *Dunaliella salina* as natural antioxidant at different

FA	FF			CSF			CSF + 0.5pp		
	1 day	20 days	90 days	1 day	20 days	90 days	1 day	20 days	90 days
C14 :0	4.65±0.19^{a}	5.09±1.12^{a}	6.14±1.78^{b}	5.72±0.25^{a}	7.72±1.12^{b*}	8.32±0.67^{c**}	5.89±0.25^{a}	6.41±1.52^{b}	7.92±1.55
C16 :0	16.4 ±0.89^{a}	20.46±1.52^{b}	22.19±2.5^{c}	17.65 ±2.14^{a}	18.23±1.95^{b*}	21.29±1.48^{c}	16.5 ±0.87^{a}	17.57±2.4^{b**}	20.76±2.4
C18 :0	2.76 ±0.66^{a}	4.01±1.73^{b}	7.15±3.45^{c}	2.92 ±0.78^{a}	4.57±0.73^{c}	7.89±0.47^{c}	3.19±0.78^{a}	3.73±0.47^{b}	6.37±1.51
Total SFA	**26.44±2.03^{a}**	**33.97±3.12^{c}**	**40.49±2.30^{c}**	**29.00±3.41^{a**}**	**34.42±3.27^{b}**	**44.19±3.06^{c***}**	**29.2±2.10^{a**}**	**30.98±2.92^{a}**	**40.67±2.9**
C16 :1n-7	6.75 ±1.51^{a}	8.80±1.55^{a}	9.984±2.47^{c}	7.82 ±1.51^{a*}	9.41±1.59^{b*}	12.06±3.02^{c**}	6.40±0.46^{a}	7.37±1.42^{b}	10.12±1.2
C18 :1n-9	12.23±2.40^{a}	15.40±2.05^{a}	17.95±3.22^{b}	12.85±2.74^{a}	13.78±2.02^{b*}	16.08±0.87^{c*}	13.53±1.13^{a}	14.31±3.05^{b}	17.31±2.8
C18 :1n-7	2.90±0.50^{a}	4.25±0.58^{b}	5.12±2.72^{c}	3.51±0.78^{a*}	4.12±0.68^{b}	6.43 ±0.18^{c*}	3.81±0.27^{a*}	4.21±0.72^{b}	6.87±1.36
Total MUFA	**24.77±2.05^{a}**	**33.23±2.56^{b}**	**40.47±3.67^{c}**	**27.54±2.31^{a**}**	**31.32±3.73^{b*}**	**39.69±2.73^{c}**	**26.72±1.58^{a*}**	**29.70±3.41^{b*}**	**40.03±3.2**
C18 :2n-6	4.68±0.97^{a}	5.40±1.96^{b}	7.32±1.51^{b}	5.69±0.82^{a*}	3.78±1.25^{b***}	3.02±1.78^{b***}	6.32±2.48^{a**}	4.92±1.63^{b}	3.96±0.69
C20 :4n-6 (ARA)	11.24±2.35^{a}	12.23±2.65^{b}	13.05±2.29^{c}	12.44±7.78^{a}	11.22±2.42^{b*}	11.23±1.65^{a*}	9.98±1.75^{a*}	7.78±1.57^{b**}	9.52±1.38
C20 :5n-3 (EPA)	7.93 ±1.46^{a}	3.49±0.51^{b}	1.22±0.54^{c}	8.57 ±1.33^{a*}	4.98±1.94^{b*}	1.74±2.31^{a}	5.67±0.36^{a**}	3.98±1.72^{b}	3.21±0.42
C22 :5n-3	1.78±0.23^{a}	1.27±0.72^{a}	0.78±0.06^{b}	2.42±0.27^{a**}	1.63±0.12^{a}	1.02±0,05^{a}	1.31±0.68^{a}	0.97±0.09^{b}	0.98±0.08
C22 :6n-3 (DHA)	13.68±2.78^{a}	8.78±2.86^{b}	1.67±0.57^{c}	12.63±2.56^{a*}	8.46±1.41^{b}	6.98±2.97^{c***}	14.85±2.35^{a*}	10.37±1.52^{b**}	7.21±0.33
PUFA n-3	23.39±1.15^{a}	13.54±1.09^{c}	3.67±0.08^{c}	23.62±3.06^{a}	15.07±0.76^{c**}	9.74±0.63^{c***}	21.83±2.58^{a*}	15.32±2.13^{c**}	11.40±1.3
PUFA n-6	15.92±1.22^{a}	17.63±1.11^{b}	20.37±1.98^{c}	18.12±0.97^{a**}	15.00±1.35^{a*}	14.25±1.53^{b***}	16.30±1.00^{a}	12.70±1.18^{b**}	13.48±1.2
Total PUFA	**42.33±1.32^{a}**	**32.69±2.78^{b}**	**24.09±2.09^{c}**	**45.77±3.02^{a*}**	**33.32±2.78^{b}**	**25.34±3.12^{c}**	**37.86±3.12^{a***}**	**31.99±3.12^{b}**	**26.04±3.0**

Results are illustrated as mean ± SD

Difference between FF and CSF conditions are presented at 5% (*p < 0.05, **p < 0.01 and ***p < 0.001).

Difference between the storage days are presented by a < 0.05, b < 0.01 and c < 0.001.

Fatty acid composition in fresh and hot smoked S. lucioperca **fillets**

Comparing fatty acid composition of hot smoked and fresh *S. lucioperca* was given in Table 2. Results showed that in hot smoked fillets, PUFA were by far the major FA class (between22-46% of total FA) followed by SFA and MUFA that ranged from 28 to 44% and from 27 to 41% respectively. Comparing the fresh and hot smoked fillets, our data revealed significant increases of SFA, MUFA and PUFA mainly during the first day of storage ($p < 0.01$). Similarly, PUFA n-3 was the major PUFA group and mainly represented by EPA and DHA that showing significant increases by ≥ 1.5-fold up when compared with the fresh fillets. Additionally, PUFA n-6 showed a significant enhancement in hot smoked fillets after 1 day of storage by 25% as compared to the long term storage showing statistical decreases of PUFA n-6 mainly ARA ($p < 0.01$). Overall, there was a tendency for significant decreases of SFA and MUFA against the increases of PUFA for mixed zander with + 0.5pp and + 1pp of *D. salina* in comparison to fresh one ($p < 0.05$).Significant differences were observed for some particular PUFA n-3 as EPA, and DHA acids, showing a statistical increase in zander fillets mixed with + 0.5pp and + 1pp after 20 and 90days of storage when comparing to the fresh ones ($p < 0.01$). Furthermore, C18:2n-6 the major PUFA n-6 acid tended to increase during the two first storage period ($p < 0.01$) and decreased significantly after long term storage period by 69% and 21% for fillets mixed with + 0.5pp and + 1pp of *D. salina* respectively when compared to the fresh one. Concerning the ARA, its amount remained statistically invariable during one and twenty days of storage compared to the fresh fillets, however, significant reductions were observed after 90 days in both storage fillets with graded *D. salina*.

Lipid peroxidation indices in fresh and smoked S. lucioperca **fillets**

Lipid peroxidation indices comprising PV, TBARS and FFA measured in fresh and smoked *S.lucioperca* fillets are presented in Fig. 2 (A.B and C). According to our results, significant changes were noted among the studied period and in function of smoking conditions. When comparing fresh fillets among the studied storage period, we observed that PV, TBARS and FFA increased significantly after 20 ($p < 0.01$) and 90 ($p < 0.001$) days. Similar variations were reported for cold smoking ad hot smoking fillets as evidence by statistical augmentation of these peroxidation indices mainly after 90 days of storage when comparing to the fresh fillets ($p < 0.001$).

Additionally, differences between fresh fillets and smoking were recorded showing that these two processes decreased significantly the levels of lipid peroxidation indices ($p < 0.05$). Also, the addition of + 0.5pp and + 1pp of *D. salina* in the smoked fillets have significant effect after 20 and 90days of storage showing decreases of PV, TBARS and FFA levels as compared to the fresh fillets ($p < 0.01$).

Table 2
Fatty acid composition of fresh (FF) and hold smoked (HSF) *Sander lucioperca* with *Dunaliella salina* as natural antioxidant at differen

FA	FF			HSF			HSF + 0.5pp		
	1 day	20 days	90 days	1 day	20 days	90 days	1 day	20 days	90 days
C14 :0	4.65±0.19^{a}	5.09±1.12^{a}	6.14±1.78^{b}	5.28±0.63^{a*}	6.92±1.51^{b*}	9.01±1.44^{c***}	4.69±0.51^{a}	5.08±1.03^{b}	6.27±1.0
C16 :0	16.40 ±0.89^{a}	20.46±1.52^{b}	22.19±2.5^{c}	16.98±3.08^{a}	17.86±2.44^{b**}	22.18±1.55^{c}	15.21±2.45^{a*}	16.12±2.76^{b***}	20.36±1.
C18 :0	2.76 ±0.66^{a}	4.01±1.73^{b}	7.15±3.45^{c}	3.26±0.92^{a}	4.13±0.87^{b}	7.41±0.51^{c}	3.12±0.84^{a}	4.01±0.56^{a}	6.81±0.3
Total SFA	**26.44±2.03^{a}**	**33.97±3.12^{c}**	**40.49±2.30^{c}**	**28.66±3.24^{a**}**	**32.90±3.15^{b}**	**44.61±4.05^{c***}**	**26.05±2.54^{a}**	**28.97±2.69^{b**}**	**38.66±3.**
C16 :1n-7	6.75 ±1.51^{a}	8.80±1.55^{a}	9.984±2.47^{c}	8.43±1.26^{a**}	8.78±1.21^{a}	13.76±2.58^{c**}	7.21±0.96^{a}	7.92±1.26^{a}	11.32±2.
C18 :1n-9	12.23±2.40^{a}	15.40±2.05^{a}	17.95±3.22^{b}	11.46±2.52^{a}	12.02±2.15^{b}	17.45±2.41^{c}	10.23±1.78^{a}	11.12±2.27^{b}	14.36±2.
C18 :1n-7	2.90±0.50^{a}	4.25±0.58^{b}	5.12±2.72^{c}	4.36±0.89^{a***}	4.99±0.38^{b}	7.16±0.23^{c**}	3.78±0.27^{a*}	4.97±0.12^{b}	6.46 ±0.4
Total MUFA	**24.77±2.05^{a}**	**33.23±2.56^{b}**	**40.47±3.67^{c}**	**27.49±2.72^{a**}**	**29.73±4.21^{b}**	**41.75±2.86^{c}**	**24.34±2.15^{a}**	**27.59±2.76^{b**}**	**36.26±3.**
C18 :2n-6	4.68±0.97^{a}	5.40±1.96^{b}	7.32±1.51^{b}	6.21±0.74^{a***}	4.21±1.13^{a}	2.27±0.14^{b***}	7.04±0.66^{a***}	6.54±0.76^{a*}	4.31±0.2
C20 :4n-6 (ARA)	11.24±2.35^{a}	12.23±2.65^{b}	13.05±2.29^{c}	13.25±2.31^{a**}	12.08±1.31^{b}	8.97±1.13^{a***}	11.78±2.58^{a}	11.12±1.26^{a}	7.33±1.0
C20 :5n-3 (EPA)	7.93 ±1.46^{a}	3.49±0.51^{b}	1.22±0.54^{c}	7.12±1.15^{a}	5.96±1.26^{b**}	1.21±0.27^{c}	7.68±1.08^{a}	6.96±1.37^{b***}	4.95±0.8
C22 :5n-3	1.78±0.23^{a}	1.27±0.72^{a}	0.78±0.06^{b}	2.51±0.14^{a**}	2.04±0.17^{a*}	1.02±0.01^{b}	3.07±0.27^{a**}	2.47±0.24^{a**}	1.64±0.0
C22 :6n-3 (DHA)	13.68±2.78^{a}	8.78±2.86^{b}	1.67±0.57^{c}	12.41±2.22^{a}	11.09±1.28^{b***}	6.14±2.37^{c***}	13.61±2.52^{a}	12.79±1.09^{b***}	9.26±1.9
PUFA n-3	23.39±1.15^{a}	13.54±1.09^{c}	3.67±0.08^{c}	22.06±1.14^{a}	19.11±1.67^{b***}	8.37±0.78^{c***}	24.37±2.08^{a*}	22.23±1.53^{b***}	15.86±1.
PUFA n-6	15.92±1.22^{a}	17.63±1.11^{b}	20.37±1.98^{c}	19.46±1.42^{a**}	16.31±1.27^{b*}	11.25±0.99^{c***}	18.83±1.66^{a**}	17.67±2.04^{a}	11.64±1.
Total PUFA	**42.33±1.32^{a}**	**32.69±2.78^{b}**	**24.09±2.09^{c}**	**46.71±4.31^{a**}**	**40.05±3.51^{b**}**	**22.29±2.05^{c}**	**49.29±4.57^{a***}**	**45.80±2.48^{b***}**	**32.19±2.**

Results are illustrated as mean ± SD

Difference between FF and HSF conditions are presented at 5% (*p < 0.05, **p < 0.01 and ***p < 0.001).

Difference between the storage days are presented by a < 0.05, b < 0.01 and c < 0.001.

Free radical (DPPH) activities in fresh and smoked S. lucioperca **fillets**

DPPH activities in fresh and smoked *S. lucioperca* fillers were reported in Fig. 3. Our results showed inhibition of the DPPH activities during 20 and 90 days when compared to the first day of storage in fresh and all smoked fillets ($p < 0.001$). The important inhibition was recorded after 90 days of storage mainly in cold smoked and hot smoked fillets ($p < 0.001$). Thus, the results show a positive correlation between the percentage of addition and the antioxidant activity, which proves the effect of polyphenyol on the antioxidant activity.

Because of the large number of nutrients, including protein, long-chain omega-3 polyunsaturated fatty acids (n-3 PUFAs), vitamins and minerals, fishes are widely considered as a healthy and balanced diet for dietary uptake. According to the World Health Organization guidelines, it is recommended that fish is consumed two to three times a week in order to prevent life style diseases [10].

Nevertheless, fish is highly perishable since it is extremely vulnerable to oxidation resulting in the development of off-odor and -flavor and subsequent spoilage development [11]. Thus, it is of need to develop or improve preservation and processing techniques to keep a high quality of aquatic products when marketing. The smoking process and the use of *D. salina* extract can be solution to extend shelf life of fishes.

The increases in proteins and lipids after both cold and hot smoking could be explained by the water loss associated to the evaporation during this mechanism. These data are in line with those of [12]. On the other hand, our experiment showed that proteins amounts remained stable in fresh and in cold smoked fillets during the storage period. Therefore protein in hot smoked fillets decreased after 20 and 90 days of storages. This last finding is mainly due to

the autolytic enzymes activities causing spoilage [13]. This decrease in protein, associated with the storage time, could be also explained by the microbial spoilage [14].

Our results revealed decreases of lipids in fresh and smoked fillets after 20 and 90 days of storages. This finding could be attributed to hydrolysis of some lipid fractions [15].

In addition *Dunaliella salina* increases of the amounts of lipids in cold and hot smoked fillets.

D. salina is a rich source of polyphenols, which have very interesting antioxidant properties. This secondary metabolite could stop lipid degradation of this kind of fish fillets, by eliminating free radicals [16].

Also we evaluated the TVBN which is considered as an important parameter allowing us to evaluate the quality of fish product. Our results demonstrated that the TVBN increases progressively and significantly in fresh and smoked fillets after 20 days and remaining highly after 90 days of storage. Our study is in agreement with the work of [17] who finds that TVBN increased gradually during the period of storage of smoke fish. This may be due to continuous degradation of protein to total volatile base nitrogen. Smoking decreased the amounts of TVBN. In this respect, we must keep in mind that production of dimethylamine, trimethylamine and ammonia is associated to destructive effect of microorganisms and is considered as TVBN [18]. Due to the effect of smoking and D. salina on microorganisms, this combined process lead to decreasing TVBN.

We observed that PV, TBARS and FFA increased after 20 and 90 days of storage. Both cold and hot smoking processes decreased these three parameters. Alternatively, polyphenols of D. salina which is an interesting antioxidant which decreases PV, TBARS and FFA content in smoked (cold and hot) fish fillet. It delays oxidation of lipid due to its polyphenols content. These data are in line with those of [19] which find that *Dunaliella salina* inhibit lipid oxidation since the first month of storage.

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) activity was widely used as an easy way for evaluating antioxidant properties. 90 days of storage in cold smoked group had the highest DPPH radical scavenging activity. The most previous research works deals the smoking treatments as an efficient process for preserving; however, at our knowledge none had studied its antioxidant activity. In this context, smoked fish was noticed to be resistant to oxidative rancidity. Smoking fish generate phenols which has a high antioxidant activity. Difference in phenols content between traditional and non-traditional methods, were not significant. Therefore temperature affected phenols level. So cold smoked fish content more antioxidants and this explained the improvement in DPPH. Whereas, at concentrations below 1 pp of *Dunaliella salina* polyphenols extract, no improvement was detected (compared to 0,5pp and to control groups) in DPPH activity.

The effect of adding microalgae was shown by [20] when he added 3 species of microalgae in the ingredients of fish burger and showed a positive correlation between the quantity of added microalgae and the activity antioxidant and has also shown that this activity depends on the species of microalgae which have different contents and physico-chemical properties of pigments. The high concentrations of oxygen and light, can cause structural damage to living organisms through the oxidative effect, but this is not the case for microalgae because they are able to protect themselves against this situation by the production of enzymatic antioxidants and not enzymatic. Indeed, microalgae are rich in natural pigments capable of improve the efficiency of the light energy used by microalgae and protect them against solar radiation and related effects.

Overall, fresh *S. lucioperca* represented a fatty acid profile dominated by the PUFA similar to that of other freshwater species namely *Lates niloticus*, *Mormyrops deliciosus* and *Hydrocynus forkahlii*. The major identified SFA and MUFA were respectively palmitic acid (C16:0) and oleic acid (C18:1n-9) which is in accordance with other studies on aquatic species [2]. In addition, freshwater fish meat is known to contain high levels of MUFA especially oleic acid, which plays significant role in human health [21].

The SFA contents increased significantly after the smoking process for all the tested storage periods and this was closely related to the increases of C14:0, C16:0 and C18:0, which were higher than those of fresh zander fish [1]. The same for most MUFA and PUFA, which was mainly due to water loss by evaporation during both hot and cold smoking [1].

However, the significant decreases in MUFA and PUFA contents can be explained by their oxidation during the smoking process and the storage.

Essential fatty acids namely EPA and DHA are necessary for human well-being, in fact, the American Heart Association recommends taking 500 mg of EPA and DHA per day. Omega-3 acids prevent heart disease and have anti-atherosclerotic effect. They have been proven to reduce mortality of patients with coronary artery disease [22].

In this study, results revealed that (EPA + DHA) contents in both cold and hot smoked zander fish can meet the daily requirements.

Despite their benefits, hot smoked processed products can lead to excessive shrinkage which may decrease the yield and the nutritive value of fish [23]. So, the optimization of time and temperature in smoking process technology is extremely important to ensure a good quality product during storage. For this, natural extracts can be used to reduce the smoking scale time/temperature.

In this study, we hypothesized that the combination of smoking treatment and *D. salina* extract incorporation on fish fillets could provide a more nutritive final product.

In fact, results showed that it reduced significantly both SFA and MUFA contents and increased polyunsaturated fatty acids and mainly omega-3 and omega-6 ($p < 0.05$).

Since antioxidants play an important role in food technology due to their usefulness against lipid oxidation, the interest in finding natural sources of antioxidants is increasing and the search for new natural sources is also becoming more important. Nowadays, there is a huge interest in the potential use of marine natural sources to obtain bioactive substances and D. *salina* extracts seems to be an effective natural antioxidant.

This microalgae is known to have antioxidant effect since it contains high amounts of polyphenols and β-carotene [24]. Some studies compared the free radical scavenging effect of algal extracts of *D. salina* with some commercially carotenoids and showed that the algal extract showed higher activity than industrial carotenoids [25].

Thus, we can conclude that due to its beneficial fatty acid composition and antioxidant properties, *D. salina* can be a safe food additive and can be used in food industry due to its functional activities. It can be also used as a new source of essential fatty acids.

The comparison of hot and cold smoking process of fish fillet shows that the hot process is more appropriate because of the higher essential fatty acids level. It should also be noted that the hot smoking process is faster than the cold process.

Conclusion

Zander fish seems to be suitable for hot smoking technology especially with the incorporation of *D. salina* extract. Thus, this product could be used to provide a new and alternative opportunity for the consumption of this freshwater species. However, sensory analysis is needed to evaluate consumer acceptance of this new food product.

The obtained results could contribute to the further improvement of this new food product as well as the development of similar products from non exploited fish species, which can increase the current offer in the market.

Declarations

Ethical Approval and Consent to participate

Compliance with Ethical Standards

Human and Animal Ethics

Not applicable

Consent for publication

I hereby consent to Thalassas: An International Journal of Marine Sciences publishing images or other clinical and/or family history information pertaining to me (or my minor child named below)). I declare that I have read the material to be published.

Availability of supporting data

Yes

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

N.B. and S.M. and A.B.A. carried out the practical part D.T., W.R.B and S.B. carried out the statistical analyses, they prepared the figures and the tables and the writing of text. J.-P. Q and M. T. participated in writing, design and coordination to draft the manuscript. All authors read and revised the manuscript.

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Figures

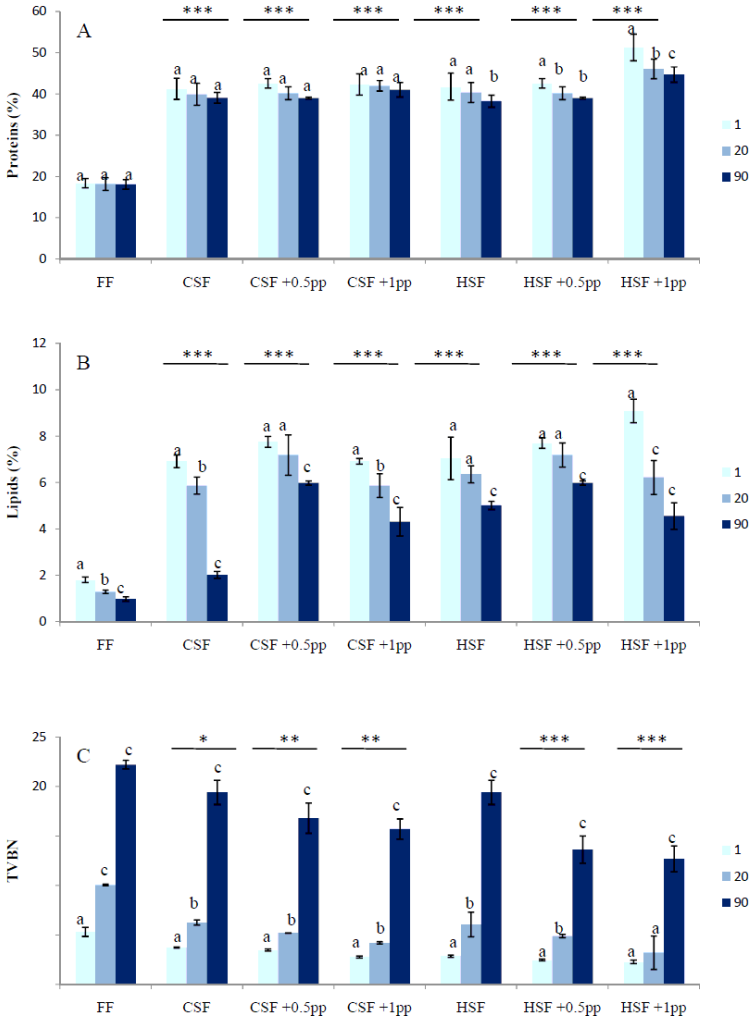

Figure 1

Amounts of proximate composition in fresh and smoked *S. lucioperca* fillets

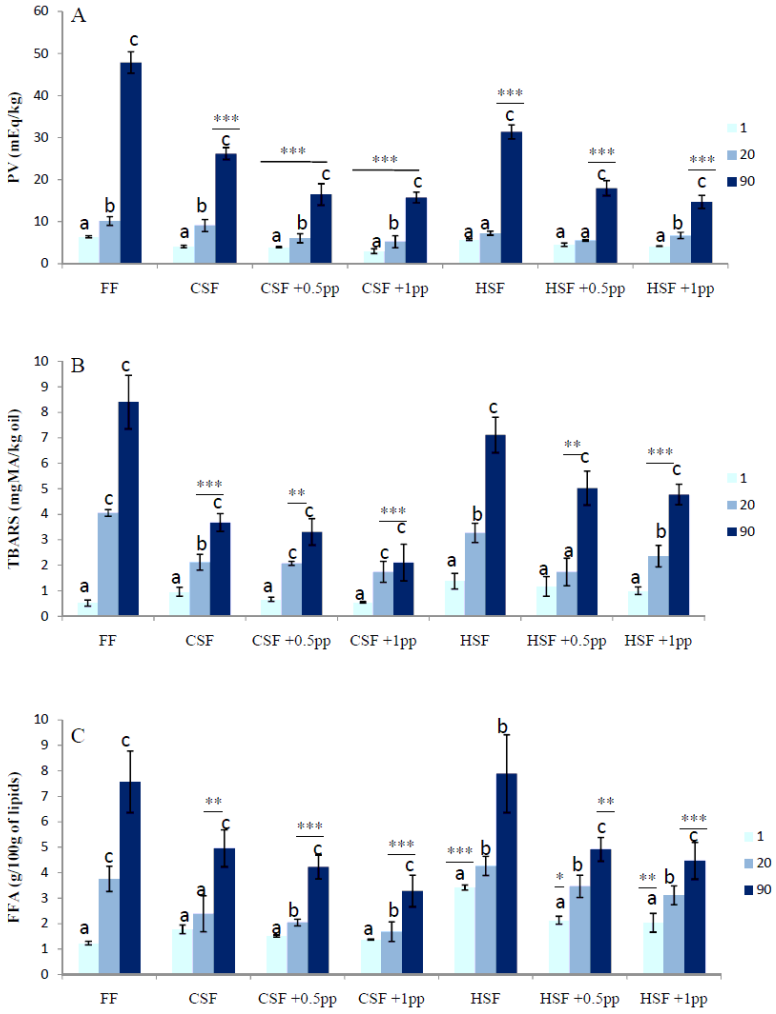

Figure 2

Amounts of proteins, lipids and total volatile basic nitrogen in fresh and smoked S. lucioperca fillets

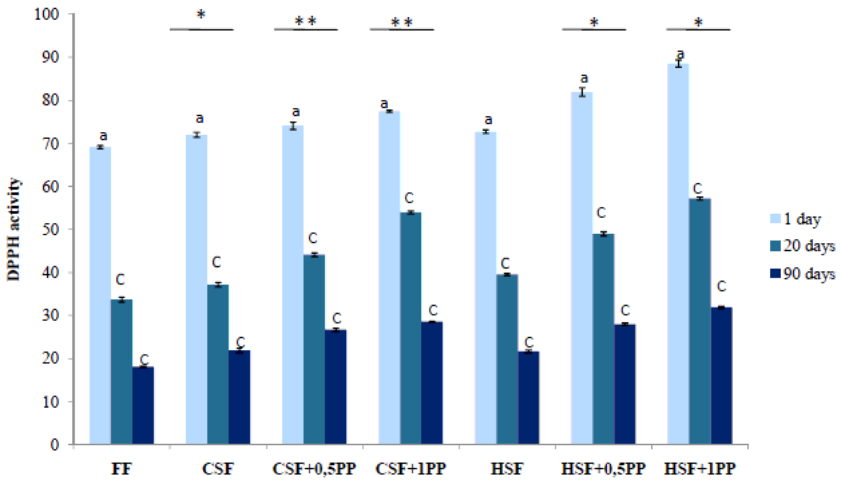

Figure 3

DPPH activities in fresh and smoked S. lucioperca fillets