International Journal of Food Science +Technology

Original article

Smoking and polyphenols' addition to improve freshwater mullet (*Mugil cephalus*) fillets' quality attributes during refrigerated storage

Olfa Bouzgarrou,[1] Nadia El Mzougui[2] & Saloua Sadok[1]*

1 Laboratore de Biodiversité et Biotechnologie Marines, Institut National des Sciences et Technologies de la Mer (INSTM), Carthage, Tunisia
2 Laboratoire du Milieu Marin, INSTM, Carthage, Tunisia

(Received 1 May 2015; Accepted in revised form 11 August 2015)

Summary The combined effects of hot smoking and added quince polyphenol (0.8% and 1.6% v/w) on the quality attributes of mullet (*Mugil cephalus*) fillets were evaluated. Biochemical parameters that were monitored in the fillets during 75 days of storage (4 ± 1 °C) included trimethylamine (TMA-N), volatile basic nitrogen (TVB-N), fatty acids and amino acids. Data were submitted to principal component analysis (PCA) which revealed that the smoking process combined with polyphenols' treatment was negatively correlated with TVB-N, TMA-N, lipids, indices of lipid quality (index of atherogenicity (IA); index of thrombogenicity (IT)) and storage time. Health concern related to the flesh content of polycyclic aromatic hydrocarbons (PAHs) following hot smoking was also evaluated, and analysis showed that the sum of 4 of the PAHs (chrysene, benzo(b)fluoranthene, benz(a)anthracene and BaP) remained far below the maximum limit set by the European Commission. Sensory analysis revealed that the smoked mullet fillet treated with a low dose of polyphenols was a very acceptable new product.

Keywords Biochemical parameters, polycyclic aromatic hydrocarbons, polyphenols, principal components analysis, smoked fish.

Introduction

Mullet (*Mugil cephalus*) is one of the most widely distributed seafood fish species in the world. Commonly, it is found in association with shallow weed beds and bare substrates in coastal waters and estuaries. In Tunisia, mullet has been introduced into freshwater reservoirs and currently is present in most inland dammed lakes (Zaouali, 1981) with the highest annual landings (34.12%) of the freshwater fish total landings (~350 tonnes p.a.) (DGPA, 2012). Freshwater mullet is not appreciated well by Tunisian consumers because of its undesirable taste and flavour, and thus, it has a limited national consumption. The mullet biomass, however, has a potential revenue generation as it can be turned into an attractive value-added fish product and smoking technology can offer such a marketing option for this species.

Traditionally used as a preserving technology, smoking nowadays is used for its sensory characteristics and has become a means of yielding a diversity of high value-added products (Gómez-Guillén *et al*., 2009; Sikorski & Sinkiewicz, 2014). The preserving effect of smoking results from the consecutive or simultaneous action of several factors including thermal inactivation of the spoilage microflora, water activity, pH and the concentration of antimicrobial and antioxidant smoke components in the product (Miler & Sikorski, 1990; Doe, 1998). In all cases, the smoked products are not sterile and may be subjected to alteration. Recently, the intensive use of natural bioactive compounds, extracted from plants, is being used in combination with processing technology to improve the preservation of seafood products (Attouchi & Sadok, 2012; Coutinho de Oliveiraa *et al*., 2015; Li *et al*., 2015). However, studies related to the use of natural antimicrobials/antioxidants as additional hurdles to preservation of seafood by smoking are still rare (Erkan, 2012; Gómez-Estaca *et al*., 2014).

In this context, the objective of this work was to develop a smoked product with adequate sensory properties, and nutritional and biochemical quality over the storage period. As an innovation, natural polyphenol extract (from quince fruit *Cydonia oblonga Miller*) was added to fish fillets to improve the preservation of the freshwater fish product. Such combined effects on mullet fillets were assessed by the determination of chemical, biochemical and sensorial indices in the fresh and smoked fillets. The smoking procedure, however,

*Correspondent: Fax: +216-71735848;
e-mail: salwa.sadok@instm.rnrt.tn

doi:10.1111/ijfs.12955

can lead to the production of aromatic polycyclic hydrocarbons (PAHs) that are carcinogenic and genotoxic substances (Simko, 2002). Such issues were also considered in this study by evaluating the PAHs in the fish fillets before and after smoking.

Materials and method

Biological materials

Mullet (Mugil cephalus)
The fish used (20 kg) were obtained from the 'Wed Abid' Lake, NW Tunisia. Following their capture, they were immediately ice-stored in insulated boxes and delivered to the laboratory. The fish were subsequently gutted, washed, headed and filleted. Tissue samples for subsequent biochemical analyses were collected randomly from six different fillets.

Quince (Cydonia oblonga Miller)
Fresh quince fruits (*Cydonia oblonga Miller*) were obtained from a local market in Tunis (Tunisia) and were used for total polyphenol (PP) extraction and total phenolic content determination according to Fattouch *et al.* (2007, 2008) with a slight modification. For the quince, the pulp parts of the washed and hand-peeled fruits were homogenised and mixed with 40 mL of cold acetone/water (3:1 v/v). The mixture was sonicated for 20 min and centrifuged at 10 000 ***g*** for 15 min at room temperature. The supernatants were collected, pooled and concentrated to dryness under nitrogen flux. The residue was dissolved in sterile water. Aqueous total polyphenol extract was used as described below.

Smoking procedure

In the seafood-processing plant, the fish used for hot smoking were previously hand-filleted and then dry-salted on grids for 30 min in a temperature-controlled room (10 ± 1 °C). For salting, fish fillets were entirely covered with cooking salt according to the plant's processing procedure. When the dry salting was completed, the salt was removed by careful rinsing with tap water (15 °C). The fillets were separated into three lots: (i) control fillets without any additive; (ii) on which polyphenols extracted from quince peels were spread on each side of each fillet using a micropipette to achieve a final concentration 0.8% PP (v/w); and lot (3) as for (2) but with a final concentration of 1.6% PP (v/w). All three lots of fillets were then smoked for 2 h at 80 °C using a Raucher FV.V2 HEIZUNG smoking cabinet. After cooling to 4 °C, the smoked fillets were vacuum-packed and stored at 2 ± 1 °C for 75 days. For biochemical and sensorial analysis, flesh samplings were made on day 1 and on the following 7, 21, 42 and 75 days of refrigerated storage of the smoked vacuum-packed fillets. All muscle samples were stored at −80 °C until analysis.

Sample preparation for biochemical analyses

For the following tests, 1 g of tissue was taken from a fish fillet (n = 6 fillets from each lot) and homogenised (DI-25; IKA, Staufen, Germany) on ice in 2 mL ultrapure water for 1 min. Perchloric acid (0.250 mL – 6% solution) was added and the extract then homogenised for further 2 min. Homogenates were centrifuged at 12 000 ***g*** for 15 min, and the supernatants used for the determination of total volatile basic nitrogen (TVB-N), trimethylamine (TMA-N) and free amino acids, measured as ninhydrin-positive substances (NPS).

Determination of total volatile basic nitrogen

The total volatile base (TVB-N) was determined by flow injection analysis according to the method of Ruiz-Capillas & Horner (1999).

Determination of trimethylamine

The trimethylamine (TMA-N) was determined by flow injection analysis according to the method of Sadok *et al.* (1996).

Determination of total free amino acids

Total free amino acids were measured as ninhydrin-positive substances (NPS) according to the method of Sadok *et al.* (1995).

Lipid extraction and fatty acids composition

Lipid extraction
For total lipid extraction, 1 g of tissue (n = 6 for each group of mullet) was extracted according to the method of Folch *et al.* (1957) using chloroform:methanol (2:1). The aliquot of chloroform layer was evaporated to dryness under nitrogen, and the lipids were quantified gravimetrically.

Fatty acids analysis and indices of lipid quality

Fatty acids analysis
Fatty acid methyl esters (FAMEs) were obtained using the method described by Metcalfe *et al.* (1966). A fraction of extracted lipid was saponified with 0.5 N NaOH in methanol followed by methylation in 14% boron trifluoride in methanol (BF_3/MeOH). The methylated sample was then extracted with n-hexane. All of these reactions were performed in triplicate for each sample.

All chemicals were obtained from Sigma-Aldrich-Fluka Company Ltd. (Poole, Dorset, UK).

The resulting methylesters were analysed by gas chromatography using an Agilent Technologies Chromatograph 6890 N (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionisation detector, a splitless injector and a polar Innowax 30M silica capillary column (0.25 mm i.d * 30 m length * 0.25 μm film thickness, Agilent Technologies, J&W Scientific, Folsom, CA, USA). The temperature of the injector and detector was 220 and 275 °C, respectively. Helium was used as a carrier gas with a flow rate of 1.5 mL min^{-1}.

Peaks were identified by comparing their retention times with FAMEs standards (Supelco, Bellafonte, PA, USA). The sequences of fatty acids were ranged according to their chromatographic retention times, and the relative values were given as percentages of the total FAMEs.

Indices of lipid quality

Index of atherogenicity (IA) and index of thrombogenicity (IT) were calculated, respectively, from the data on fatty acid composition according to the method of Ulbritch & Southgate (1991):

1 Index of atherogenicity (IA): This indicates the relationship between the sum of the main saturated and unsaturated fatty acids classes. The following equation was applied:

$$\mathrm{IA} = \frac{(4 \times C14:0) + C16:0 + C18:0}{\sum \mathrm{MUFA} + \sum \mathrm{PUFA}\ n-6 + \sum \mathrm{PUFA}\ n-3}.$$

2 Index of thrombogenicity (IT): This is defined as the relationship between the pro-thrombogenetic (saturated) and the antithrombogenetic fatty acids (MUFAs, PUFAs n−6 and PUFAs n−3). The following equation was applied:

$$\mathrm{IT} = \frac{(C14:0 + C16:0 + C18:0)}{(0.50 \times \mathrm{MUFA}\ n-6 + 3 \times \mathrm{PUFA}\ n-3 + (\mathrm{PUFA}\ n-3/\mathrm{PUFA}\ n-6))}.$$

Quantitative determination of polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons were determined according to the UNEP/IOC/IAEA (1995) method.

Sample preparation

Mullet muscle samples, previously stored at −80 °C, were lyophilised and then hand-ground using mortar and pestle in liquid nitrogen before treatment.

Extraction procedure

The hexane, dichloromethane (DCM) and methanol (MeOH) used were high-purity solvents (Burdick and brand Jackson). Anhydrous sodium sulphate (Na_2SO_4), active aluminium oxide (90) and silica gel 60 for column chromatography used for cleaning were supplied by Fluka. A PAH standard mixture was provided by International Atomic Energy Agency (IAEA).

Appropriate blanks were analysed with each set of analyses. Sample comparisons were made with reference material (IAEA 406) for quality control purposes.

Freeze-dried fish muscle sample (5 g) were placed in a cellulose cartridge that previously had been methanol-cleaned. Each fish sample was extracted with 200 mL methanol for 8 h. Before starting the extraction, 50 μL of internal standard (Cadalene) was added to each sample. Each extract was saponified with KOH solution (7M) and heated for 2 h at 37 °C. Hexane (80 mL) and 20 mL of saturated NaCl solution were then added to each sample and mixed mechanically for 20 min. The organic phase was recovered and re-extracted with 50 mL of hexane and saturated NaCl solution and mechanically mixed for another 15 min. This step was repeated twice. The organic phase was evaporated using a sample concentrator vacuum (miVac Duo Quanttro Geneva) and concentrated to 1 mL under nitrogen flux.

Adsorption chromatographic separation

The preseparation of aliphatic hydrocarbons and aromatic hydrocarbons was carried out by adsorption chromatography on a column packed with 30 g of silica, 80 g of alumina and 1 g of Na_2SO_4. The concentrated extract (1 mL) was transferred to the column, and a first elution was carried out with 20 mL of hexane (F1) which contained aliphatic hydrocarbons. Aromatic hydrocarbons (F2) were next eluted with 30 mL of a mixture containing 90% hexane and 10% dichloromethane. This fraction was concentrated to 10 μL under nitrogen flux.

Instrumental analysis

The analyses were made using a GC-MS (gas chromatograph coupled to a mass spectrometer) 'type Varian 4000'. The column used was a CP 8944 fused capillary column (30 m long × 0.32 mm i.d. ×0.25 μm film thickness).

Helium was used as the carrier gas with a flow rate of 1 mL min^{-1}. A splitless injection mode was used. Nitrogen was used as the carrier gas with a flow rate of 1 mL min^{-1}. The injector was maintained at

290 °C. All injection volumes were 1 μL in the splitless mode. The column temperature was initially held at 60 °C for 2 min and ramped to 290 °C at a rate of 3 °C min, and then, the temperature was held constant at 300 °C for 10 min. Injections of 1 μL of sample each were performed in the split mode, and the split valve was opened after 0.40 min.

Sensory analysis

Sensory analysis for smoked mullet fillets was performed according to the 'Recommended Laboratory Methods for Assessment of Fish Quality' (Woyewoda *et al*., 1986). Sixty inexperienced juries have assessed the attributes of the smoked product. The juries scored the fillets for general acceptability, flavour, odour, juiciness and texture using a four-point hedonic scale (3, dislike extremely to 0, like extremely).

Statistical analyses

For each lot and at each sampling time, the results are presented as mean ± standard deviation (SD) of (n) fillets.

The values for the chemical composition of the fresh fillets and for the smoked fillets at different times of storage were compared by two-way analysis of variance (ANOVA) (general linear model (GLM)). If significant differences ($P < 0.05$) between means were obtained, Tukey's honest significant test was used to differentiate between means. Statistics were performed using SPSS 20 software for windows (SPSS Inc., Chicago, IL, USA). The chemical values, as well as the respective perceptual changes through processing, were also explored by principal component analysis (PCA) (Martens & Næs, 1989). This was done to visualise, and thus improve, the interpretation of the relative changes in chemical composition found in the different fillets. PCA was carried out by the multivariate statistical software (The Unscrambler version 9.8, CAMO Software AS, Oslo, Norway).

For sensory analyses, one-way ANOVA was performed. If significant differences ($P < 0.05$) between means were obtained, Tukey's honest significant test was used to differentiate between means.

Results and discussion

Biochemical analyses

Total volatile basic nitrogen

The initial TVB-N concentration in freshwater *M. cephalus* muscle samples was 8.2 ± 0.53 mg N/100 g – a level that was lower than values reported in other studies for marine *M. cephalus* (Horsfall *et al*., 2006; Orak & Kayışoğlu, 2008; Mostafa & Salem, 2015). This difference may be attributed to the origin of the fish as marine fish muscles contain higher amount of nonprotein nitrogen precursor of post-mortem TVB-N formation (Waarde, 1988).

Following smoking, the TVB-N levels decreased significantly ($P < 0.05$) – possibly due to a washing effect during salting and subsequent water loss during the smoking process. Previous study showed that hot smoking reduces the accumulation of TVB-N in the muscle (Antonios & Michel, 2004), as a consequence of the reduction in water activity and deposition of smoke components, such as phenols which have been identified as major components of smoke (Sérot & Lafficher, 2003).

Subsequently and during refrigerated storage, TVB-N increased significantly ($P < 0.05$) in smoked and vacuum-packed fillets (Fig. 1). TVB-N levels, however, remained significantly lower than the range of values (30–45 mg per 100 g) that are commonly found in good-quality smoked products (EC, 2005).

An enhanced inhibition of TVB-N formation in mullet muscle during refrigerated storage was obtained by the addition of quince polyphenols to the fillets, and this effect was PP dose-dependent (Fig. 1). The reduction in the TVB-N in the PP-treated fillets may be attributed to the antimicrobial effect of polyphenols as explained (above) and reported in other studies (Antonios & Michel, 2004; Li *et al*., 2015).

Trimethylamine nitrogen

Changes in TMA-N levels of mullet fillet during 75 days of refrigerated storage at 4 °C are shown in Fig. 2. Initially, the TMA-N content of mullet fillets was low (1.03 ± 0.11 mg N per 100 g), indicating the

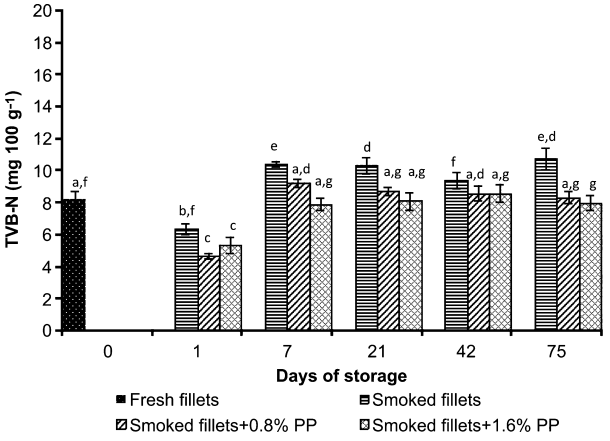

Figure 1 Effect of different concentrations of polyphenols and smoking on total volatile basic nitrogen during storage at 4 °C. Data are mean ± standard deviation ($n = 6$ in each case). Means within the same row with different superscript are significantly different ($P < 0.05$).

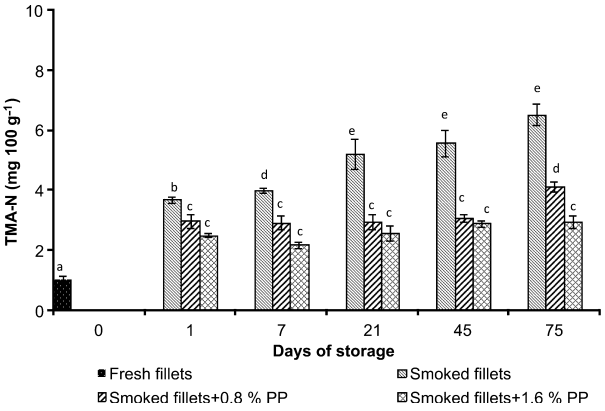

Figure 2 Effect of different concentrations of polyphenols and smoking on trimethylamine nitrogen during storage at 4 °C. Data are mean ± standard deviation ($n = 6$ in each case). Means within the same row with different superscript are significantly different ($P < 0.05$).

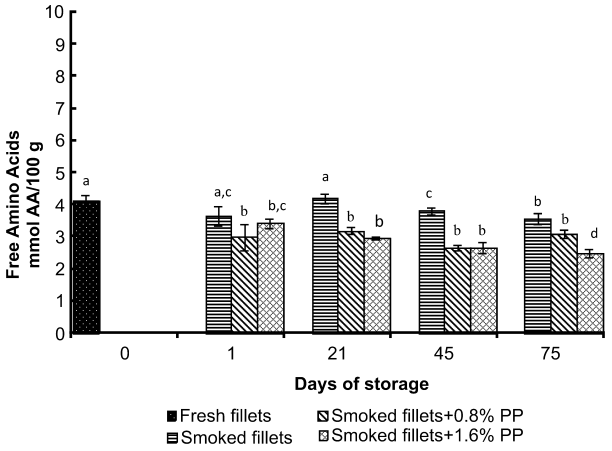

Figure 3 Effect of different concentrations of polyphenols and smoking on free amino acids during storage at 4 °C. Data are mean ± standard deviation ($n = 6$ in each case). Means within the same row with different superscript are significantly different ($P < 0.05$).

good quality of the product, but also the freshwater origin of the fish. Thus, TMA, which is a pungent volatile amine often associated with the typical 'fishy' odour of spoiled seafood, originates from the enzymatic/bacterial reduction of trymethylamine oxide (TMAO), a compound naturally present in the living tissue of marine fish species, but found at significantly lower (absent, in some cases) levels in freshwater organisms such as *M. cephalus* (Chung & Chan, 2009).

Throughout refrigerated storage, TMA-N content increased significantly in control-smoked samples and in PP-treated fillets but at much lower levels. In all cases, TMA contents remained below the critical values (10–12 mg per 100 g) established as indicator of spoilage in smoked fish and fresh fish (Hansen *et al.*, 1995; EC, 1995, respectively). The polyphenols' inhibitory effect was markedly significant towards the end of storage as TMA levels in PP-treated/smoked fillets (2.97 ± 0.2 mg TMA per 100 g) were significantly ($P < 0.05\%$) lower than in the control lot (6.54 ± 0.37 mg TMA per 100 g). Such findings are in agreement with those of Erkan (2012) who found an inhibitory effect of garlic and thyme oils on the microbial activity in smoked/essential-oil-treated trout. In this study, the lower production of TMA-N may be attributed to the antimicrobial properties of polyphenols on TMAO reduction (Hattori *et al.*, 1990; Fattouch *et al.*, 2007).

Free amino acid (FAA) variation

The initial concentration of FAAs, measured as ninhydrin-positive substances (NPS) in fresh fillets, was 4.15 ± 0.17 mmol FAA per 100 g (Fig. 3). Smoking was not found to affect FAA formation ($P > 0.05$) as it is a process where appropriate combination of temperature and time is sufficient to cause the complete coagulation of the proteins in the fish-flesh-limiting FAA liberation. However, polyphenols were shown to have a significant inhibitory effect ($P < 0.05$) on FAA formation during refrigerated storage. This could be due to the antibacterial effects of polyphenol extracts on peptide degradation as shown by Haslam *et al.* (1992) and Hattori *et al.* (1990). Moreover, our findings support those of Ruiz-Capillas & Moral (2001) that FAA variation can be used to investigate the effect of factors affecting fish quality.

Fat content and fatty acid composition

Fresh fillets were found to have a high relative lipid content (6.83 ± 0.94%) compared with the 1.3 ± 0.12% reported for raw golden grey mullet fillets (Ghelichpour & Shabanpour, 2011). This large variation in relative lipid contents may be due to a variety of factors including fishing area, fishing season, spawning period (Nielsen *et al.*, 2005) or fish size and gender (Rajasilta, 1992). The lipid content of fillets was significantly affected ($P < 0.05$) by hot smoking (Fig. 4) with smoked fillets having a higher lipid content than fresh ones (11.47% ± 1.69 and 5.25 ± 1%, respectively). The fat content increased after smoking as a consequence of muscle water loss as reported by Espe *et al.* (2002). Quince polyphenols' addition seemed to limit such loss as significantly ($P < 0.05$) lower lipid levels were found in treated mullet fillet lots. However, increased polyphenols' content had no significant ($P > 0.05$) effects on their lipid variation.

Fatty acid composition

All quantitative estimations of the mean fatty acid (FA) concentrations in fresh, smoked fillets and smoked fillets treated with 0.8 and 1.6% PP are given

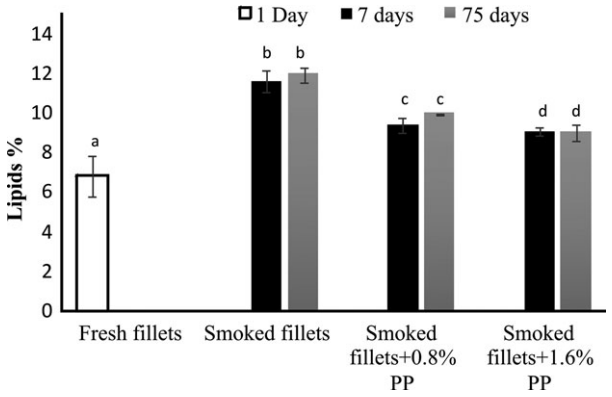

Figure 4 Effect of different concentrations of polyphenols and smoking on lipid contents during storage at 4 °C. Data are mean ± standard deviation ($n = 6$ in each case). Means within the same row with different superscript are significantly different ($P < 0.05$).

in Table 1. In fresh *M. cephalus* muscle, saturated fatty acids (28.72%) represent the bulk of the total identified FA, followed by the monounsaturated fatty acids (MUFAs; 28.27%) and the polyunsaturated fatty acids (PUFAs; 26.67%). Although the mullet in this study is of freshwater origin, their fatty acid rankings are in accordance with the results found for marine mullet (Özogul & Özogul, 2007; Kose *et al.*, 2010; Kumaran *et al.*, 2012). Within these groups, the major fatty acids were palmitic acid (C16:0 = 21.8%), palmitoleic acid (C16:1 w7 = 17.9%) and oleic acid (C18:1 w9 = 6.69%), respectively (Table 1). These results are in agreement with previous studies on fatty acids in *Mugil cephalus* (Kumaran *et al.*, 2012). The sum of SFA of fish fillets increased following smoking, but was slightly affected by the addition of polyphenols and by the storage period. However, in this study, the sums of the freshwater mullet SFA, recognised as a health risk factor (Ulbrich & Southgate, 1991), were found to be lower than that found in marine mullets (Özogul & Özogul, 2007; Kumaran *et al.*, 2012). On the other hand, MUFAs showed an important increase in smoked fish fillets, which may be due to the dehydrating effect caused by smoking or to the degradation of some PUFA to MUFA. Considering the PUFA

Table 1 Changes in fatty acid composition (%) in fresh mullet fillets (F), smoked fillets (S) and smoked fillets with the different polyphenols' treatments (S + 0.8% PP) and (S + 1.6% PP) in cold-stored (7 and 75 days)

Fatty acids	F	S (7 days of storage)	S + 0.8% PP (7 days of storage)	S + 1.6% PP (7 days of storage)	S (75 days of storage)	S + 0.8% PP (75 days of storage)	S + 1.6% PP (75 days of storage)
C14:0	$5.75 \pm 0.15^{a,b}$	6.31 ± 0.07^{a}	$6.74 \pm 0.84^{a,c}$	$6.71 \pm 0.27^{a,c}$	$8.90 \pm 0.46^{a,c}$	$9.10 \pm 0.08^{a,c}$	10.2 ± 1.09^{c}
C16:0	$21.8 \pm 0.34^{a,b}$	23.14 ± 0.24^{a}	23.53 ± 0.51^{a}	22.23 ± 1.13^{a}	21.59 ± 0.56^{a}	17.49 ± 1.4^{b}	20.35 ± 0.87^{c}
C16:1 n-7	17.9 ± 0.1^{a}	16.7 ± 0.87^{a}	19.93 ± 0.07^{a}	$20.3 \pm 0.48^{a,b}$	20.3 ± 2.5^{a}	23.08 ± 0.43^{b}	22.23 ± 2.27^{b}
C16:2 n-4	2.3 ± 0.15^{a}	1.19 ± 0.29^{b}	0.53 ± 0.03^{b}	0.73 ± 0.29^{b}	0.64 ± 0.05^{b}	1.03 ± 0.32^{b}	0.9 ± 0.2^{b}
C16:3 n-4	2.34 ± 0.09^{a}	1.6 ± 0.1^{b}	1.76 ± 0.12^{b}	1.67 ± 0.18^{b}	1.57 ± 0.06^{b}	1.6 ± 0.33^{b}	0.67 ± 0.32^{c}
C18:0	$1.75 \pm 0.3^{a,c}$	2.88 ± 0.03^{b}	2.45 ± 0.01^{a}	$1.84 \pm 0.08^{a,c}$	2.01 ± 0.67^{a}	$2.18 \pm 0.02^{a,b,c}$	1.29 ± 0.29^{c}
C18:1 n-9	6.69 ± 0.21^{a}	11.13 ± 0.31^{b}	10.5 ± 0.1^{b}	11.26 ± 0.46^{b}	$8.23 \pm 0.09^{a,c}$	$8.87 \pm 1.55^{b,c}$	$7.74 \pm 0.41^{a,c}$
C18:1 n-7	$3.42 \pm 0.39^{a,c,d}$	2.88 ± 0.23^{b}	$2.45 \pm 0.01^{b,c,d}$	2.84 ± 0.1^{c}	2.22 ± 0.19^{a}	2.63 ± 0.48^{b}	2.32 ± 0.13^{d}
C18:2 n-6	3.97 ± 0.06^{a}	$5.1 \pm 0.09^{b,c}$	$4.62 \pm 0.21^{b,c}$	$4.13 \pm 0.2^{a,c}$	$4.36 \pm 0.11^{a,c}$	4.46 ± 0.57^{c}	3.89 ± 0.06^{a}
C18:3 n-4	0.19 ± 0.02^{a}	0.08 ± 0.00^{b}	00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
C18:3 n-3	4.77 ± 0.06^{a}	$5 \pm 0.07^{a,b}$	5.78 ± 0.07^{b}	$5.28 \pm 0.29^{a,b}$	5.02 ± 0.15^{a}	5.74 ± 0.24^{a}	$5.67 \pm 0.16^{a,b}$
C18:4 n-3	2.11 ± 0.13^{a}	1.27 ± 0.04^{a}	$1.98 \pm 0.41^{a,b}$	1.25 ± 0.13^{c}	$1.72 \pm 0.07^{b.d}$	$1.92 \pm 0.19^{b,d}$	$2.10 \pm 0.16^{c,d}$
C20:1 n-9	0.5 ± 0.01^{a}	0.4 ± 0.11^{a}	0.42 ± 0.08^{a}	0.48 ± 0.14^{a}	0.34 ± 0.05^{a}	0.49 ± 0.11^{a}	0.50 ± 0.10^{a}
C20:4 n-6	2.07 ± 0.13^{a}	2.17 ± 0.09^{a}	1.93 ± 0.45^{a}	2.34 ± 0.42^{a}	1.68 ± 0.32^{a}	1.95 ± 0.26^{a}	2.00 ± 0.22^{a}
C20:4 n-3	0.48 ± 0.01^{a}	0.5 ± 0.01^{a}	0.49 ± 0.04^{a}	0.42 ± 0.05^{a}	0.39 ± 0.03^{a}	0.45 ± 0.02^{a}	0.49 ± 0.03^{a}
C20:5 n-3	5.83 ± 0.08^{a}	3.02 ± 0.07^{b}	3.32 ± 0.12^{b}	3.03 ± 0.29^{b}	2.71 ± 0.32^{b}	3.44 ± 0.19^{b}	3.37 ± 0.05^{b}
C22:5 n-3	1.45 ± 0.08^{a}	1.4 ± 0.06^{a}	1.49 ± 0.15^{a}	1.36 ± 0.01^{a}	0.96 ± 0.07^{a}	1.10 ± 0.03^{a}	1.38 ± 0.18^{a}
C22:6 n-3	1.1 ± 0.01^{a}	$1.21 \pm 0.0b$	2.01 ± 0.17^{a}	$1.56 \pm 0.07^{a,b}$	$1.12 \pm 0.20^{a,b}$	1.35 ± 0.20^{a}	1.53 ± 0.27^{a}
TOTAL	83.67 ± 0.5^{a}	87 ± 0.08^{b}	86.6 ± 0.36^{b}	86.76 ± 1.58^{b}	$86.52 \pm 1.64^{b,c}$	$85.94 \pm 1.99^{b,c}$	$87.2 \pm 0.89^{b,c}$
∑ PUFA	26.67 ± 0.29^{a}	22.09 ± 0.13^{b}	21.76 ± 1.37^{b}	21.83 ± 1.09^{b}	$20.37 \pm 0.^{a,b}$	21.83 ± 1.99^{b}	$22.94 \pm 0.17^{b.}$
∑ MUFA	$28.27 \pm 0.24^{a,c}$	$36.46 \pm 1.49^{b,a}$	32.53 ± 1.1^{b}	36.6 ± 1.13^{b}	$30.23 \pm 1.66^{b.c}$	$32.89 \pm 1.81^{b.c}$	$34.6 \pm 0.86^{b.c}$
∑ SFA	28.72 ± 0.3^{a}	32.39 ± 0.28^{b}	32.53 ± 1.11^{b}	$29.76 \pm 1.17^{a,b}$	$30.71 \pm 0.08^{a,b}$	28.39 ± 1.02^{a}	$29.82 \pm 0.05^{a,b}$
ΣPUFA n-3	15.57 ± 0.1^{a}	$12.39 \pm 0.23^{a,b}$	$14.43 \pm 0.41^{a,b}$	12.34 ± 0.75^{b}	$11.80 \pm 0.85^{a,b}$	$14.69 \pm 0.08^{b,c}$	14.67 ± 0.37^{c}
ΣPUFA n-6	$6.04 \pm 0.13^{a,c}$	$7.27 \pm 0.01^{a,b,c}$	$5.08 \pm 1.89^{b,c}$	6.47 ± 0.55^{c}	6.4 ± 0.34^{a}	$6.41 \pm 0.83^{b,c}$	$5.89 \pm 0.27^{a,c}$
IA	0.93 ± 0.02^{a}	0.91 ± 0.02^{a}	1.01 ± 0.09^{a}	0.92 ± 0.02^{a}	1.22 ± 0.28^{a}	$1.07 \pm 0.03^{a,b}$	1.13 ± 0.06^{b}
IT	0.44 ± 0.01^{a}	0.53 ± 0.03^{a}	0.48 ± 0.01^{a}	$0.51 \pm 0^{a,b}$	0.58 ± 0.07^{b}	0.43 ± 0.01^{a}	$0.47 \pm 0.05^{a,b}$

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; IA, index of atherogenicity; IT, index of thrombogenicity.
Mean in the same column with different alphabetical letters is significantly different ($P < 0.05$).

profile, the relative proportion of the n-3 fatty acids (15.75%) in fresh mullet fillets was lower (21.7%) than value found in its congener marine mullet (Özogul & Özogul, 2007). Among the omega-3 FA, eicosapentaenoic (EPA: C20:5 n-3) was relatively the most abundant polyunsaturated fatty acid (5.83%) and moderate levels of docosahexaenoic acid (DHA: C22:6 n-3) occurred. Both FA contents are lower than values reported in others studies (Özogul & Özogul, 2007; Kumaran *et al*., 2012) for marine *M. cephalus*. Such difference may be due to fish origin with different environmental conditions including salinity and temperature (Khériji *et al*., 2003).

Following smoking, EPA (5.83 ± 0.08%) decreased significantly ($P < 0.05$) to 3.02 ± 0.07% in fillets and was accompanied by an increase in the concentration of monounsaturated FA such as C22:1 (Table 1). However, hot smoking did not affect significantly the omega-6 fish contents; similar results were found in other studies that show that PUFA degradation during hot smoking is significant but it is more pronounced for omega-3 than omega-6 (Kolakowsk, 2010). In this study, the effect of polyphenols on lipid stability appears towards the end of the refrigerated storage as revealed by PUFA levels in PP-treated fillets being significantly higher than those in the control lot (Table 1).

When screening bioactive nutrients of health benefit, IA and IT were identified among the various nutritional indices (Kalogeropoulos *et al*., 2008). In this study, the IA and IT (0.93 and 0.44, respectively; Table 1) were found within the range of values established for Mediterranean fish (Kalogeropoulos *et al*., 2004). These indices, however, were significantly higher than those reported for processed roe mullets known as Greek avgotaracho (Kalogeropoulos *et al*., 2008). Our results also showed a stability of the IA and a nonsignificant increase in the IT after hot smoking. Such results suggest that mullet is less affected by smoking than other species such as shrimp (Lira *et al*., 2014). Throughout 75 days of refrigerated storage, the values of the IA remained unchanged in the smoked PP-treated fillets. Such results suggest that polyphenols have an important preservative effect on the nutritional quality of mullet fillet.

In accordance with the results of Kumaran *et al*. (2012), this study showed that up to 75 days of refrigerated storage, smoked fillet still contain substantial amounts of fatty acids of health benefit.

Polycyclic aromatic hydrocarbons (PAHs)

The concentrations of PAHs (fluorene to fluoranthene) varied between 0.11 and 0.17 μg kg^{-1} (Table 2). It should be noted that cadalene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a) pyrene, indo(123 cd)pyrene, dibenz(ah)anthracene and benzo(ghi)perylene were not found in fresh or smoked samples. These PAHs are considered to be much more toxic than low molecular weight PAHs, such as fluorene. It is important to note the absence of benzopyrenes including benzo(a)pyrene (BaP) in mullet fillets before and after smoking. Such compounds are regarded as a marker of the carcinogenic PAHs. Chrysene, another polycyclic aromatic hydrocarbon, is considered to be less dangerous than benzo[a]pyrene and is detected at very low doses (0.01 μg kg^{-1}).

Table 2 Comparison of polycyclic aromatic hydrocarbons concentrations (ng g^{-1}) in fresh and smoked mullet fillets

Compounds	Before smoking	After smoking	After smoking and addition of 0.8%PP
Naphthalene	0.03	0.07	0.08
Acenaphthylene	0.25	0.32	0.38
Acenaphthene	0.10	0.11	0.21
Fluorene	0.09	0.11	0.19
Phenanthrene	0.12	0.71	0
Anthracene	0.01	0.06	0.86
Fluranthene	0.11	0.17	0.06
Pyrene	0.07	0.12	0.20
Benzo(a)anthracene	ND	ND	0.25
Chrysene	ND	0.10	0
Benzo(b)fluoranthene	ND	ND	0.22
Benzo(k)fluoranthene	ND	ND	0
Benzo(a)pyrene	ND	ND	ND
indeno(123c)pyrene	ND	ND	ND
Dibenz(ah)anthracene	ND	ND	ND
Benzo(ghi)perylene	ND	ND	ND
∑ (chrysene. benzo(b)fluoranthene. benz(a)anthracene. Benzo(a)pyrene)	ND	0.1	0.22
∑PAHs	0.82	1.8	2.5

ND, not detected.

The sum of 4 PAHs (chrysene, benzo[b]fluoranthene, benz[a]anthracene and BaP) was 0.1 μg kg^{-1} after smoking treatment and was far below the maximum limit (12 μg kg^{-1}) fixed by the European Commission (34) for smoked fish.

It should be noticed that the concentrations of PAHs in smoked mullet fillets found in the present study are much lower than those found in cold smoked sardine and dolphinfish (Gómez-Estaca *et al*., 2011) and smoked rainbow trout fillets (Visciano *et al*., 2008). The low PHAs levels found in the present study indicate a good smoking process within the plant. Hence, it was reported that PAHs content in smoked product can be affected by several factors including manual handling of the smoking process, quality of the raw material and drip loss of the fat (Karl & Leinemann, 1996). In this study, and in all lots, we noted the absence of benzo(a)pyrene and a modest increase in the sum of 4 PAHs (chrysene, benzo(b)fluoranthene, benz(a)anthracene and BaP) (0.22 μg kg^{-1}).

This sum remains below the maximum limit set by the European commission (EC, 2011).

Principal component analysis

A PCA was performed to find potential influences on mullet fillets of the smoking procedure, polyphenol treatment and storage time on the quality parameters including TVB-N, TMA-N, NPS, lipids and indexes of lipid quality (IA and IT).

Two principal components (PCs) were found to explain 62% of the variations in the data set. The scores and loadings of PC1 and PC2, representing 44% and 18% of the total variation, are given in Fig. 5. The score plot shown in Fig. 5a displayed a clear distinction between smoked fillets, smoked – 0.8% PP-treated fillets and smoked – 1.6% PP-treated fillets.

The PCA also showed a negative correlation between smoking treatment and muscle NPS levels. Along the PC1 axis, we observed that treatment with smoking is in the negative side, while the NPS is in the positive side. The smoking treatment also has an inhibitory effect on total free amino acids. PCA revealed that treatment of mullet fillets by smoking is positively correlated with the levels of lipids and also with the indices of lipid quality (IA and IT).

Along the PC2 axis, we note that treatment of smoking combined with polyphenols (0.8% and 1.6%) was negatively correlated with TVB-N, TMA-N, lipids, indices of lipid quality (IA, IT) and storage time. From this, we suggest that this treatment improved the preservation of mullet fillet by delaying proteolysis, as reflected by a decreased level of TVB-N, and by the inhibition of bacterial growth by reducing the production of TMA.

Treatment with polyphenols has an effect on the preservation of fatty acids as it inhibits the increase in IA and IT during storage. We noted that increasing the dose of polyphenols affected significantly the formations of TMA-N and TVB-N. As shown in Fig. 5b, treatment by both concentrations of polyphenols (0.8% and 1.6%) is located on both sides of PC1.

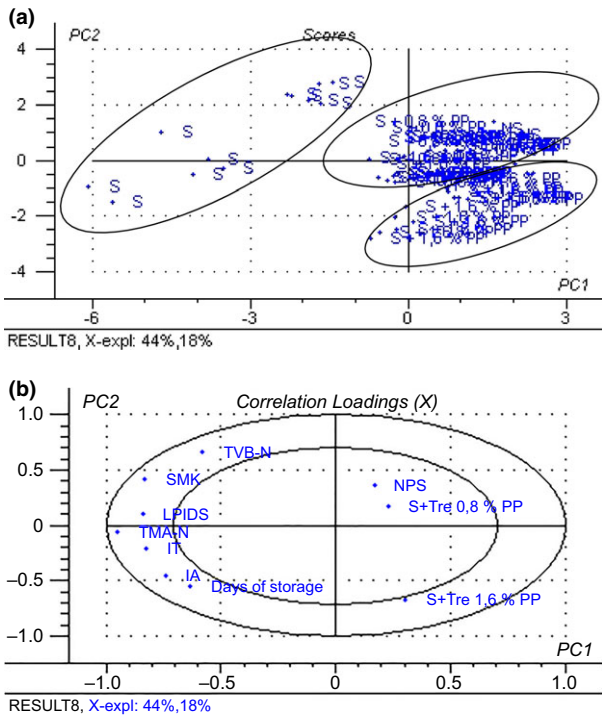

Figure 5 Score (a) and loading (b) plot from principal component analysis (PCA) of smoked mullet fillets (SMK), polyphenols treated then smoked fillet lots (S+Tre0.8% PP and S+Tre 1.6% PP) respectively with their chemical values (lipids, NPS, TMA-N and TVB-N) and relative changes during storage.

Sensory analyses

Average scores of smoked samples, and polyphenols-treated/smoked samples are shown in Table 3. The results showed that the general taste of the new product was very acceptable to consumers with the average score values <1.2 in all tested fish fillet lots. Similar results were obtained by Vasiliadou *et al.* (2005) who studied the suitability of smoking sea bream. In this study, a better acceptability was found for 0.8% PP-treated lot with a lower value (1.06). Considering the other sensory parameters, scoring was < 1.5 for all lots. Such results revealed that the Tunisian consumers have considered the smoked mullet fillets treated by a low dose of polyphenols to be a very acceptable product.

Table 3 Average scores for sensory evaluation of smoked fillets and smoked fillets with the different polyphenols' treatments (0.8% PP) and (1.6% PP) of mullet

	General acceptability	Flavour	Odour	Juiciness	Texture
Smoked fillets	1.18 ± 0.02^{a}	1.36 ± 0.02^{a}	1.27 ± 0.03^{a}	1.24 ± 0.02^{a}	1.36 ± 0.02^{a}
Smoked fillets with 0.8% PP	1.06 ± 0.02^{b}	1 ± 0.02^{b}	1.27 ± 0.03^{a}	1.24 ± 0.02^{a}	1.27 ± 0.02^{a}
Smoked fillets with 1.6% PP	1.12 ± 0.02^{a}	1.33 ± 0.03^{a}	1.48 ± 0.03^{a}	1.33 ± 0.02^{a}	1.24 ± 0.02^{a}

Scoring was as follows: 0 = excellent; 1 = good; 2 = acceptable; >2 = reject. Mean with different alphabetical letters (a, b) is significantly different ($P < 0.05$).

Conclusion

Based on chemical, biochemical and sensorial analyses, we conclude that the combined process of smoking and polyphenols' addition yields a fishery product with acceptable sensory properties throughout storage and with good quality. Such procedural combinations can, technically and economically, be used for the preservation and amelioration of the biochemical and organoleptic quality of freshwater fish and exhibit potential advantages for this fishery sector. The process of smoking constitutes a safe method as the resultant contents of benzo(a)pyrene and the sum of 4 PAHs (chrysene, benzo[b]fluoranthene, benz[a]anthracene and BaP) are always to be lower than the European legal limit.

Acknowledgment

This work was supported by the project (BIOVecQ PS1.3/08) within the cross border IEVP program (*Instument Européen de Voisinage et de Partenariat-Programme de coopération transfrontalière Italie-Tunisie-2007–2013*) funded by European Union and by a grant from the Tunisian Ministry of Agriculture (IRESA). The authors acknowledge the Society of Trident of Carthage (Tunisia) for supplying mullet samples.

References

Antonios, E.G. & Michel, G.K. (2004). Effect of salting and smoking-method on the keeping quality of chub mackerel (*Scomber japonicus*): biochemical and sensory attributes. *Food Chemistry*, **93**, 511–520.

Attouchi, M. & Sadok, S. (2012). The effects of essential oils addition on the quality of wild and farmed sea bream (*Sparus aurata*) stored in ice. *Food and Bioprocess Technology*, **5**, 1803–1816.

Chung, S.W.C. & Chan, B.T.P. (2009). Trimethylamine oxide, dimethylamine, trimethylamine and formaldehyde levels in main traded fish species in Hong Kong. *Food Additives and Contaminants: Part B*, **2**, 44–51.

Coutinho de Oliveiraa, T.L., Ramosb, A.L.S., Ramos, E.M., Piccoli, R.H. & Cristianini, M. (2015). Natural antimicrobials as additional hurdles to preservation of foods by high pressure processing. *Trends in Food Science & Technology*, **45**, 60–85.

DGPA. (2012). Fisheries statistics. Directorate General of Fisheries and Aquaculture, Tunisia.

Doe, P.E. (1998). *Fish Drying and Smoking Production and Quality*. Pp. 89–115. Lancaster: Technomic Publishing Co Inc.

European Commission (EC). (1995). *Commission of the European Community, Decision 95/ 149/EC of 8 March 1995 fixing the total volatile basic nitrogen (TVB-N) limit values for certain categories of fishery products and specifying the analysis methods to be used.* Brussels: CEC.

European Commission (EC). (2005). Commission Regulation (EU) No 2074/2005.

European Commission (EC). (2011). Commission Regulation (EU) No 835/2011 of 19 August 2011. L 215. P4.

Erkan, N. (2012). The effect of thyme and garlic oil on the preservation of vacuum-packaged hot smoked rainbow trout (*Oncorhynchus mykiss*). *Food and Bioprocess Technology*, **5**, 1246–1254.

Espe, M., Nortvedt, R., Lie, O. & Hafsteinsson, H. (2002). Atlantic salmon (*Salmo salar, L*) as raw material for the smoking industry. Effect of different smoking methods on losses of nutrients and on the oxidation of lipids. *Food Chemistry*, **77**, 41–46.

Fattouch, S., Caboni, P., Coroneo, V. *et al.* (2007). Antimicrobial activity of Tunisian quince (*Cydonia oblonga Miller*) pulp and peel polyphenolic extracts. *Journal of Agriculture and Food Chemistry*, **55**, 963–969.

Fattouch, S., Sadok, S., Raboudi-Fattouch, F. & Ben Slama, M. (2008). Damage inhibition during refrigerated storage of mackerel (*Scomber scombrus*) fillets by a presoaking in quince (*Cydonia oblonga*) polyphenolic extract. *International Journal of Food Science and Technology*, **43**, 2056–2064.

Folch, J., Lees, M. & Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipids from animal tissue. *Journal of Biological Chemistry*, **226**, 497–509.

Ghelichpour, M. & Shabanpour, B. (2011). The investigation of proximate composition and protein solubility in processed mullet fillets. *International Food Research Journal*, **18**, 1343–1347.

Gómez-Estaca, J., Gómez-Guillén, M.C., Montero, P., Sopelana, P. & Guillén, M.D. (2011). Oxidative stability, volatile components and polycyclic aromatic hydrocarbons of cold-smoked sardine (*Sardina pilchardus*) and dolphinfish (*Coryphaena hippurus*). *LWT-Food Science and Technology*, **44**, 1517–1524.

Gómez-Estaca, J., Gómez-Guillén, M.C. & Montero, P. (2014). The effect of combined traditional and novel treatments on oxidative status of dolphinfish (*Coryphaena hippurus*) and sardine (*Sardina pilchardus*) muscle lipids. *Food Science and Technology International*, **20**, 431–440.

Gómez-Guillén, M.C., Gómez-Estaca, J., Giménez, B. & Montero, P. (2009). Alternative fish species for cold-smoking process. *International Journal of Food Science and Technology*, **44**, 1525–1535.

Hansen, L.T., Gillb, T. & Huss, H.H. (1995). Effects of salt and storage temperature on chemical, microbiological and sensory changes in cold-smoked salmon. *Food Research International*, **28**, 123–130.

Haslam, E., Lilley, T.H., Warminski, E. *et al.* (1992). Polyphenol complexation, A study in molecular recognition. *ACS Symposium Series*, **506**, 8–50.

Hattori, M., Kusumoto, I.T., Namba, T., Ishigami, T. & Hara, Y. (1990). Effect of tea polyphenols on glucan synthesis by glucosyltransferase from *Streptococcus mutans*. *Chemical and Pharmaceutical Bulletin*, **38**, 717–720.

Horsfall, M. Jr, Kinigoma, B.S. & Spiff, A.I. (2006). Evaluation of the levels of total volatiles bases and trimethylamine formed in fish stored at low temperature. *Bulletin of the Chemical Society of Ethiopia*, **20**, 155–159.

Kalogeropoulos, N., Andrikopoulos, N.K. & Hassapidou, M. (2004). Dietary evaluation of Mediterranean fish and mollusks pan-fried in virgin olive oil. *Journal of the Science of Food and Agriculture*, **84**, 1750–1758.

Kalogeropoulos, N., Nomikos, T., Chiou, A., Fragopoulou, E. & Antonopoulou, S. (2008). Chemical composition of greek avgotaracho prepared from mullet (*Mugil cephalus*): nutritional and health benefits. *Journal of Agriculture and Food Chemistry*, **56**, 5916–5925.

Karl, H. & Leinemann, M. (1996). Determination of polycyclic aromatic hydrocarbons in smoked fishery products from different smoking kilns. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, **202**, 458–464.

Khériji, S., El Cafsi, M., Masmoudi, W., Castell, J.D. & Romdhane, M.S. (2003). Salinity and temperature effects on the lipid composition of mullet sea fry (*Mugil cephalus*, Linne, 1758). *Aquaculture International*, **11**, 571–582.

Kolakowsk, A. (2010). Fish lipids. In: *Chemical and Functional Properties of Food Compounds Series*, 2nd edn (edited by Z.E. Sikorski & A. Kolakowsk). Pp. 273–313. Lancaster: CRC Press Taylor & Francis Group.

Kose, S., Koral, S., Ozogul, Y. & Tufan, B. (2010). Fatty acid profile and proximate composition of Pacific mullet (*Mugil so-iuy*) caught in the Black Sea. *International Journal of Food Science and Technology*, **45**, 1594–1602.

Kumaran, R., Ravi, V., Gunalan, B., Murugan, S. & Sundramanickam, A. (2012). Estimation of proximate, amino acids, fatty acids and mineral composition of mullet (*Mugil cephalus)* of Parangipettai, Southeast Coast of India. *Advances in Applied Science Research*, **3**, 2015–2019.

Li, J., Hui, T., Wang, F. *et al.* (2015). Chinese red pepper (*Zanthoxylum bungeanum* Maxim.) leaf extract as natural antioxidants in salted silver carp (*Hypophthalmichthys molitrix*) in dorsal and ventral muscles during processing. *Food Control*, **56**, 9–17.

Lira, G.M., Barros Silva, K.W., Figueirêdo, B.C. & Bragagnolo, N. (2014). Impact of smoking on the lipid fraction and nutritional value of seabob shrimp (*Xiphopenaeus kroyeri*, *Heller,* 1862). *LWT-Food Science and Technology*, **58**, 183–187.

Martens, H. & Næs, T. (1989). *Multivariate Calibration*. Chichester, UK: WILEY.

Metcalfe, L.D., Schimitz, A.A. & Pelka, J.R. (1966). Rapid preparation of fatty acids esters from lipids for gas chromatographic analysis. *Analytical Chemistry*, **38**, 514–515.

Miler, K.M.B. & Sikorski, Z.E. (1990). Smoking. In: *Seafood: Resources, Nutritional Composition, and Preservation* (edited by Z.E. Sikorski). Pp. 163–180. Boca Raton, FL: CRC Press.

Mostafa, A.A. & Salem, R.H. (2015). Characterization of microbiological and nutritional variations in processed mullet (*Mugil cephalus*) Fish. *International Journal of Microbiological Research*, **6**, 108–122.

Nielsen, D., Hyldig, G., Nielsen, J. & Nielsen, H.H. (2005). Lipid content in herring (*Clupea harengus L.*) influence of biological factors and comparison of different methods of analyses: solvent extraction, Fatmeter, NIR and NMR. *Journal of Food Science & Technology*, **38**, 537–548.

Orak, H.H. & Kayışoğlu, S. (2008). Quality changes in whole, gutted and filleted three fish species (*Gadus euxinus, Mugil cephalus, Engraulis encrasicholus*) at frozen storage period (–26°C). *Acta Scientiarum Polonorum Technologia Alimentaria*, **7**, 15–28.

Özogul, Y. & Özogul, F. (2007). Fatty acid profiles of commercially important fish species from the Mediterranean, Aegean and Black Seas. *Food Chemistry*, **100**, 1634–1638.

Rajasilta, M. (1992). Relationships between food, fat, sexual maturation, and spawning time of Baltic herring *(Clupea harengus membras)* in the Archipelago Sea. *Canadian Journal of Fish and Aquatic Sciences*, **49**, 644–654.

Ruiz-Capillas, C. & Moral, A. (2001). Production of Biogenic amines and their potential use as quality control indices for hake (*Merluccius merluccius*) stored in ice. *Journal of Food Science*, **66**, 1030–1032.

Ruiz-Capillas, C. & Horner, W.F.A. (1999). Determination of the trimethylamine and total volatile basic nitrogen in flesh fish by flow Injection analysis. *Journal of the Science of Food and Agriculture*, **14**, 1982–1986.

Sadok, S., Uglow, R.F. & Haswell, S.J. (1995). Determination of ninhydrine positive substances in haemolymph and seawater. *Analyst*, **120**, 2097–2099.

Sadok, S., Uglow, R. & Haswell, S.J. (1996). Determination of trimethylamine in fish, by flow injection analysis. *Analytica Chimica Acta*, **321**, 69–74.

Sérot, T. & Lafficher, C. (2003). Optimisation of solid phase microextraction coupled to gas chromatography for the determination of phenolic compounds in smoked herring. *Food Chemistry*, **82**, 513–519.

Sikorski, Z.E. & Sinkiewicz, I. (2014). *Smoking | Traditional, in Encyclopedia of Meat Sciences*, 2nd edn (edited by C. Devine & M. Dikeman). Pp. 321–327. New York: Churchill Livingstone, Elsevier Ltd.

Simko, P. (2002). Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. *Journal of Chromatography B*, **770**, 3–18.

Ulbritch, T.L.V. & Southgate, D.A.T. (1991). Coronary heart disease. Seven dietary factors. *Lancet*, **338**, 985–999.

UNEP/IOC/IAEA. (1995). Determination of petroleum hydrocarbons in selected marine organisms. Reference methods for marine pollution studies. N° 72 UNEP.

Vasiliadou, S., Ambrosiadis, I., Vareltzis, K., Fletouris, D. & Gavriilidou, I. (2005). Effect of smoking on quality parameters of farmed gilthead sea bream (*Sparus aurata L.*) and sensory attributes of the smoked product. *European Food Research Technology*, **221**, 232–236.

Visciano, P., Perugini, M., Conte, F. & Amorena, M. (2008). Polycyclic aromatic hydrocarbons in farmed rainbow trout (*Oncorhynchus mykiss*) processed by traditional flue gas smoking and by liquid smoke flavorings. *Food and Chemical Toxicology*, **46**, 1409–1413.

Waarde, A.V. (1988). Biochemistry of non-protein nitrogenous compounds in fish including the use of amino acids for anaerobic energy production. *Comparative Biochemistry and Physiology*, **91B**, 207–228.

Woyewoda, A.D., Shaw, S.J., Ke, P.J. & Burns, B.G. (1986). *Recommended Laboratory Methods for Assessment of Fish Quality*. *Canadian Technical Report of Fisheries and Aquatic Science No. 1448*. Pp. 2–5, 41–49, 65–72, 125–134. Halifax, N.S., Canada: Minister of Supply and Services Canada.

Zaouali, J. (1981). Problems of aquaculture: brackish water and aquaculture potential. *Archives of Pasteur Institut of Tunis*, **58**, 93–103.