Fatty acid composition, chemical composition and processing yield of traditional hot smoked common carp (*Cyprinus carpio*, L)

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Received: September 2015 Accepted: August 2016

Abstract

The main objective of this study was to describe the proper technological process of hot smoking of common carp as well as to determine the yield of the final product and also proximate and fatty acid composition of hot-smoked common carp meat. The average yield of smoked common carp was 82.19% based on weight of eviscerated and descaled fish or 47% based on weight of live fish. The smoking process reduced moisture content by 19% and increased protein by 35% and lipid by 28%. Increase of cholesterol level from 50 to 51.93% during the smoking process was not statistically significant. Unsaturated fatty acid content was found to be higher in smoked than in raw common carp, mainly due to the increase in the contents of both monounsaturated fatty acids and polyunsaturated fatty acids. These values were higher compared to those of saturated fatty acids which were almost the same in raw (25.38%) and in smoked (25.5%) carp. Furthermore, n-3 content was not affected by the smoking process and the n-3/n-6 ratio. The results of the present research indicate that hot smoking process affects the chemical and fatty acid composition of traditionally hot smoked common carp.

Keywords: Common carp, Fatty acids, Manufacturing, Yield, Proximate composition

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Introduction

Consumption of fish meat is increasing worldwide, primarily due to the fact that fish meat is recommended as an important component in healthy human nutrition, mainly due to its high content of n-3 highly unsaturated fatty acids (n-3 HUFA) (Balk *et al.,* 2006) and, undoubtedly, fish meat represents the best source of these nutrients in human nutrition (Topic Popovic *et al.,* 2012; Sarma *et al.,* 2013). Therewith, freshwater fish meat contains high levels of monounsaturated fatty acids (MUFA), especially oleic acid (OA) (Ćirković *et al.,* 2012; Ljubojević *et al.,* 2013a), which also plays significant role in human health (Lopez-Huertas, 2010; Gillingham *et al.,* 2011). Furthermore, consumption of fish meat is encouraged for the high protein content of high biological value, presence of essential amino acids, minerals and vitamins (Hathwar *et al.,* 2012). Moreover, in line with the modern course aimed at revitalizing and promoting traditional food manufacturing processes, autochthonous fish products made from locally available fish species are receiving significance. With the appropriate technology of manufacturing and with the use of good raw fish material regarding its chemical and fatty acid composition, it is possible to produce traditionally hot smoked fish with a favorable chemical content and fatty acid profile. The market for traditional hot smoked common carp certainly exists and the present results should contribute to increased production of this food as well as to the development of similar products from fish meat. Development of new fish products could expand and diversify offers of that type of food and to contribute to better sale of fish, not only in traditional fish markets, but also in retail stores and supermarkets. Common carp is usually sold as a raw or live fish and there is a need for development of technology which will enable the presence of various fish products in the market. Technological processes of processing, preservation and storage are different for fish meat and for the mammalian meat (Okanović *et al.,* 2013a,b). For proper manufacturing of fish products, knowledge about chemical composition and characteristics of raw fish meat is very important in order to apply the most appropriate technology procedures adjusted to certain fish species. Recently, fish sausages of common carp meat and also of meat of other cyprinid fish species were developed (Okanović *et al.,* 2013a,b). This represents a great advance, but there is still a need for new products and improvements of the existing ones. Smoking is a traditional method of fish preservation and has considerable economic importance worldwide. The application of smoking for the extension of the shelf-life is a process of great interest, given that fish meat generally spoils fast and easily. Further, it also can improve taste of the final product and it lends color and flavor to the finished products as well

as having a bacteriostatic and antioxidant role (Kristinsson *et al.*, 2008). Salting has also a noticeable preservative effect, mainly due to the reduction in water activity, which leads to the prevention of the growth of spoilage microorganisms and the formation of a more membranous surface which also inhibits microorganism growth (Frangos *et al.*, 2010). Hot-smoking is a traditional pasteurizing treatment and the preservative effect of that treatment depends on the composition and preparation of raw material, temperature, relative humidity, as well as density and composition of the smoke and the smoking time (Kolodziejska *et al.*, 2002). The proximate and lipid composition of smoked fish depends particularly on their contents and composition in the raw fish used for smoking. Additional factors affecting the lipid composition of smoked fish are the preparation of the raw material for smoking, smoking itself as well as storage of smoked fish. Until now, many studies have been conducted to evaluate different characteristics of traditional and modern cold or hot smoked fish and other marine food throughout the world (Kolodziejska *et al.*, 2002; Goulas and Kontominas, 2005; Franco *et al.*, 2010; Colakoglu *et al.*, 2011; Lira *et al.*, 2014). To our best knowledge, no information exists on the procedure of traditional smoking of common carp, yield, as well as proximate and fatty acid composition of smoked common carp. Therefore, the main objective of this study was to describe the proper technological process of hot smoking of common carp as well as to determine the yield of the final product as well as proximate composition and fatty acid profile of hot-smoked common carp meat.

Materials and methods

Fish samples

Fresh whole common carp (average weight of 2850 g) was obtained from the fishery farm Ečka, Lukino Selo, Republic of Serbia and transported to the fish processing facilities (Agropapuk, Kuzmin, Republic of Serbia) in a refrigerator, at 4°C. The fish were slaughtered and the scales and guts were removed. The fish were then washed in clean fresh water.

Salting and smoking of the fish

The fish were dry salted at room temperature using refined NaCl. The ratio between fish and salt weight was approximately 20. After finishing dry salting, excess salt was taken off. Then the fish were kept at room temperature for 30 minutes and after that the fish were smoked. Fish were subsequently washed in clean water and dried. The fish were then hot smoked for 12 hours at temperatures above 60°C. For the hot smoking, hard wood as well as wood sawdust were used, in order to produce enough smoke. After smoking, fish was cooled (at 4°C, for 4 hours), and then packed.

The yield of smoking process was determined by using 21 fish. Calculation of eviscerating, descaling,

dressing percentage, smoking yields and losses of total smoking process were based on live weight of fish.

Chemical analysis

The following measurements were taken to analyze the chemical composition of the hot smoked common carp: protein, water, total fat, ash, total fatty acid and cholesterol concentrations. Water, crude protein, crude fat and ash contents were determined according to methods described by Spirić *et al*. (2010). Water content of samples of hot smoked common carp was determined after drying the samples at 105ºC to constant weight for 24 h. Crude protein content was determined by Kjeldahl method using Kjeltec Auto 1030 Analyzer, Tecator, Sweden, and ash was determined after burning at 550±25°C. Crude lipid in flesh of hot smoked common carp was also analyzed using the Soxhlet System with ether as solvent.

Lipid extraction and fatty acid analysis

Total lipids were extracted by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA). A homogenate of the sample mixed with diatomaceous earth was extracted with a mixture of n-hexane and iso-propanol (60:40 v/v) in a 33 mL extraction cell at 100°C and nitrogen pressure of 10.3 MPa (Spirić *et al.,* 2010). Then, the solvent was removed under stream of nitrogen in the Dionex Solvent Evaporator 500 at 50 °C until dry. The fat extract was further used for fatty acid determination. Fatty acid methyl esters (FAME) were prepared by trans-esterification using trimethylsulfonium hydroxide (Spirić *et al.,* 2010). The Shimazu Gas Chromatograph (GC-2010) (Kyoto, Japan) used for FAME determination was equipped with a split injector, fused silica cyanopropyl HP-88 column (length 100 m, i.d. 0.25 mm, film thickness 0.20 μm, J&W Scientific, USA), and flame ionization detector and workstation (Shimadzu GC Solution ver. 2.3). The injector temperature was 250°C and detector temperature was 280°C. The carrier gas was nitrogen at a flow rate of 1.33 mL min^{-1} and injector split ratio of 1:50. Injected volume was 1 μL. The chromatographic peaks in the samples were identified by comparing relative retention times of FAME peaks with peaks in a Supelco 37 Component FAME mix standard (Supelco, Bellefonte, USA) as described by Spirić *et al.* (2010). The relative amount of each fatty acid methyl ester was expressed as a percentage of the total amount of fatty acids in the analysed sample.

Cholesterol determination

Direct saponification method (Maraschiello *et al.,* 1996) has been used for cholesterol determination in fish muscle. In short, cholesterol in fish fillets (from dorsal body parts) was measured using HPLC/PDA system (Waters 2695 Separation module/Waters photodiode array

detector, USA), on a Phenomenex Luna C18 (2) reverse phase column, 150 mm x 3.0 mm, 5µm particle size, with C18 analytical guard column, 4.0 x 2.0 mm (Bligh and Dyer 1959). Detection was performed at 210 nm and the total analysis lasted 10 min.

Statistical analysis

The present study was carried out according to a completely random design with two treatments (raw and smoked common carp). The data were submitted to an analysis of variance (Statistica Version 14.0; StatSoft Inc., Tulsa, USA), and the significance was evaluated using the student t test at a level of significance of 95%.

Results

Average yield of smoked common carp was 82.19 % based on weight of eviscerated and descaled fish or 47% based on weight of live fish (Table 1).

Table 1: Average values of fish weight and yield, losses occurring during the smoking process of common carp (*Cyprinus carpio*) (n=21)

	ẋ	Sd	Se	Iv	Cv
Weight of whole fish (g)	2850	64.65	14.11	2700 - 2980	2.28
Weight of eviscerated and descaled fish (g)	1852.9	74.82	16.33	1701-1966.8	4.04
Dressing percentage (%)	65	1.64	0.36	62-68	2.52
Weight of hot smoked fish (g)	1523.1	67.45	14.72	1374.4-1632.4	4.43
Yield of smoked fish (%)	82.19	0.93	0.2	80.5-84	1.13
Smoking process loss (%)	17.81	0.93	0.2	16-19.5	5.23
Total loss (%)	53.42	1.53	0.33	50.53-56.1	2.87

Note: ẋ-mean value; Sd–standard deviation; Se-standard error; Cv –coefficient of variation; Iv – interval of variation

The smoking process reduced moisture content from 78.30% to 63.46 (by 19%) and increased protein by 35 %, lipid by 28% and ash content from 1.07 to 4.55%. The increase in cholesterol level from 50 to 51.93% during the smoking process was not statistically significant (Table 2).

Table 2: Chemical composition and cholesterol content of raw and traditionally hot smoked common carp.

	Raw common carp	Smoked common carp
Moisture content (g/100 g, dry weight)	78.30±0.1[a]	63.36±0.06[b]
Protein content (g/100 g, dry weight)	17.40±0.10[b]	26.65±0.13[a]
Fat content (g/100 g, dry weight)	3.41±0.1[b]	5.00±0.02[a]
Ash content (g/100 g, dry weight)	1.07±0.03[b]	4.55±0.03[a]
Cholesterol (mg/100g)	50.00±0.42	51.93±1.48

Note: Means in the same row with different letters are significantly different (p<0.05). Values are mean and standard deviation of 8 samples assessed in triplicate. * Statistical significance at the level of p<0.05

In the fresh common carp a total of 19 fatty acids were identified, whereas 19 fatty acids were identified in the smoked common carp. In the present study, unsaturated fatty acid content (USFAs) was found to be higher in smoked than in raw common carp, mainly due to increasing contents of both MUFA and PUFA, and these values were higher compared to those of saturated fatty acids (SFA) (Table 3). In raw and smoked common carp, palmitic acid (16:0) was the main component of SFA folowed by stearic acid (18:0) and there were no significant diference in contents of these fatty acids between raw and smoked carp (Table 3). Oleic acid (C18:1n-9) has been noted as a dominant MUFA in both fresh (32.42%) and smoked (34.05%) samples. Among the n-3 PUFAs, eicosapentaenoic (EPA) (20:5n-3), docosapentaenoic acid (DPA; 22:5n-3) and docosahexaenoic acid (DHA) (22:6n-3) were the dominant fatty acids in fresh and smoked samples. Linoleic acid (LA, C18:2n-6) was the dominant n-6 fatty acid in fresh (19.68%) as well as in smoked (19.46%) common carp. In the present study, SFA content was almost the same in raw (25.38%) and in smoked (25.5%) carp, while contents of MUFA and PUFA significantly increased after smoking. Furthermore, n-3 content was not affected by the smoking process as well as by n-3/n-6 ratio.

Table 3: Fatty acid composition (g/100 g, dry weight) of the raw and traditionally hot smoked common carp.

Fatty acids	Raw common carp	Smoked common carp
C14:0	0.77±0.03[b]	1.15±0.05[a]
C15:0	0.25±0.05	0.25±0.05
C16:0	18.82±0.1	18.51±0.2
C16:1	5.28±0.1[b]	9.2±0.1[a]
C17:0	0.38±0.08[b]	0.4±0.05[a]
C18:0	5.16±0.05	5.09±0.03
C18:1cis-9	32.42±0.13[b]	34.05±0.08[a]
C18:1cis-11	2.82±0.14[a]	0
C18:2n-6	19.68±0.11	19.46±0.15
C20:0	0[b]	0.11±0.01$_a$
C18:3n-6	0.2±0.02[b]	0.35±0.05[a]
C18:3n-3	2.21±0.1	2.31±0.1
C20:1	2.46±0.05[a]	2.13±0.06[b]
C20:2n-6	0.61±0.04	0.65±0.06
C20:3n-6	0.86±0.05	0.53±0.04[b]
C20:3n-3	0.67±0.03[a]	0.28±0.03[b]
C20:4	1.16±0.05[b]	2.34±0.07[a]
C20:5n-3	1.16±0.05	1.16±0.08
C22:5n-3	0.57±0.03[b]	0.68±0.03[a]
C22:6n-3	1.19±0.01[b]	1.42±0.07[a]
SFA	25.38±0.07	25.51±0.16
MUFA	42.99±0.21[b]	45.38±0.13[a]
PUFA	28.31±0.15[b]	29.17±0.08[a]
n-3	5.81±0.01	5.84±0.13
n-6	22.50±0.15[b]	23.32±0.21[a]
n-3/n-6	0.26±0.002	0.25±0.01
n-6/n-3	3.87±0.03	3.99±0.12
PUFA/SFA	1.12±0.01[b]	1.14±0.01[a]
USFA/SFA	2.81±0.01[b]	2.92±0.01[a]

Note: Means in the same row with different letters are significantly different ($p< 0.05$). Values are mean and standard deviation of 8 samples assessed in duplicate.

* Statistical significance at the level of $p<0.05$; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-3 PUFA, polyunsaturated fatty acids from the n-3 family; n-6 PUFA, polyunsaturated fatty acids from the n-6 family. USFA, unasturated fatty acids

Discussion

Hot - smoking method affected weight losses of smoked fillet in the present study as also noted by Franco *et al.* (2010). These authors found that the process losses were significantly higher in hot-smoking process in comparison with the process of cold smoking.

Sigurgisladottir *et al.* (2000) suggested that the weight loss is due to dehydration during smoking and it varies between 10 and 25% depending on several factors, including origin of raw material, characteristics of final products and parameters used in the process, especially time and temperature.

Ljubojević *et al.* (2012) and Okanović *et al.* (2013a,b) noted that quality of raw fish is highly important for yield and quality of the final product. Rora *et al.* (1999) found that type of raw material, manual processing method, method of salting, and smoking conditions (temperature, moisture, air flow rate, drying) are determinant factors on the weight variation during smoking. Yield of final product is of great technological as well as economical importance. Nutrition, age, weight, seasonal variation and physiological phase have great influence on yield (Ljubojević *et al.*, 2012). Therefore, those factors that might affect analysis should be avoided. Processing methods have a significant effect on the nutritional value of fish, and considerable diferences in moisture, protein, lipid and ash content have been reported previously (Türkkan *et al.*, 2008). In the present study, smoking process reduced moisture content and increased protein, lipid and ash content. According to results obtained by Goulas and Kontominas (2005) the hot-smoking process resulted in a significant reduction in moisture content of chub mackerel samples, and this reduction corresponds to loss of the moisture content of the raw samples from 21.2 to 22.4%. Cardinal *et al.* (2001) noted that industrial specifications for "smoked finished products" commonly recommend a moisture content in the fish flesh of less than 65%, which is in agreement with obtained results of 63.36%. The results of our study show that moisture content in common carp is higher in comparison with the findings of Kolodziejska *et al.* (2002), who reported that the mean moisture content of smoked mackerel was 56.7%.

According to results reported by Franco *et al.* (2010) hot-smoking process reduced moisture and incresed crude protein, lipid and ash contents. This was also reported by Colakoglu *et al.* (2011) after hot-smoking of thornback ray (*Raja clavata*) and spiny dog fish (*Squalis acanthias*).

Proximate composition of raw fish flesh is of great technological importance since it affects yield, odour, flavor, texture and fat oxidation stability. Nutrition, weight, age, season, rearing system affect proximate composition of fish flesh (Ćirković *et al.*, 2012; Ljubojević *et al.*, 2013b, 2014, 2015).

Franco *et al.* (2010) found significant differences between smoked and non-smoked fillets of *Brycon cephalus* where smoking process reduced moisture content from 72.9% to 58.5% and increased protein from 20.7% to 28.07%, lipid from 3.37 to 8.09% and ash from 1.25 to 3.28% (by 3.28%)

which is in agreement with our results. These authors suggested that increase of ash content in smoked fish fillets is probably due to absorption of sodium chloride by the muscle durng salting. Lipid increase observed in smoked carp is caused by moisture reduction due to smoking. This is in accordance with previous findings, which reported a reverse correlation between the fat and water contents, which is common for many fish species (Żmijewski *et al.*, 2006; Ljubojević *et al.*, 2013c).

Stearic acid is known to be a very stable saturated fatty acid (Žilić *et al.*, 2010) and little changes in its content may have been originated by the effects of high temperature of the hot smoking which further leads to an elevation of the MUFA. It should be mentioned that stearic acid (C18:0) is regarded as a neutral fatty acid which has no effect on blood cholesterol levels in human in contrast to myristic acid and palmitic acid, which have a strong influence (Hodson *et al.*, 2008). The effects of processing techologies on fatty acid composition of fish flesh have been examined earlier. However, to our knowledge, there are no published studies on the fatty acid composition of traditionally hot smoked common carp in the literature. In the study conducted by Colakoglu *et al.* (2011) after hot-smoking of thornback ray (*Raja clavata*) and spiny dog fish (*Squalis acanthias*) C16:0 increased significantly, while no significant differences in total MUFA and C18:1n-9 between fresh and smoked fish were observed. Besides, no significant differences were noted in MUFA and C18:1 n-9 contents of salmon after heat treatment (Larsen *et al.*, 2010). Colakoglu *et al.* (2011) observed that total PUFA content in marinated and smoked fish decreased significantly compared to that in fresh fish. Ohshima *et al.* (1996) also found decreases in PUFA levels of salmon after grilling and Özden (2005) noted that PUFA content in marinated anchovy and rainbow trout were lower than that in fresh fish. On the other hand, Stolyhwo *et al.* (2006) reported that smoking techniques did not significantly affect PUFA content in Atlantic mackerel. Observed differences may be species specific or due to variations in hot processing methods that may have a profound effect on the stability of PUFAs. Colakoglu *et al.* (2011) noted that DHA and EPA were significantly lower in processed compared with fresh fish. Moreover, Beltran and Moral (1991) reported that hot smoking of sardine fillets resulted in small, but statistically significant decreases in EPA and DHA contents, while accordiing to the results of the present study, content of EPA was the same in raw and smoked common carp, while DHA content increased slightly, but was statistically significant. According to Colakoglu *et al.* (2011), the n-3 content decreased, but differences were not significant in smoked thornback ray, while n-6 contents of the smoked thornback ray were significantly lower than that in fresh fish. Colakoglu *et al.* (2011) noted that in the spiny dogfish n-3 and n-6 were significantly lower in

smoked fish compared to those of fresh samples and that n-3:n-6 ratio increased after smoking.

In the present research, n-6/n-3 ratio values of both raw and smoked common carp were below 4.0, which is in accordance with the recommendation of Simopoulos (2008) for human nutrition.

The results of the present research indicate that hot smoking process affects the chemical and fatty acid composition of traditionally hot smoked common carp. Data on yield of final products are very important for fish processing and analysis of economic feasibility of production and processing. According to the results of this research, with the appropriate selection of raw fish material and convenient/adequate manufacturing process, it is possible to produce the traditional hot smoked common carp with a favorable chemical content and with respectable and satisfactorily healthy fatty acid composition. There is a high market demand for hot smoked common carp exists, and obtained results could contribute to the further improvement of this product as well as the development of similar products from fish meat, which would increase the current offer of fish and fish products in the market.

Acknowledgements

This research was funded within the project "The influence of the quality of the food components for cyprinid fish species on the quality of meat, losses and the profitability of production", TR 31011, by the Ministry of Education, Science and Technological Development, Republic of Serbia.

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