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Comparative Effect of Different Smoking Process on Biochemical, Microbial and Sensory Characteristics of Pacific Oyster *Crassostrea gigas*

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Abstract: Changes in biochemical, sensory and microbiological quality of pacific oyster *Crassostrea gigas* after smoking process and during chill storage were investigated. N-3 and N-6 polyunsaturated fatty acids levels were 0.81 and 0.11 g 100 g^{-1} fresh oyster, respectively. After processing, all fatty acid groups increased with higher levels (10.37 g 100 g^{-1} oyster) in oyster smoked at 125°C. However, storage period didn't have any effect on fatty acid contents. So, saturated, monounsaturated and polyunsaturated fatty acids (4.22; 2.08 and 3.80, 100 g oyster) contents remain unchanged after storage. Based on sensory data, hot smoked samples were perceived more acceptable than other samples. Smoking process significantly lessened the levels of different strains and pseudomonas was completely eradicated. On the 14th day, the microbial load remains constant with the emergence of pseudomonas and staphylococcus. Afterwards a significant increase ($p<0.05$) was observed in all lots for mesophilic and psychrophilic bacteria at the end of storage.

Key words: Oyster, smoking, chilling, quality change, fatty acids

INTRODUCTION

Fish and shellfish are nutritious foods that constitute desirable components of a healthy diet. Seafood, including oysters is rich in Polyunsaturated Fatty Acids (PUFAs) such as Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) (Cruze-Romero *et al*., 2008). However, they are very prone to oxidation that is also favoured by storage period. Oxidative degradation of lipids in seafood product during processing and subsequent storage directly affects their quality including flavour, colour, odour, texture and nutritional value (Kumolu-Johnson *et al*., 2010; Chaijan *et al*., 2006).

As fresh seafood, oysters have a short shelf-life which causes substantial practical problems for its distribution. Various food preservation techniques have been utilized to improve the microbial safety and extend the shelf-life of seafood in general including freezing, chemical preservation, smoking and modified atmosphere packaging. Smoking is probably the oldest method used for seafood preservation as a result of the combined effects of dehydration, antimicrobial and antioxidant activity of several of the smoke constituents (formaldehyde, carboxylic acids, phenols) (Doe, 1998; Leroi and Joffraud, 2000; Rorvik, 2000).

In addition, smoking process was particularly efficient in the shelf-life extension of sea food characterized by high initial load of microflora ($2.2\text{-}3.3\times10^5$ CFU g^{-1} of mesophiles, $0.05\text{-}3.01\times10^5$ CFU g^{-1} of psychrophiles, 4.2×10^3 CFU g^{-1} of *Staphylococcus micrococcus*, $3\text{-}4.5\times10^5$ CFU g^{-1} of salmonellae) (Mbarki *et al*., 2009; Adebayo-Tayo and Ogunjobi, 2008; Duman *et al*., 2007; Bilgin *et al*., 2008), high level of polyunsaturated fatty acids (>40% of total fatty acids) and rapid sensory deterioration (Selmi and Sadok, 2008; Mbarki *et al*., 2009).

The microflora of molluscan shellfish is more variable than that of fish. It's closely related to the aquatic habitat and varies with factors such as salinity, environmental conditions, bacterial load in the water, water temperature, diet and chilling conditions. The dominant groups of bacteria found on fish and shellfish stored under refrigeration are Pseudomonas, Vibrionaceae, *Shewanella putrefaciens* and Moraxella/Acinetobacter and to a lesser extent, aeromonas and Psychrobacter (Cao *et al*., 2009a).

In the same way, packaging of fresh fishery products after treatment is considered essential in order to prevent microbial contamination during refrigerated storage. Vacuum packaging is widely used in the food

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industry because of its effectiveness in reducing microbial counts and oxidative reactions in the product at relatively low cost (Gopal *et al*., 1999). Consequently, smoking treatment is a barrier which has the potential for seafood preservation when applied in combination with processes such as vacuum packaging at low temperature.

Effects of smoking on oyster odour, taste and flavour have been studied with sensory panels of varying degrees of training. In spite of its importance for new products acceptability and marketing, consumer studies are rather limited. Only limited information is available also in the literature on the shelf-life and microbial flora of Pacific oyster smoked at different temperatures. Microbial flora analysis could gain better perception of the spoilage mechanism which could lead to methods for prolonging the shelf-life and encourage the marketability of oysters.

This study deals with the effects of different smoking procedures and chilled storage period on pacific oysters *Crassostrea gigas* as assessed by microbiological and chemical parameters. Hedonic tests were also performed to assess consumers' perceptions and preferences towards different smoking temperatures.

MATERIALS AND METHODS

Samples collection and preparation: Pacific oysters *Crassostrea gigas* of commercial size measuring 10-12 cm in shell length were collected from the bivalve purification centre M.A. TRAD (Bizerte, Tunisia) in May 2009. Oysters belonging to the same caught samples were kept in ice and transported to the laboratories of High Institute of Fisheries and Aquaculture of Bizerta where they were rapidly scrubbed, rinsed and the meat was aseptically extracted using a sterile knife. The freshly extracted meats were separated in to two lots: the first one was used for raw material, the second lot was processed according to the three smoking methods (S1: Smoking at 45°C; S2: Smoking at 85°C; S3: Smoking at 125°C) described in Fig. 1. All smoking lots were vacuum-packed in polyethylene bags and then stored in a refrigerator at 2-4°C.

In the commercial conditions, chilled smoked products are discarded after one month due to sanitary precautions. Hence, the samples were stored for 4 weeks and the tissue sampling from each lot was performed on days 0, 7, 14, 21, 28.

Proximate composition: Moisture was determined according to the AOAC (1990) Method by drying in oven at 105°C (n = 6). Ash content was determined by burning samples (n = 6) for 12 h in a furnace at 525°C according to

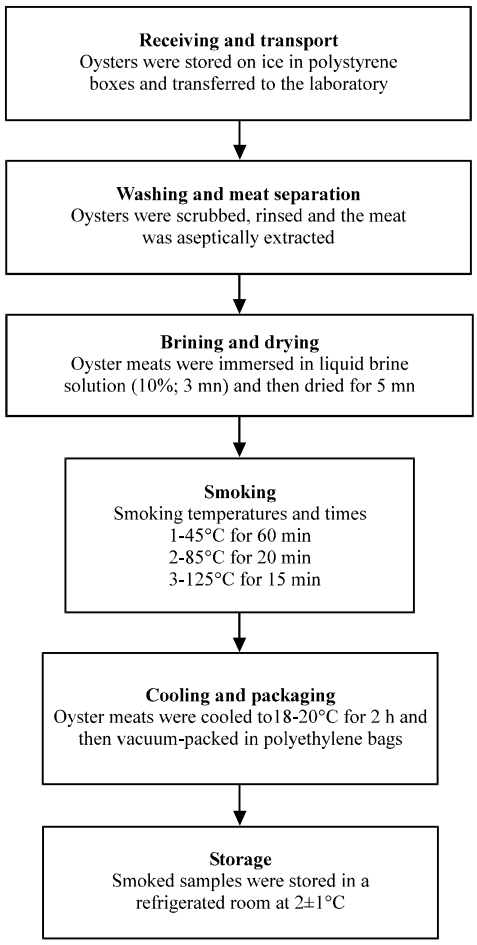

Fig. 1: Fow diagram of the production of smoked pacific oyster

the AOAC (1995) Method. Total protein content in the homogenized samples was determined using the spectrometric method as described by Lowry *et al*. (1951). Carbohydrate content was determined according to the method of DuBois *et al*. (1956). Total lipids were extracted according to the method of Bligh and Dyer (1959) by chloroform/methanol (1:2). The aliquot of chloroform layer was evaporated to dryness under nitrogen and the lipids were quantified gravimetrically.

Liquid-Holding Capacity (LHC): LHC of oyster samples was evaluated using the centrifugation test as described by Gomez-Guillen *et al*. (2000) with slight modification. Samples (10 g) were weighed and placed in a tube with a weighted filter paper (w_1). The tubes were centrifuged at 500 g for 10 min at 10°C and the wet paper was weighted (w_2) before drying at 50°C to constant weight (w_3). S was the weight of oyster sample:

$$\text{Liquid loss (\%)} = \frac{100\times(w_1-w_2)}{S}$$

$$\text{Water loss (\%)} = \frac{100\times(w_2-w_3)}{S}$$

$$\text{Fat loss (\%)} = \frac{100\times(w_3-w_1)}{S}$$

All losses were expressed as percentage of muscle wet weight.

Fatty acid analysis: Fatty Acids Methyl Esters (FAMEs) were obtained by the method described by Metcalfe *et al*. (1966). A fraction of the lipid extract was saponified with 0.5 N NaOH in methanol followed by methylation in 14% boron trifluoride in methanol (BF3-/MeOH). The resulting methyl esters were analysed (n = 4) using an Agilent Gaz Chromatograph System 6890 N equipped with a flame ionization detector, a splitless injector and a polar INNOWAX fused silica capillary column (30×0.25 mm i.d.×0.25 mL film thickness). The temperature of the injector and the detector were 250 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 comparison of their retention times with PUFA 3 FAMEs Standards (SUPELCO).

Thiobarbituric acid reactive substances TBARS: The TBARS was determined according to the AOCS (1998) Method. This procedure allows the direct determination of TBARS in oils and fats without preliminary isolation of secondary oxidation products. Oil sample was solubilized in 1-butanol, mixed with 0.2% TBA in 1-butanol, incubated 2 h in a 95°C water bath and cooled under tap water. The absorbance was measured at 532 nm against a corresponding blank (a reaction with all the reagents and treatments except the oil). Results were expressed as mg malonaldehyde/kg of oil (n = 3).

Microbiological analyses: Microbiological counts were made on fresh and smoked samples in the 1st, 14th and 28th days. The 10 g of sample were taken aseptically into a sterile blender containing sterile peptone water (0.1% w/v) and blended for 4 min at low speed. Volumes of 0.1 mL of decimal dilutions of these homogenates were incubated in the different culture media.

Mesophile and psychrophilic counts were determined using Plate Count Agar (Biokar Diagnostics, Beauvais, France), after incubation for 48 h at 30°C and for 8-10 days at 5°C, respectively. Besides, researchers made the counts of Pseudomonads (NF V 04-504:04, 1998) using Cetrimide Agar (Fluka Biochemika, Steinheim, Switzerland); *Staphylococcus aureus* (NF V 08-057-1, 1994) using Baird-Parker Agar (Biokar Diagnostics, Beauvais, France) and vibrio using TCBS Agar (Thiosulfate Citrate Bile Salts Sucrose Agar). Microbiological data were transformed into logarithms of the number of colony forming units (log CFU/g).

Sensory assessment: The sensory quality of smoked oyster was evaluated at each smoking temperature and sampling time (days 0, 7, 14, 21 and 28) by 76 member consumer panel from the staff and students of the High Institute of Fisheries and Aquaculture of Bizerte. Prior to sensory assessment, samples were removed from the refrigerator and held for 20 min at room temperature. Samples were cut, marked with random three-digit codes and then presented to each panelist in random order. Panelists were asked to evaluate all four parameters (odour, taste, colour and texture) of oyster meat using a 9 point hedonic scale (1 = dislike extremely and 9 = like extremely).

Statistical analysis: Statistical analysis was performed using SPSS Software, Version 10.0.5. The differences among the mean values were processes by Duncan's test (95% confidence interval) with one-way ANOVA.

RESULTS AND DISCUSSION

Proximate composition: Table 1 shows the changes of proximate composition of fresh and smoked oysters during chill storage. In the data, moisture (81.53%), protein (10.52%), lipid (4.13%), carbohydrate (2.36%) and ash (1.74) contents of fresh pacific oysters are comparable

Table 1: Change in proximate composition of pacific oyster *Crassostrea gigas* during different smoking processes and following 28 days of chill storage

			Storage time (day)		
Parameters	Fresh oyster	Smoked method	0	14	28
Moisture	81.53±0.31^{a}	S1	58.44±0.43^{b1}	57.74±0.35^{b1}	58.97±0.51^{b1}
	-	S2	50.17±0.52^{b2}	50.59±0.54^{b2}	51.24±0.65^{b2}
	-	S3	43.87±0.48^{b3}	43.74±0.46^{b3}	43.43±0.47^{b3}
Lipid	4.13±0.21^{a}	S1	9.56±0.32^{b1}	9.18±0.21^{b1}	10.13±0.37^{b1}
	-	S2	11.48±0.29^{b2}	11.41±0.27^{b2}	10.75±0.53^{b1}
	-	S3	12.14±0.42^{b2}	13.39±0.36^{b3}	13.21±0.39^{b2}
Protein	10.52±0.63^{a}	S1	23.86±0.27^{b1}	22.93±0.16^{b1}	21.74±0.36^{b1}
	-	S2	28.86±0.22^{b2}	28.11±0.27^{b2}	27.48±0.42^{b2}
	-	S3	32.17±0.35^{b3}	31.08±0.32^{b3}	31.29±0.23^{b3}
Carbohydrate	2.36±0.26^{a}	S1	6.18±0.09^{b1}	6.31±0.11^{b1}	5.31±0.25^{b1}
	-	S2	6.84±0.13^{b12}	6.89±0.24^{b2}	6.93±0.33^{b2}
	-	S3	7.49±0.16^{b2}	7.21±0.14^{b2}	6.84±0.22^{b2}
Ash	1.74±0.07^{a}	S1	3.43±0.14^{b1}	3.37±0.16^{b1}	3.58±0.12^{b1}
	-	S2	3.52±0.11^{b1}	3.55±0.21^{b1}	3.63±0.09^{b1}
	-	S3	3.68±0.09^{b1}	3.47±0.05^{b1}	3.61±0.08^{b1}

S1: Smoking at 45°C; S2: Smoking at 85°C; S3: Smoking at 125°C. Means with the same letter within line are not significantly different (p>0.05). Means with the same number within row are not significantly different (p>0.05)

to those found in pacific oysters and others shellfish species (Cruz-Romero *et al*., 2007, 2008). Among seafood products, pacific oyster demonstrates an exceptional nutritional value in the human diet being rich in polyunsaturated fatty acids, minerals and vitamins (Cruz-Romero *et al*., 2008; Adebayo-Tayo and Ogunjobi, 2008).

After smoking process, the high increase of lipid, protein and ash levels were due especially to the water losses after processing. The moisture contents decrease with the increase of smoking temperature. So, smoked oysters products lessen with 28.3, 38.5 and 46.2% after processing at 120, 85 and 45°C, respectively. These findings are in accordance with those of Goulas and Kontominas (2005) and Kumolu-Johnson *et al*. (2010) who reported in oyster *Crassostrea* sp. and catfish *Clarias gariepinus* final moisture of about 25.3 and 26.5% after oven drying (127°C) and hot smoking (100°C) processes, respectively.

Industrial specifications for smoked finished products generally recommend water content in the smoked flesh of <65% (Cardinal *et al*., 2001). This is in agreement with the values of all smoked oysters (43.87-58.44%).

Liquid-Holding Capacity (LHC): Changes in Liquid Loss (LL), Fat Loss (FL) and Water Loss (WL) of fresh and smoked oysters during storage are shown in Table 2. Independently of smoking temperatures, the water losses of fresh sample (8.81%) were higher than those of all smoked oysters (0.5-0.53%), however, fat loss remain unchanged after processing. Such results were in accordance with those found by others researchers (Gomez-Guillen *et al*., 2000; Rora *et al*., 2003) who reported that the water loss in salmo fillet decreased significantly during smoking process.

Liquid and fat losses increased significantly with the increase of storage time to reach 1.39-1.78 and 0.88-1% after 28 days of chill storage, respectively. The higher losses were recorded in the samples smoked at 45°C. It was reported that fat loss increases with storage time as occurred in smoked Atlantic salmon fillets due to collagen denaturation caused by cold-smoking (Rora *et al*., 2003). Similarly, Ofstad (1995) suggested also that the liquid loss of fish muscle increases due to the denaturation of collagen during storage.

Fatty acids analysis: Fatty acid profile of oyster during different smoking processes and after 28 days of chill storage is shown in Table 3. In fresh oyster, saturated

Table 2: Liquid holding capacity (expressed as % loss of wet weight) of pacific oyster *Crassostrea gigas* during different smoking processes and following 28 days of chill storage

			Storage time (day)		
Parameters	Fresh oyster	Smoked method	0	14	28
Liquid	9.13±0.39^{a}	S1	0.82±0.04^{b1}	1.34±0.03^{c1}	1.78±0.06^{d1}
		S2	0.77±0.05^{b1}	1.27±0.03^{c12}	1.53±0.05^{d12}
		S3	0.75±0.02^{b1}	1.11±0.02^{c2}	1.39±0.03^{d2}
Water	8.81±0.24^{a}	S1	0.51±0.05^{b1}	0.60±0.04^{b1}	0.79±0.05^{c1}
		S2	0.53±0.04^{b1}	0.61±0.05^{b1}	0.60±0.04^{b2}
		S3	0.5±0.040^{b1}	0.55±0.07^{b1}	0.51±0.07^{b2}
Fat	0.32±0.05^{a}	S1	0.31±0.02^{b1}	0.73±0.05^{c1}	1.00±0.03^{d1}
		S2	0.24±0.01^{b1}	0.66±0.01^{c1}	0.91±0.06^{d1}
		S3	0.25±0.05^{b1}	0.56±0.03^{c1}	0.88±0.05^{d1}

S1: Smoking at 45°C; S2: Smoking at 85°C; S3: Smoking at 125°C. Means with the same letter within line are not significantly different (p>0.05). Means with the same number within row are not significantly different (p>0.05)

Table 3: Fatty acid profile (expressed as g fatty acid/100 g edible oyster) of total oyster *Crassostrea gigas* lipids during different smoking processes and following 28 days of chill storage

		Smoked oysters (0 day)			Smoked oysters (28 days)		
Fatty acid	Fresh oyster	45°C	85°C	125°C	45°C	85°C	125°C
C14:0	0.10±0.01^{a}	0.29±0.02^{b}	0.40±0.01^{c}	0.38±0.02^{c}	0.26±0.04^{b}	0.41±0.03^{c}	0.43±0.03^{c}
C16:0	1.09±0.06^{a}	2.64±0.07^{b}	3.02±0.04^{c}	3.27±0.06^{cd}	2.61±0.08^{b}	2.76±0.40b^{c}	3.43±0.05^{d}
C18:0	0.33±0.02^{a}	0.62±0.05^{b}	0.62±0.04^{b}	0.61±0.02^{b}	0.72±0.04^{b}	0.73±0.05^{b}	0.68±0.02^{b}
Total SFAs	1.52^{a}	3.55^{b}	4.04^{c}	4.26^{cd}	3.59^{b}	3.91^{bc}	4.54^{d}
C16:1n-7	0.14±0.01^{a}	0.37±0.03^{b}	0.40±0.02^{b}	0.39±0.02^{b}	0.34±0.02^{b}	0.37±0.03^{b}	0.42±0.03^{b}
C18:1n-9	0.39±0.02^{a}	0.83±0.06^{b}	0.89±0.02^{b}	1.21±0.04^{c}	0.85±0.03^{b}	0.77±0.05^{b}	1.36±0.02^{c}
C18:1n-7	0.08±0.01^{a}	0.20±0.04^{b}	0.19±0.01^{b}	0.33±0.02^{c}	0.18±0.01^{b}	0.18±0.04^{b}	0.26±0.03^{bc}
C20:1n-9	0.06±0.00^{a}	0.21±0.03^{b}	0.18±0.01^{b}	0.15±0.03^{b}	0.18±0.02^{b}	0.15±0.01^{b}	0.13±0.04^{b}
Total MUFAs	0.67^{a}	1.61^{b}	1.66^{b}	2.08^{c}	1.55^{b}	1.47^{b}	2.17^{c}
C16:2n-4	0.12±0.02^{a}	0.30±0.02^{b}	0.25±0.01^{b}	0.35±0.02^{c}	0.27±0.01^{b}	0.21±0.02^{b}	0.28±0.03^{bc}
C18:2n-6	0.08±0.01^{a}	0.22±0.02^{b}	0.24±0.03^{b}	0.32±0.03^{b}	0.19±0.03^{b}	0.21±0.05^{b}	0.27±0.02^{b}
C16:3n-4	0.05±0.01^{a}	0.03±0.01^{a}	0.05±0.00^{a}	0.05±0.01^{a}	0.02±0.01^{a}	0.15±0.01^{b}	0.06±0.01^{a}
C18:3n-3	0.19±0.01^{a}	0.43±0.02^{b}	0.61±0.02^{cd}	0.68±0.02^{d}	0.38±0.01^{b}	0.53±0.03^{c}	0.72±0.01^{d}
C18:4n-3	0.19±0.03^{a}	0.55±0.02^{b}	0.73±0.01^{c}	0.89±0.03^{d}	0.53±0.01^{b}	0.77±0.02^{c}	0.83±0.03^{dc}
C20:4n-6	0.02±0.01^{a}	0.12±0.01^{b}	0.11±0.00^{b}	0.09±0.01^{b}	0.10±0.03^{b}	0.10±0.01^{b}	0.08±0.01^{b}
C20:4n-3	0.03±0.01^{a}	0.05±0.02^{a}	0.05±0.02^{a}	0.09±0.02^{a}	0.03±0.00^{a}	0.08±0.02^{a}	0.07±0.02^{a}
C20:5n-3	0.23±0.02^{a}	0.60±0.02^{b}	0.72±0.05^{bc}	0.78±0.04^{c}	0.61±0.02^{b}	0.63±0.04^{bc}	0.73±0.02^{c}
C22:5n-3	0.01±0.00^{a}	0.03±0.01^{a}	0.04±0.00^{a}	0.02±0.01^{a}	0.02±0.00^{a}	0.03±0.01^{a}	0.04±0.01^{a}
C22:6n-3	0.17±0.03^{a}	0.52±0.03^{b}	0.51±0.04^{b}	0.75±0.05^{c}	0.50±0.02^{b}	0.64±0.05^{bc}	0.73±0.05^{c}
Total PUFAs	1.09^{7a}	2.85^{b}	3.31^{c}	4.03^{d}	2.65^{b}	3.35^{cd}	3.81^{d}

SFAs: Saturated Fatty Acids; MUFAs: Monounsaturated Fatty Acids; PUFAs: Polyunsaturated Fatty Acids; S1: Smoking at 45°C; S2: Smoking at 85°C; S3: Smoking at 125°C. Means with the same letter within line are not significantly different (p>0.05)

fatty acids SFAs (1.52 g/100 g oyster) constitute the majority of the fatty acids pool followed by PUFAs (1.08 and 100 g oyster) and monounsaturated fatty acids MUFAs (0.67 g/100 g oyster). N-3 and n-6 PUFAs levels were 0.81 and 0.11 g/100 g fresh oyster, respectively in which 22:6n-3, 18:3n-3, 18:4n-3 and 20:5n-3 were the prominent PUFAs. Such findings are in accordance with those found by others researchers (Friedman and Moe, 2006) who reported a high level of n-3 PUFAs in Mediterranean clams.

Significant differences ($p<0.05$) were found in fatty acid content during and between the different smoking processes. Therefore, all fatty acid groups increased ($p<0.05$) after processing with higher levels (10.37 g/100 g oyster) in oyster smoked at 125°C. Independently of smoking temperature, storage period has any effect ($p>0.05$) on fatty acid contents. So, SFAs (4.22 and 100 g oyster), MUFAs (2.08 and 100 g oyster) and PUFAs (3.80 and 100 g oyster) contents remain unchanged after 28 days of chill storage. Such results are in agreement with the data found in fatty acids profile of smoked atlantic salmon during refrigerated storage (Rora *et al*., 2003). These results suggest also the efficiency of smoking process and that smoked oyster is a good source of n-3 and n-6 fatty acids.

PUFAs are especially sensitive to oxidation and degradation phenomena both by enzymatic and chemical oxidation which produces a great variety of volatile compounds (Coutron-Gambotti and Gandemer, 1999; Kolakowska, 2002). Such compounds affect nutritional quality, wholesomeness, safety, colour, flavour and texture. In the data, the stability of PUFAs content in smoked oyster may be due to the combined effect of dehydration and antioxidant activity of the smoke constituents (formaldehyde, carboxylic acids, phenols) (Leroi and Joffraud, 2000; Rorvik, 2000) and the vacuum packaging at low temperature.

Thiobarbituric acid reactive substances: Figure 2 shows the effects of smoking processes and storage time on the formation of thiobarbituric acid reactive substances in oyster lipid. Results indicate that the TBARS levels in fresh lipid oyster increased significantly ($p<0.05$) after smoking and as the storage time increased. The initial value of TBARS was 0.17 mg MA kg^{-1} oil suggesting that lipid oxidation did not occur during post-mortem handling to some extent. This value increased significantly ($p<0.05$) to 0.35, 0.36 and 0.39 mg MA kg^{-1} after smoking at 120, 85 and 45°C, respectively indicating the formation of secondary lipid oxidation products such as aldehydes and others volatiles compounds (Kolakowska, 2002).

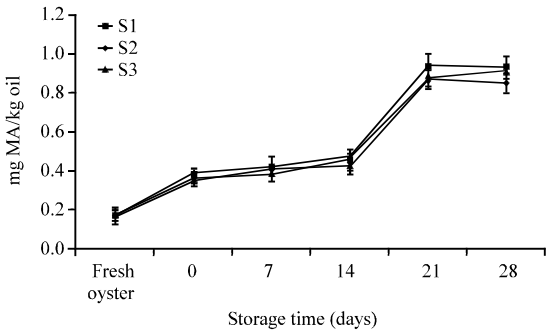

Fig. 2: TBARS values evolution in the muscle of pacific oyster *Crassostrea gigas* during chilled storage as influenced by different smoking temperature. TBARS: Thiobarbituric Acid Reactive Substances; S1: Smoking at 45°C; S2: Smoking at 85°C; S3: Smoking at 125°C; MA: Malonaldehyde. Bars indicate standard deviations from quadruplicate determinations

Such increase may be attributed to the partial dehydration of fish and to the increased oxidation of unsaturated fatty acids as a result of smoking at relatively high temperatures. These findings are in agreement with those reported by Goktepe and Moody (1998) and Goulas and Kontominas (2005) who observed an increase in TBARS of raw catfish and chub mackerel after smoking.

TBARS levels of smoked samples remained constant up to 14 days of chilled storage with no significant differences between the three smoked methods. This fact can be attributed to the antioxidant activity of phenolic constituents of smoke deposited onto the oyster, the chilling condition and to the oxygen barrier properties of the polyethylene bags used in the packages. Thereafter, a significant increase was observed in all lots to reach 0.85, 0.91 and 0.93 mg MDA kg^{-1} after processing at 120, 85 and 45°C, respectively at the end of storage. The final TBARS values of all smoked samples did not exceed the value of 1-2 mg MA kg^{-1} which is usually regarded as the limit beyond which the seafood will normally develop an objectionable odour and taste (Connell, 1990).

Microbiological analyses: The initial loads of mesophiles, psycrophyles and pseudomonas in fresh oyster were 4.11, 5.23 and 0.74 log CFU g^{-1}, respectively. Staphylococcus and Vibrio bacteria haven't been observed in the initial load (Table 4).

The higher level of bacteria in fresh oyster could be due to the fact that deposits feeders are known to ingest sediments and use organic matters and microorganisms in the sediment as food (Jeffrey, 1995). This result was more or less different with previous studies (Cao *et al*., 2009a),

since the microbial flora of freshly caught oysters reflects the microbial flora in the surrounding environment and can be influenced by many factors such as seasonal period, gathering method, etc. Although, the initial microbial counts of fish and shellfish are generally between 2-5 log CFU g^{-1} (Cao *et al*., 2009b).

Smoking at 45, 85 and 125°C significantly lessened the levels of different strains and pseudomonas was completely eradicated ($p<0.05$). Total mesophilic bacteria drops to the interval 3.11-3.47 log CFU g^{-1} and total psychrophilic bacteria drops to the interval 3.87-4.43 log CFU g^{-1}. Such results were in agreement with Bilgin *et al*. (2008) and Colakoglu (2004) which stated that the hot smoking techniques reduced significantly the microbial content of seabream *Sparus aurata*, *Rutilus rutilus* and *Coregenus* sp. compared to fresh fillets.

On the 14th day, the microbial load remains constant with the emergence of pseudomonas and staphylococcus. Afterwards a significant increase was observed in all lots for mesophilic and psychrophilic bacteria to reach 4.62-5.53 and 4.38-4.74 log CFU g^{-1}, respectively at the end of storage. Similar behaviour can be observed in the works reported by Bilgin *et al*. (2008).

It was reported that 6 log CFU g^{-1} is considered as the upper acceptability limit for marine species (Erkan, 2007) and the shelf-life of seafood is limited by the growth and biochemical activities of Gram-negative, psychrotrophic strains of *Pseudomonas*, *Achromabacter*, *Flavobacterium* and *Moraxella* species in presence of atmospheric oxygen (Huss, 1994). Hence, smoking combined with vacuum packaging is an interesting method of preservation to reduce qualitatively and quantitatively the microbial population in fishery products (Mendes *et al*., 2005).

Sensory assessment: Sensory evaluation was examined after smoking processes and once a week over 28 days and their results are presented in Table 5. The differences in all sensory parameters measured over a period of 28 days were significant ($p<0.05$). However, there was no significant difference ($p>0.05$) in odour and colour within the 1st 21 days after different smoking processes and the appearance of smoked samples were scored as excellent.

Collected data showed that hot smoked samples (85 and 120°C) were perceived to be significantly ($p<0.05$) more acceptable than other samples, independent of storage time. The majority of consumers related their choice to the specific taste and the soft juicy texture. Texture and juiciness of muscle foods strongly influences and drives the perception of consumers (Szczesniak, 1991; Risvik, 1994). On the same way, Risvik (1994) proposed a simplified model for understanding texture where water/fat perceptions and structure perception (described as juiciness and tenderness) are orthogonal phenomena by which most of the other textural characteristics can be explained.

In general, as the time of storage progressed, the sensory properties of smoked oyster decreased with

Table 4: Changes in microbial counts in the muscle of pacific oyster *Crassostrea gigas* after smoking and storage at 2±1°C

			Storage time (day)		
Bacteria (log CFU/g)	Fresh oyster	Smoked method	0	14	28
Total mesophilic count	4.11	S1	3.47	3.53	4.74
		S2	3.11	3.18	4.52
		S3	3.14	3.21	4.38
Total psychrophilic bacteria	5.23	S1	4.43	4.63	5.53
		S2	4.09	4.17	4.76
		S3	3.87	3.96	4.62
Pseudomonas	0.17	S1	-	0.44	1.07
		S2	-	0.38	0.83
		S3	-	0.41	0.79
Staphylococcus	-	S1	-	0.31	0.48
		S2	-	0.22	0.34
		S3	-	0.25	0.37
Vibrio	-	S1	-	-	0.21
		S2	-	-	0.11
		S3	-	-	0.13

S1: Smoking at 45°C; S2: Smoking at 85°C; S3: Smoking at 125°C

Table 5: Sensory evaluation of vacuum packaged pacific oyster *Crassostrea gigas* during different smoking processes and following 28 days of chill storage

		Storage time (days)				
Parameters	Smoked method	0	7	14	21	28
Odour	S1	7.03±0.19^{a}	7.16±0.08^{a}	7.11±0.13^{a}	7.13±0.22^{a}	5.66±0.26^{b}
	S2	7.76±0.11^{a}	7.83±0.13^{a}	7.69±0.20^{a}	7.74±0.17^{a}	6.04±0.21^{b}
	S3	7.53±0.14^{a}	7.49±0.22^{a}	7.57±0.13^{a}	7.71±0.11^{a}	6.22±0.19^{b}
Taste	S1	5.44±0.24^{a}	5.53±0.19^{a}	5.39±0.09^{a}	4.66±0.11^{b}	4.03±0.34^{b}
	S2	6.93±0.26^{a}	6.85±0.09^{a}	6.89±0.13^{a}	6.33±0.07^{b}	5.87±0.28^{b}
	S3	6.82±0.22^{a}	6.77±0.17^{a}	6.91±0.18^{a}	6.14±0.23^{b}	6.27±0.19^{b}
Colour	S1	6.66±0.11^{a}	6.52±0.24^{a}	6.63±0.16^{a}	6.11±0.16^{a}	4.89±0.36^{b}
	S2	6.97±0.27^{a}	6.83±0.20^{a}	6.77±0.09^{a}	6.85±0.33^{a}	5.22±0.33^{b}
	S3	7.06±0.10^{a}	7.11±0.27^{a}	6.96±0.10^{a}	6.89±0.21^{a}	5.13±0.21^{b}
Texture	S1	6.13±0.26^{a}	6.22±0.14^{a}	5.49±0.12^{b}	5.36±0.17^{b}	5.09±0.17^{b}
	S2	7.83±0.14^{a}	7.88±0.18^{a}	7.19±0.14^{b}	6.36±0.12^{b}	6.42±0.12^{b}
	S3	7.62±0.18^{a}	7.55±0.13^{a}	7.59±0.17^{a}	6.58±0.12^{b}	6.54±0.12^{b}

S1: Smoking at 45°C; S2: Smoking at 85°C; S3: Smoking at 125°C. Means with the same letter within line are not significantly different ($p>0.05$)

lowers values obtained at lower smoking temperature (45°C). Thereafter, the general taste reached 4.92, 5.89 and 6.04 after processing at 45, 85 and 120°C, respectively at the end of storage. Such finding may attribute to the best deposition of smoke compounds (formaldehyde, carboxylic acids, phenols) at higher temperatures (Cardinal *et al*., 2001). Goulas and Kontominas (2005) reported also that the sensory scores of smoked chub mackerel *Scomber japonicus* in 50% smoke/50% air is higher than those smoked at 100% air.

It is also important to note the effectiveness of vacuum packaging in reducing microbial counts and oxidative reactions which affects the sensory seafood quality (Mbarki *et al*., 2009).

CONCLUSION

Results of the present study showed that smoking process had a significant effect on microbial load and fatty acid content. It was found that processing cause immediately 1 log reduction in mesophilic bacteria and increase n-3 and n-6 fatty acids content. Based on sensory evaluation, all smoking process led to an appreciated product with better sensory characteristics (taste and texture) especially oysters smoked at 125°C. Such treatments can technically and economically used as a safe method for preservation and exhibit potential advantages for shellfish industry, especially in hot countries.

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REFERENCES

AOAC, 1990. Official Methods of Analysis of the Association of Analytical Chemists. 16th Edn., Association of Official Analytical Chemists, Washington, DC.

AOAC, 1995. Official Methods of Analysis of the Association of Official Analytical Chemists. 16th Edn., Vol. 1, AOAC International, Arlington, VA., USA.

AOCS, 1998. Official Method Cd 19-90, 2-Thiobarbituric Acid Value-Direct Method. In: Official Methods and Recommended Practices of the American Oil Chemists' Society, Firestone, D. (Ed.). 5th Edn., Association of Official Analytical Chemists, Champaign, IL., pp: 19-90.

Adebayo-Tayo, B.C. and A.A. Ogunjobi, 2008. Comparative effects of oven drying and sun drying on the microbiological, proximate nutrient and mineral composition of *Trypanotonous* spp. (Periwinkle) and *Crassotrea* spp. (Oyster). Electron. J. Environ. Agric. Food Chem., 7: 2856-2862.

Bilgin, S., M. Unlusayin, L. Izci and A. Gunlu, 2008. The determination of the shelf life and some nutritional components of gilthead seabream (*Sparus aurata* L., 1758) after cold and hot smoking. Turk. J. Vet.Anim. Sci., 32: 49-56.

Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.

Cao, R., C.H. Xue, Q. Liu and Y. Xue, 2009a. Microbiological, chemical and sensory assessment of Pacific oysters (*Crassostrea gigas*) stored at different temperatures. Czech J. Food Sci., 27: 102-108.

Cao, R., C.H. Xue and Q. Liu, 2009b. Changes in microbial flora of Pacific oysters (*Crassostrea gigas*) during refrigerated storage and its Shelf-life extension by chitosan. Int. J. Food Microbiol., 131: 272-276.

Cardinal, M., C. Knockaert, O. Torrissen, S. Sigurgisladottir and T. Morkore *et al*., 2001. Relation of smoking parameters to the yield, color and sensory quality of smoked Atlantic salmon (*Salmo salar*). Food Res. Int., 34: 537-550.

Chaijan, M., S. Benjakul, W. Visessanguan and C. Faustman, 2006. Changes of lipids in sardine (*Sardinella gibbosa*) muscle during iced storage. Food Chem., 99: 83-91.

Colakoglu, F.A., 2004. The effects of processing techniques on microflora of roach (*Rutilus rutilus*) and whitefish (*Coregenus* sp). Turk. J. Vet. Anim. Sci., 28: 239-247.

Connell, J.J., 1990. Methods of Assessing and Selecting for Quality. In: Control of Fish Quality, Connell, J.J. (Ed.). Fishing News Books, Oxford, pp: 122-150.

Coutron-Gambotti, C. and G. Gandemer, 1999. Lipolysis and oxidation in subcutaneous adipose tissue during dry-cured ham processing. Food Chem., 64: 95-101.

Cruz-Romero, M., A.L. Kelly and J.P. Kerry, 2007. Effects of High-pressure and heat treatments on physical and biochemical characteristics of oysters (*Crassostrea gigas*). Innovative Food Sci. Emerg. Technol., 8: 30-38.

Cruz-Romero, M.C., J.P. Kerry and A.L. Kelly, 2008. Fatty acids, volatile compounds and colour changes in High-pressure-treated oysters (*Crassostrea gigas*). Innovative Food Sci. Emerg. Technol., 9: 54-61.

Doe, P.E., 1998. Fish Drying and Smoking Production and Quality. In: Basic Principles, Doe, P.E. (Ed.). Technomic Publishing, Lancaster, PA., pp: 13-45.

DuBois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.

Duman, M., B. Patir, E. Duman and O.I. Ilhak, 2007. The effects of salt and storage temperature on microbiological changes in Hot-smoked mirror carp (*Cyprinus carpio* L.). Pak. J. Biol. Sci., 10: 3002-3005.

Erkan, N., 2007. Sensory, chemical and microbiological attributes of sea bream (*Sparus aurata*): Effect of washing and ice storage. Int. J. Food Properties, 10: 421-434.

Friedman, A. and S. Moe, 2006. Review of the effects of omega-3 supplementation in dialysis patients. Clin. J. Am. Soc. Nephrol., 1: 182-192.

Goktepe, I. and M.W. Moody, 1998. Effect of modified atmosphere packaging on the quality of smoked catfish. J. Muscle Foods, 9: 375-389.

Gomez-Guillen, M.C., P. Montero, O. Hurtado and A.J. Borderias, 2000. Biological characteristics affect the quality of farmed atlantic salmon and smoked muscle. J. Food Sci., 65: 53-60.

Gopal, T.K.S., J. Joseph and K.K. Balachandran, 1999. Development of Fish Products Employing Hurdle Technology. In: Preservation of Food by Hurdle Technology, Gopal, T.K.S., J. Joseph and K.K. Balachandran (Ed.). DFRL, Mysore, pp: 93-103.

Goulas, A.E. and M.G. Kontominas, 2005. Effect of salting and Smoking-method on the keeping quality of chub mackerel (*Scomber japonicas*): Biochemical and sensory attributes. Food Chem., 93: 511-520.

Huss, H.H., 1994. Assurance of seafood quality. FAO Fisheries Technical Paper, Food and Agriculture Organization of the United Nations Rome, pp: 298-334.

Jeffrey, S.L., 1995. Marine Biology, Function, Biodiversity, Ecology. Oxford University Press Inc., USA., pp: 394-399.

Kolakowska, A., 2002. Lipid Oxidation in Food Systems. In: Chemical and Functional Properties of Food Lipids, Sikorski, Z. and A. Kolakowska (Eds.). CRC Press, London, UK., pp: 133-165.

Kumolu-Johnson, C.A., N.F. Aladetohun and P.E. Ndimele, 2010. The effects of smoking on the nutritional qualities and shelf-life of *Clarias gariepinus* (BURCHELL 1822). Afr. J. Biotechnol., 9: 73-76.

Leroi, F. and J.J. Joffraud, 2000. Salt and smoke simultaneously affect chemical and sensory quality of cold-smoked salmon during 5C storage predicted using factorial design. J. Food Protect., 63: 1222-1227.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Mbarki, R., N. ben Miloud, S. Selmi, S. Dhib and S. Sadok, 2009. Effect of vacuum packaging and low-dose irradiation on the microbial, chemical and sensory characteristics of chub mackerel (*Scomber japonicas*). Food Microbiol., 26: 821-826.

Mendes, R., H.A. Silva, M.L. Nunes and J.M.A. Empis, 2005. Effect of Low-dose irradiation and refrigeration on the microflora, sensory characteristics and biogenic amines of Atlantic horse mackerel (*Trachurus trachurus*). Eur. Food Res. Technol., 221: 329-335.

Metcalfe, L.D., A.A. Schmitz and J.R. Pelka, 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem., 38: 514-515.

Ofstad, R., 1995. Microstructure and Liquid-holding capacity in cod (*Gadhus morhua* L.) and salmon (*Salmo salar*) muscle: Effects of heating. Ph.D. Thesis, University of Tromso, Norway.

Risvik, E., 1994. Sensory properties and preferences. Meat Sci., 36: 67-77.

Rora, A.M.B., C. Regost and J. Lampe, 2003. Liquid holding capacity, texture and fatty acid profile of smoked fillets of Atlantic salmon fed diets containing fish oil or soybean oil. Food Res. Int., 36: 231-239.

Rorvik, L.M., 2000. *Listeria monocytogenes* in the smoked salmon industry. Int. J. Food Microbiol., 62: 183-190.

Selmi, S. and S. Sadok, 2008. The effect of natural antioxidant (*Thymus vulgaris* Linnaeus) onflesh quality of tuna (*Thunnus thynnus* (Linnaeus)) during chilled storage. Pan-Am. J. Aquat. Sci., 3: 36-45.

Szczesniak, A., 1991. Textural perceptions and food quality. J. Food Quality, 14: 75-85.