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Fatty acid composition of fresh and smoked Black and Caspian Sea sprat, *Clupeonella cultriventris* (Nordmann, 1840) treated with different salt composition

E. Zakipour Rahimabadi*[1, 2], S. Faralizadeh[1], A.A. Khanipour[3]

1. Department of Fisheries, Faculty of Natural Resources, University of Zabol, Zabol, Iran

2. Department of Fisheries, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, Guilan, Iran

3. Iranian Fisheries Science Research Institute. Inland Waters Aquaculture Research Center Agricultural Research Education and Extension Organization (AREEO), Bandar Anzali, Iran.

** Corresponding author's E-mail: e_zakipour@yahoo.com*

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ABSTRACT

The effect of salting by different salt composition on fatty acid profile studied in hot smoked Black and Caspian Sea sprat, *Clupeonella cultriventris*. After initial prepare activities, samples were exposed to brining process within two salt concentrations including of 100 % NaCl (treatment 1 or T_1) and 75 % NaCl- 25 % KCl (treatment 2 or T_2) under 15% brine solution for 3 hrs. The smoking process included turning the samples into drying and hot smokes by slow and high rates of smoking machine (Atmoos) set and packaged. Fatty acid profiles of treated samples were compared with fresh fish samples (control). Smoking process decreased the content of SFA and contemporary increased the content of PUFA in comparison to control samples. These changes were slightly higher in T_2. N-3/n-6 ratio of control samples (7.30) was increased in T_1 samples (7.71) and decreased in T_2 samples (6.86) after smoking process. Atherogenic index (AI) value was decreased after smoking process in both treatments with higher decrement for T_2. Thrombogenic index (TI) value was also decreased after smoking process in both treatments. Partial sodium replacement did not affect fatty acid composition, PUFAs content, AI and TI values.

Key words: *Clupeonella cultriventris*, Fatty acid profile, Sodium replacement, Hot smoking.

INTRODUCTION

Smoking is a traditional fish preservation method that also provides a specific flavor and color to the fish. It also increases the shelf-life of fish as a result of combination of salting, drying and deposition of smoke components. Salting or brining is one of the smoking steps which are necessary for its functional properties such as flavor, texture and preservation (Fuentes *et al.* 2010).

It is well documented that nutritional values of food is affected by processing. For example, impurities in the salt, during brining and also drying and heating during the process effect on lipid oxidation in seafood (Stołyhwo *et al.* 2006). In addition, salt content and the type of salt used in salted and smoked seafood are other problems regarding to health issues.

Excessive dietary sodium intake, which is higher than recommended in many countries, causes high blood pressure, leads cardiovascular disease, renal disease, gastric cancer, decreased bone mineral density and possibly obesity (Devine *et al.* 1995; Tsugane *et al.* 2004; Cutler & Roccella 2006; Liem *et al.* 2011). Because of growing awareness about these kinds of diet related diseases and improving the quality and increasing the healthy aspects of final products, in recent years, there has been a tendency for sodium reduction in foods. Replacing sodium with potassium, which is also called smart salt

(Sarkkinen *et al.* 2011), is the most common way to reduce the amount of sodium in human dietary (Geleijnse *et al.* 2007; Bidlas & Lambert 2008; Fuentes *et al.* 2010; Fuentes *et al.* 2011). Sodium and potassium have similar properties and partial replacement of NaCl by KCl has not been linked with major of the diet-related diseases and does not adversely effect on quality, sensory and shelf life of smoked Black and Caspian Sea sprat (Faralizadeh *et al.* 2015; Faralizadeh *et al.* 2016) and smoked sea bass (Geleijnse *et al.* 2007; Fuentes *et al.* 2010; Fuentes *et al.* 2011).

The sensory analysis of smoked Black and Caspian Sea sprat shows that 25% sodium replacement by potassium is feasible for this product (Faralizadeh *et al.* 2015).

However, complete replacement of NaCl by KCl has not been recommended due to stronger bitterness and metallic taste and also some harmful effects to individuals (Gillette 1985; Smith & Van der Klaauw 1995; Appel *et al.* 2006).

The smoking process causes changes in fatty acid composition of food due to oxidation (Beltrán & Moral 1991).

Although the effects of sodium replacement on quality, sensory and shelf life of fish products, has been well studied, there is not through knowledge about this replacement on fatty acid composition of fish.

Therefore, the aim of this work was to study the effects of the sodium chloride replacement by potassium chloride on fatty acid composition of hot smoked Black and Caspian Sea sprat.

MATERIALS AND METHODS

Raw material

45 kg of freshly Black and Caspian Sea sprat, *Clupeonella cultriventris* was purchased from a fishing boat in Anzali, Guilan Province, Iran in November 2012.

The fish were placed in CSW boxes (containing 25 % ice powder, 15 % sea water and 60 % fish) and transported to the National Fish Processing Research Center (Anzali, Iran) within 2 h.

They were immediately washed, headed, eviscerated and re-washed.

Salting and hot smoking of fish

The fish were brined in 15 % salt solution for 3 h at 5 °C in two different treatments: salt solution with 100 % of NaCl (T_1) and 25 % KCl/ 75 % NaCl (T_2).

Then, the fish were washed with tap water for 1 min and air-dried on stainless steel drainer at 40 °C. Hot smoked were prepared according to specific way of fish smoking of National Fish Processing Research Center with an industrial smoking machine – Atmoos (Germany) using *Fagus orientalis* wood. At the first step, the fish samples were surface dried in Atmoos at 40 °C for 50 min to reduce the moisture content from 80 to 60 %. During the next three hours the temperature was gradually increased to 60 °C, 75 °C and finally 85 °C. The entire smoking process took about 4 hours. Finally, the smoked fish were cooled.

Lipid extraction and fatty acid analysis

The procedure used for the lipid extraction was based on Bakar *et al.* (2008). About 20 g of fish muscle were homogenized in a warring blender for 2 min with a mixture of 20 ml chloroform and 50 ml methanol. One volume of chloroform (20 ml) and distilled water (20 ml) were added to the mixture and blended for 30 sec, respectively.

The homogenate was then filtered, and transferred to a reparatory funnel for phase separation. The lower fraction was collected and filtered. It was then transferred to a rotary evaporator for evaporation. Lipid samples were converted to their constituent fatty acid methyl esters by the method of Metcalf *et al.* (1996). Analysis of fatty acid methyl esters was performed on a Shimadzu GC (Shimadzu, 17A, Japan) with a bpx 7 column (30 m x 0.25 mm, film thickness) and quantified by FID detector. The split ratio was 10:1. The GC conditions were as follows: injection port temperature was 250 °C; flame ionization detector temperature was 280 °C.

The oven was set at an initial temperature of 160 °C for 6 min and then raised to 200 °C at 20 °C.min^{-1} and held at 200 °C for 5 min. The carrier gas was helium.

The sample amount injected for each analysis was 1 ml. Samples were manually injected into the GC port. Compounds were identified by comparison with the retention times of known standards.

Nutritional quality

The propensity of fresh and smoked Black and Caspian Sea sprat, *Clupeonella cultriventris* tissue to promote the incidence of coronary heart disease, atherogenic (AI) and thrombogenic (TI) indices were calculated by using the Barrento *et al.* (2010) equations (see below Eq.).

$$AI = \frac{[12{:}0 + (4 \times 14{:}0) + 16{:}0]}{[\sum \text{MUFA} + \sum \text{PUFA (n}-6) + (\text{n}-3)]}$$

$$TI = \frac{(14{:}0 + 16{:}0 + 18{:}0)}{[(0.5 \times \sum \text{MUFA}) + (0.5 \times \sum \text{PUFA (n}-6) + \left(3 \times \sum \text{PUFA (n}-3) + \left(\frac{\text{n}-3}{\text{n}-6}\right)\right)]}$$

Statistical analysis

The data were analyzed using the one way analysis of variance test (ANOVA).

The Tukey's test was used for mean comparison when a significant variation was found by the ANOVA test. The significance of results was at 5%. The software used was Minitab, release 13.

RESULTS AND DISCUSSION

Table 1 shows the fatty acids compositions of *C. cultriventris*. A total of twenty nine fatty acids were detected using GC in raw and smoked samples. In raw (control) samples, MUFA was the most abundant group of fatty acids followed by SFA and PUFA. A similar pattern was found in sturgeon species (Badiani *et al.* 1996; Nikoo *et al.* 2010), while different patterns were reported by Öksüz & Özyilmaz (2010) and Stołyhwo *et al*. (2006) for Black Sea Anchovy and Baltic sprats, respectively. In Black Sea Anchovy, SFA was the most abundant group of fatty acids followed by PUFA and MUFA and in Baltic sprats, PUFA was the most abundant group of fatty acids. Environmental factors, such as diet, season and temperature as well as biological differences such as age, sex and sizes are known factors which affect the lipid content and fatty acid composition (Sigurgisladóttir & Pálmadóttir 1993).

In the raw samples, the amount of fatty acids in the descending order were C18:1> C16:0> C22:6 n-3. The smoked samples using two different salt compositions showed similar changes in group of fatty acids and the same patterns as control. Although in both smoked samples, MUFA was the most abundant group of fatty acids followed by SFA and PUFA, the smoking decreased the content of SFA and at the same time increased the content of PUFA. These changes were slightly higher in T_2 (25 % KCl/ 75 % NaCl). The amount of fatty acids in both smoked samples at descending order were also C18:1c > C16:0> C22:6 n-3c.

Heterogeneous changes were found in fatty acid concentration after smoking of samples cured by two different salt compositions. Some distinct trends with regard to alterations in fatty acid composition were as follows: the content of C16:0 and C14:0 of SFAs and C18:1c of MUFAs decreased; while the content of C20:5 n-3c (EPA) and C22:6 n-3c (DHA) of PUFAs increased after smoking. The increase in PUFA content (especially increases in EPA and DHA) in this work, disagrees with the known results about the smoked sardine reported by Beltrán & Moral (1991) and in milkfish reported by Swastawati (2004). Conversely, some authors recorded practically no change in the fatty acid composition of fish after smoking (Bhuiyan *et al.* 1986).

It is mentioned that diet of fish and proximate composition of raw fish (Røra *et al.* 2002), smoking duration (Swastawati 2004) and method of smoking (Adeyemi *et al.* 2013; Bouriga *et al.* 2012) are important factors affecting the fatty acid composition of smoked fish.

Higher PUFAs content after smoking may be explained by the fact that SFA and MUFA are largely represented in neutral lipids and are more prone to migration from food during process (Enser *et al.* 1996; Badiani *et al.* 2002). A ratio of n-3/n-6 ratio in control (7.30) increased in T_1 after smoking (to 7.71) while decreased in T_2 (to 6.86). In fact, after smoking, the content of n-3 PUFAs and also n-6 PUFAs increased in both treatments.

The increment of n-3 PUFAs was slightly higher in T_1, while the increment of n-6 PUFAs was slightly higher in T_2 which affected the n-3/n-6 ratio (Table 2). The results found by Adeyemi *et al.* (2013) shows different effects on the n-3/n-6 ratio of *Trachurush trachurus* using different smoking method. The n-3/n-6 ratio of *T. trachurus* increased after charcoal smoking and decreased after wood smoking, respectively. MUFA+PUFA/SFA ratio increased from 1.47 in control samples to 1.78 and 1.73 in T_1 and T_2, respectively. The C22:6/C16:0 ratios also increased after smoking in both treatments (Table 2).

Table 1. Fatty acid composition (g.100g^{-1} fatty acids) of smoked *Clupeonella cultriventris* with different salt at salting process.

Fatty acid	Control	Salt composition	
		NaCl (100 %)	NaCl/ KCl (75 % /25 %)
Dodecanoic acid (C12:0)	0.00 ± 0.00	0.11 ± 0.00 ^{a}	0.11 ± 0.00 ^{a}
Tridecanoic acid (C13:0)	0.07 ± 0.00 ^{a}	0.00 ± 0.00	0.00 ± 0.00
Tetradecanoic acid (C14:0)	5.03 ± 0.14 ^{a}	4.88 ± 0.04 ^{a}	4.88 ± 0.14 ^{a}
Pentadecanoic acid (C15:0)	0.97 ± 0.04 ^{a}	0.82 ± 0.04 ^{a}	0.87 ± 0.04 ^{a}
Hexadecanoic acid (C16:0)	26.41 ± 0.75 ^{a}	22.47 ± 0.71 ^{b}	23.95 ± 0.42 ^{ab}
Heptadecanoic acid (C17:0)	0.26 ± 0.01 ^{b}	0.00 ± 0.00	1.23 ± 0.01 ^{a}
Octadecanoic acid (C18:0)	5.45 ± 0.21 ^{a}	3.99 ± 0.08 ^{b}	3.70 ± 0.07 ^{b}
Icosanoic acid (C20:0)	0.24 ± 0.00 ^{b}	2.42 ± 0.18 ^{a}	0.14 ± 0.03 ^{b}
Docosanoic acid (C22:0)	0.71 ± 0.04 ^{a}	0.35 ± 0.20 ^{a}	0.77 ± 0.01 ^{a}
Tetracosanoic acid (C24:0)	0.26 ± 0.03 ^{a}	0.41 ± 0.08 ^{a}	0.31 ± 0.01 ^{a}
∑ SFA	39.40	35.45	35.96
(Z)-Tetradec-9-enoic acid (C14:1)	0.70 ± 0.07 ^{a}	0.68 ± 0.03 ^{a}	0.00 ± 0.00
cis-10-Pentadecenoic acid (C15:1)	0.20 ± 0.08 ^{a}	0.14 ± 0.00 ^{a}	0.19 ± 0.06 ^{a}
Hexadec-9-enoic acid (C16:1)	5.88 ± 0.25 ^{b}	6.00 ± 0.28 ^{b}	7.24 ± 0.17 ^{a}
cis- 10-Heptadecenoic acid (C17:1)	0.91 ± 0.14 ^{a}	1.17 ± 0.21 ^{a}	1.05 ± 0.07 ^{a}
(E)-Octadec-9-enoic acid (C18:1 t)	0.87 ± 0.07 ^{a}	0.34 ± 0.01 ^{b}	0.38 ± 0.14 ^{b}
(9Z)-Octadec-9-enoic acid (C18:1 c)	30.29 ± 0.72 ^{a}	27.40 ± 0.21 ^{ab}	26.57 ± 0.88 ^{b}
(Z)-Eicos-11-enoic acid (C20:1)	0.15 ± 0.04 ^{b}	0.47 ± 0.01 ^{b}	2.20 ± 0.21 ^{a}
(Z)-Docos-13-enoic acid (C22:1)	0.06 ± 0.00 ^{b}	2.15 ± 0.27 ^{a}	0.05 ± 0.00 ^{b}
(Z)-Tetracos-15-enoic acid (C24:1)	0.37 ± 0.04 ^{b}	0.56 ± 0.03 ^{a}	0.36 ± 0.04 ^{b}
∑ MUFA	39.43	38.92	38.04
(9E,12E)-Octadeca-9,12-dienoic acid (C18-2 n-6 t)	0.15 ± 0.01 ^{a}	0.13 ± 0.00 ^{ab}	0.10 ± 0.01 ^{b}
(9Z,12Z)-9,12-Octadecadienoic acid (C18-2 n-6 c)	1.52 ± 0.42 ^{a}	2.01 ± 0.01 ^{a}	2.32 ± 0.28 ^{a}
all- cis- 9,12,15- Octadecatrienoic acid (ALA, C18:3 n-3 c)	1.26 ± 0.14 ^{a}	0.16 ± 0.14 ^{b}	1.38 ± 0.04 ^{a}
all-cis-6,9,12-Octadecatrienoic acid (GLA, C18:3 n-6 c)	0.11 ± 0.00 ^{a}	0.13 ± 0.01 ^{a}	0.11 ± 0.05 ^{a}
all-cis-6,9,12,15-Octadecatetraenoic acid (C18:4 n-3 c)	1.88 ± 0.28 ^{a}	0.00 ± 0.00	0.00 ± 0.00
all-cis-11,14-Eicosadienoic acid (C20:2 n-6 c)	0.15 ± 0.07 ^{a}	0.08 ± 0.00 ^{a}	0.15 ± 0.01 ^{a}
all-cis-5,8,11,14-Eicosatetraenoic acid (C20:4 n-6 c)	0.32 ± 0.04 ^{a}	0.41 ± 0.04 ^{a}	0.39 ± 0.07 ^{a}
all-cis-5,8,11,14,17-Icosapentaenoic acid (C20:5 n-3 c)	4.51 ± 0.71 ^{a}	6.16 ± 0.71 ^{a}	5.74 ± 0.07 ^{a}
all-cis-7,10,13,16,19-docosapentaenoic acid (C22:5 n-3 c)	0.32 ± 0.03 ^{a}	0.48 ± 0.07 ^{a}	0.34 ± 0.00 ^{a}
all-cis-docosa-4,7,10,13,16,19-hexaenoic acid (C22:6 n-3 c)	8.46 ± 0.64 ^{b}	14.48 ± 0.32 ^{a}	13.62 ± 0.72 ^{a}
∑ PUFA	18.68	24.04	24.15
Other fatty acids	2.47 ± 0.14 ^{a}	2.47 ± 0.24 ^{a}	1.95 ± 0.14 ^{a}

Values are means and S.D. of duplicate; Means with the same letter within a row were not significantly different at P<0.05 level.

The atherogenic (AI) and thrombogenic (TI) indices in control samples were 0.80 and 0.47, respectively (Table 2). Values obtained in this study are slightly higher than results reported

from other seafood such as horse mackerel (*Trachurus trachurus*) and bouge (*Boops boops*) by Orban *et al.* (2011), raw roes of blue fin tuna (*Thunnus thynnus* L.) by Garaffo *et al.* (2011), for red porgy (*Pagrus pagrus*) by García-Romero *et al.* (2014) probably due to smoking process; while it is lower than other animal foods like goat milk (Osmari *et al.* 2011). Atherogenic index decreased after smoking in both treatments with higher decrement for T_2. Thrombogenic index was also decreased after smoking in both treatments (Table 2).

These results disagree with the results found by Garaffo *et al.* (2011) who report an increased AI and TI values for cured products of roes in blue fin tuna.

Decreases in SFAs content after smoking of Black Sea anchovy could be reason of decreasing in AI and TI.

Table 2. Significant ratios in fatty acid composition of smoked *Clupeonella cultriventris* with different salt at salting process.

Ratios	Control	Salt composition	
		NaCl (100 %)	NaCl/ KCl (75 %/25 %)
∑ n-3	16.43	21.28	21.08
∑ n-6	2.25	2.76	3.07
∑ n-3/ ∑ n-6	7.30	7.71	6.86
∑ EPA+DHA	12.97	20.64	19.36
MUFA+PUFA/SFA	1.47	1.78	1.73
C22:6/C16:0	0.32	0.64	0.57
AI	0.80	0.76	0.69
TI	0.47	0.23	0.35

The Black and Caspian Sea sprat is a rich source of n-3 long chain polyunsaturated fatty acids and has a good nutritional value regarding to n-3/n-6 ratio as well as AI and TI values. Pepping (1999) pointed out that the human body's optimal balance between omega-6 and omega-3 fatty acids is a 2:1 to 4:1 ratio.

So, higher n-3/n-6 ratio of the fish can help this ratio balance in human body which has been changed in recent decades due to changes.

The atherogenic (AI) and thrombogenic (TI) indices, as proposed by Barrento *et al.* (2010), give an indication of the attitude of a composite diet or a single food to protect from atherosclerosis and platelets aggregation. Good values of atherogenicity (AI) and thrombogenicity (TI) indices of the Black and Caspian Sea sprat also could promote a positive effect on nutritional value for human consumption.

Salting process of the Black and Caspian Sea sprat by two different salt compositions followed by smoking provide good parameters with higher PUFAs content and lower AI and TI values in both treatments (Tables 1- 2).

Partial replacement of sodium by potassium in processed fish, in addition to supplying a highly nutritive food, may be useful to achieve the recommended salt intake level of 5-6 grams per day (Sarkkinen *et al.* 2011) to treating hypertension. The results of present study showed that partial sodium replacement did not affect negatively the fatty acid profile and nutritional value of smoked Black and Caspian Sea sprat regarding to PUFAs content and AI and TI values.

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ترکیب اسید چرب کیلکای معمولی (*Clupeonella cultriventris*) تازه و دودی شور شده با نمک‌های مختلف

الف. زکی‌پور رحیم‌آبادی [۱و۲*]، س. فرعلیزاده[۱]، ع.ا. خانی‌پور[۳]

۱- گروه شیلات، دانشکده منابع طبیعی، دانشگاه زابل، زابل، ایران

۲- گروه شیلات، دانشکده منابع طبیعی، دانشگاه گیلان، صومعه‌سرا، ایران

۳- مرکز ملی تحقیقات فرآوری آبزیان، بندر انزلی، ایران

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چکیده

تاثیر ترکیب نمک مصرفی هنگام شورکردن ماهی کیلکای معمولی (*Clupeonella cultriventris*) در فرآیند دودی گرم بر ترکیب اسید چرب آن بررسی شد. برای این منظور، ماهی‌ها پس از آماده‌سازی اولیه، با استفاده از دو ترکیب نمکی شامل ۱۰۰٪ NaCl (تیمار ۱) و ۷۵-۲۵٪ KCl-NaCl (تیمار ۲) با غلظت شوری ۱۵ درصد طی ۳ ساعت آب‌نمک گذاری شدند. نمونه‌ها سپس ابتدا به صورت سطحی خشک و در مرحله بعد با سرعت‌های کند و تند دستگاه آتموس دود داده شده و بسته بندی شدند. پروفایل اسید چرب نمونه های تیمار شده با نمونه‌های ماهی تازه (تیمار شاهد) مقایسه شدند. پروسه دودی کردن سبب کاهش محتوای اسیدهای چرب غیر اشباع (SFA) و افزایش محتوای اسیدهای چرب چند غیر اشباعی (PUFA) در نمونه‌های تیمار در مقایسه با نمونه شاهد شد. این تغییرات در تیمار ۲ اندکی بیشتر بود. نسبت اسیدهای چرب امگا-۳ به امگا-۶ در نمونه شاهد (۷/۳۰) پس از دودی کردن به ترتیب در تیمار ۱ و تیمار ۲ افزایش (۷/۷۱) و کاهش (۶/۸۶) نشان داد. پروسه دودی کردن سبب کاهش شاخص آتروژنیک (AI) در هر دو تیمار شد که مقدار این کاهش در تیمار ۲ بیشتر بود. شاخص ترومبوژنیک (TI) هم پس از دودی کردن در هر دو تیمار کاهش نشان داد. جایگزینی نسبی نمک سدیم با پتاسیم بر ترکیب اسیدهای چرب، محتوای اسیدهای چرب بلند زنجیره و مقادیر آتروژنیک و ترومبوژنیک ماهی کیلکای دودی شده تاثیر نداشته است.

* مولف مسئول