



## Research article

Changes in the EPA and DHA content and lipids quality parameters of rainbow trout (*Oncorhynchus mykiss*, Walbaum) and carp (*Cyprinus carpio*, L.) at individual stages of hot smokingGrzegorz Bienkiewicz<sup>a</sup>, Grzegorz Tokarczyk<sup>b,\*</sup>, Barbara Czerniejewska–Surma<sup>a</sup>, Jacek Suryn<sup>c</sup><sup>a</sup> Department of Food Quality, West Pomeranian University of Technology, Szczecin, Papieża Pawła VI nr 3, 71-459, Szczecin, Poland<sup>b</sup> Department of Food Sciences and Technology, West Pomeranian University of Technology, Szczecin, Papieża Pawła VI nr 3, 71-459, Szczecin, Poland<sup>c</sup> Lisner Sp. z o.o., Poznań, Strzeszyńska 38/42, 60-479, Poznań, Poland

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## ABSTRACT

The aim of this study was to evaluate qualitative changes in lipids of two most popular freshwater farmed fish (rainbow trout and carp) at particular stages of hot smoking process (brining, drying, smoking and heating). In order to characterize qualitative changes, the amount of peroxides (PV), secondary oxidation products (AsV) and the degree of hydrolysis of lipids (AV) was determined during the smoking process. The studies were carried out both in the fraction of lipids extracted using chloroform-based method (free lipids), as well as the fraction extracted by Bligh and Dyer method of the 1:1 chloroform: methanol ratio (bound lipids). Heat smoking results in loss of fat, especially at the last two stages of the process and the final contents of lipids were about 18% lower in the carp samples and about 10% lower in the trout samples. The dynamics of free lipid oxidation (Ch-lipids) was much smaller than the lipids extracted using B-D method, but the trend of lipid changes was similar. There was no significant difference in the anisidine value between two species, both for free lipids (Ch-lipids) and bound lipids (B-D-lipids), except for the last stage of smoking. No statistically significant differences between the studied species were found also in the case of lipid hydrolysis level, but after the smoking process an amount of free fatty acids increased. Subsequent stages of smoking process resulted in statistically significant losses of EPA and DHA in both fish species and in both types of lipids, i.e. B-D-lipids and Ch-lipids.

## 1. Introduction

Rainbow trout (*Oncorhynchus mykiss*) is one of the most important freshwater fish species in terms of economic purposes. It is popular in Europe, as well as the whole world due to rapid growth, taste and nutritional quality of its meat. With its ongoing production, rainbow trout is the most commonly farmed freshwater fish species (Dursun and Erkan, 2014; Mahmoud and Buettner, 2017).

Carp (*Cyprinus carpio*) is the other commercially important species farmed in aquaculture (Chirwa et al., 2017; Grigorakis et al., 2018). It is particularly important for the fishing industry not only for culinary reasons, but also for maintenance of proper water retention through pond management in the Central Europe region (Hryszko and Lirski, 2017). Among the domestic farm-raised freshwater species, carp and trout are eaten most commonly (about 0.50 kg per person) (Pieńkowska and Hryszko, 2016). However, fish consumption is still significantly lower

than in other European countries. On the other hand, people reach for the processed fish products such as ready-made fish dishes more and more often (Duman and Karaton Kuzgun, 2018; Neira et al., 2019).

Smoking is one of the oldest ways to preserve food. It is a process of saturation of raw materials and semi-finished food products, which have been brined, salted, desalted as well as dried or semi-dried by smoke components (Migdał et al., 2016). The preserving effect of smoking is based on the synergistic effect of temperature, table salt and other chemical substances as well as reduced water activity. Smoke plays primarily the role of flavorings, shaping the food coloring. Additionally, it also shows antibacterial and antioxidant properties (Zachara et al., 2017). Smoke is produced in the pyrolysis process as a result of controlled combustion of wood material with limited access to atmospheric oxygen. During pyrolysis, different smoke components are formed, which are classified into two phases: a dispersion phase (composed of air and gas combustion products) and a dispersed phase.

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The latter one includes steam, pairs of organic compounds and particulates (Husain and Patang, 2018). Thus, apart from providing flavor and smell qualities, smoke can perform protective functions, improving preservation and safety of food. On the other hand, smoking as a technological treatment conceals defects and poor quality of the raw material. The work by Tokarczyk et al. (2011) studied the influence of warm smoking on sensory properties of frozen lake whitefish of low quality and low suitability for technological processing. The study shows that warm smoking had a positive influence on meat flavoring as well as visual appearance of smoked carcasses. At the same time, it was demonstrated that such features as release of water during chewing or perceptibility of rancid taste did not improve after the smoking process. However, the qualitative parameters of the lipid fraction were not determined here, which apparently was considerably responsible for the rancid taste.

Smoking as a process of fish treatment is very popular. Therefore, it is necessary to select technological parameters with utmost care in order to protect the important nutritive value of the ingredients in the best possible way. The work assumes that particular stages of fish hot smoking will have a significant influence on the qualitative changes of the lipid fraction, i.e. dynamics and rate of oxidation. Two most popular species of farm-raised fat freshwater fish were selected for the study. The aim of the study was to compare the oxidative changes of rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*) lipids at particular stages of hot smoking, depending on the method of their extraction.

## 2. Material and methods

### 2.1. Fish samples

The study was carried out on two species of farmed fish: rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*). Trout specimens (20 individuals, 35–40 cm length, 0.7–0.9 kg weight), and carp (20 individuals, 30–35 cm length, 1.2–1.4 kg weight) were obtained from an aquaculture facility in West Pomeranian province in Poland. Individuals were sacrificed in the fish farm, the gills were cut off, and then bled in a water-ice mixture. After that the fish were transported to the laboratory during 12 h under crushed ice condition. Fish to ice ratio was 1:1. In the laboratory the fish were beheaded, gutted and washed.

### 2.2. The preparation and smoking process of fish

The fish was rinsed in the laboratory and cut to form a fish steak. Subsequently, the fish was brined for 20 min in 20% NaCl solution. Afterwards, the fish was rinsed and placed on the grid in the smoking chamber. The smoking consisted of three phases:

Phase I - Drying. The fish was dried for 60 min at the temperature of 35–40 °C using forced air circulation.

Phase II - Smoking. The smoking time was 90 min and the smoking temperature was 40–60 °C. Gradient increased in proportion to the time of smoking. Smoke was produced from the pyrolysis of beech wood chips.

Phase III - Heating. After the smoking stage (90 min), the temperature of the smoking chamber was increased to 80 °C for the duration of 30 min, which made it possible to maintain a temperature above 75 °C in the tissue of smoked fish species.

After each stage, the prepared samples were cooled down to about 20 °C and taken for further determinations.

### 2.3. Extraction of lipids and determination of lipids content

Lipids were extracted:

1. By the Pokorný et al. (1975) using only one solvent: chloroform. Quantification results were expressed as grams of lipid per 100 g of muscle tissue.

2. By the Bligh and Dyer (1959) method, by employing a single-phase solubilisation of the lipids using a chloroform–methanol (1:1) mixture. Quantification results were expressed as grams of lipid per 100 g of

muscle tissue. The lipids content was determined gravimetrically, by evaporating a defined amount of extract.

### 2.4. Determination of lipids quality parameters

The peroxide value (PV) was determined in the lipid extract by peroxide reduction with ferric thiocyanate, according to Pietrzyk (1958) based on the oxidation of ferrous salt by hydroperoxides and the reaction of ferric salts with potassium isothiocyanate. The red ferric complexes formed were determined spectrophotometrically. Results were expressed as milliequivalents of oxygen per kilogram of lipids (meqO<sub>2</sub>/kg of lipids).

The anisidine value (AV) was determined in fish muscle according to the AOCS (1993) method, based on the reaction between  $\alpha$ - and  $\beta$ -unsaturated aldehydes (primarily 2-alkenals) and p-anisidine reagent. AV was expressed as 100 times the absorbance measured at 350 nm (Thermo Scientific, Genesys 20) in a 1-cm path length cuvette from a solution containing 10 mg of lipid in 1 ml of reaction medium.

Acid value (AV) was determined by titration of 0.1 N KOH in methanol, according to Polish Standard method, 2009 (PN-EN ISO 660:2009). Results were expressed as percent of free fatty acid calculated as oleic acid (% FFA).

### 2.5. Determination of fatty acids

Fatty acid methyl esters (FAME) were obtained from the tissue by alkaline hydrolysis of extract of lipids with 0.5 N sodium methylate (CH<sub>3</sub>ONa). Details of the analysis have been described in an earlier study (Domiszewski and Bienkiewicz, 2010). Next, the FAMES were separated using a gas chromatography apparatus, coupled with a mass spectrometer (Agilent Technologies 7890A) and equipped with a split/splitless type injector. Conditions of FAMES separation were as follows: column SPTM 2560, 100 m 0.25 mm ID, 0.20  $\mu$ m film, catalogue no. 24056; carrier gas – helium at a constant flow rate of 1.2 ml/min; split 1:50; injector temperature: 220 °C; detector temperature: 220 °C; programmed furnace temperature: 140 °C (5 min) increased to 240 °C at a rate of 4 °C/min; analysis time: 45 min. The qualitative interpretation of chromatograms was based on the comparison of retention times and mass spectra of the particular FAMES of the sample with those of analogous FAME standards by Sigma company (Lipid Standard). As an internal standard, C 19:0 was used.

### 2.6. Determination of water content

Moisture was determined gravimetrically after drying the material in an oven at 105 °C according to the AOAC (2002) method.

### 2.7. Determination of water activity

Water activity ( $a_w$ ) was determined in triplicate using the HygroLab C1 instrument (Rotronic, Switzerland), equipped with a HC2-AW probe, calibrated in the range 0.1–0.95 with solutions of LiCl of known activity (Labuza et al., 1976).

### 2.8. Determination of total volatile bases nitrogen

TVB-N (total volatile bases nitrogen) was determined by Conway and Byrne (1933) method according to the Commission Regulation (EC) No 2074/2005 of 5 December 2005 (Anonymous, 2005).

### 2.9. Statistical treatment

Numbers presented in tables are the mean values of triplicate analyses. The statistical analysis was based on the one-way analysis of variance, homogeneous groups were formed according to the Duncan test for  $p \leq 0.05$ . The data were statistically analysed using STATISTICA (data analysis software system) 2010 version by StatSoft Inc.

### 3. Results and discussion

#### 3.1. Changes of lipids amount at particular smoking stages

Two most popular species of farmed fish, i.e. carp and trout were evaluated. Both species can be qualified as fat fish (Skalecki et al., 2013). The studied initial samples of fish cut to form of a fish steak contained very similar amounts of lipids, i.e. 12% for trout and 11% for carp (Figure 1). Both species were characterized by high freshness quality, which was confirmed by TVB-N values (Table 1). Figure 1 shows the changes in the amount of lipid extracted from two studied fish species at each stage of smoking using two methods. The application of selective extraction with two solvents with different elution strength of the lipid component allows for separation of the extracted lipids into free lipids (Chloroform extraction - Ch) and lipids, which occurs in food together with other ingredients such as proteins (Bligh & Dyer extraction). Both free and bound lipids, designated as (B-D), were thus obtained. The application of such extractions in the studies on the interaction of lipids with other food ingredients was demonstrated by Pokorný et al. (2010) and Odoil et al. (2019). At the second stage of smoking, during which the samples were dried by the stream of hot air into the smoking chamber, a slight increase in the extractability of lipids from trout was observed compared to raw samples and the samples after brining process. At this stage, the change was caused by a greater loss of water and salt-saturated tissue (Table 1). This relation concerned both, Ch-lipid extraction and B-D-lipid extraction. The subsequent stages of smoking resulted in a greater decrease of lipid content in both species. This was caused by loss of water, but mainly due to loss of lipid in the smoking process together with the increasing of temperature. The observed differences in the rate of lipids dripping depended on the method of extraction. Ch-lipids, constituting mainly an adipose tissue component, dripped much faster as early as at the smoking stage, in comparison to B-D lipids. Lipid loss with the rise of temperature depends on its place in the tissue. Subcutaneous and ventral lipids have dripped the fastest. The differences in the distribution of lipids in rainbow trout fillet and its various properties depending on the location were presented by Bienkiewicz et al. (2013) Barbosa et al. (2017). They do not indicate only different amounts of lipids distributed in rainbow trout fillet, but also point to its different susceptibility to UV rays catalysed oxidation. Very similar changes in salmon during smoking presented Malesa-Ciećwierz et al. (2019). This can also be of a crucial importance when it comes to explanation of oxidative changes in the smoking process. The opposite relationship was presented at the last stage of the smoking process, where we can observe a significantly larger loss of B-D-lipids in comparison to Ch-lipids. Increasing of temperature up to 80 °C at the last stage of the smoking

**Table 1.** Water activity and the content of salt (NaCl), water, TVB-N in the fresh fish flesh and at the various stages of heat smoking of carp and trout.

	raw	brined	dried	smoked	heated
NaCl [%]					
trout	-	2.36 ± 0.02	2.58 ± 0.03	2.57 ± 0.02	2.8 ± 0.4
carp	-	2.29 ± 0.05	2.41 ± 0.02	2.44 ± 0.01	2.7 ± 0.2
Water [%]					
trout	70 ± 1	67 ± 1	61.3 ± 0.5	61 ± 2	55.0 ± 0.1
carp	70 ± 1	69 ± 1	66 ± 1	64 ± 1	57 ± 1
Water activity					
trout	0.986 ± 0.002	0.924 ± 0.001	0.911 ± 0.001	0.921 ± 0.003	0.958 ± 0.003
carp	0.988 ± 0.003	0.929 ± 0.002	0.912 ± 0.002	0.922 ± 0.002	0.949 ± 0.002
TVB-N [mg/100g]					
trout	7.1 ± 0.4	7.0 ± 0.2	7.4 ± 0.1	8.1 ± 0.1	9.0 ± 0.1
carp	11.1 ± 0.6	7.5 ± 0.2	7.8 ± 0.1	8.7 ± 0.5	9.1 ± 0.1

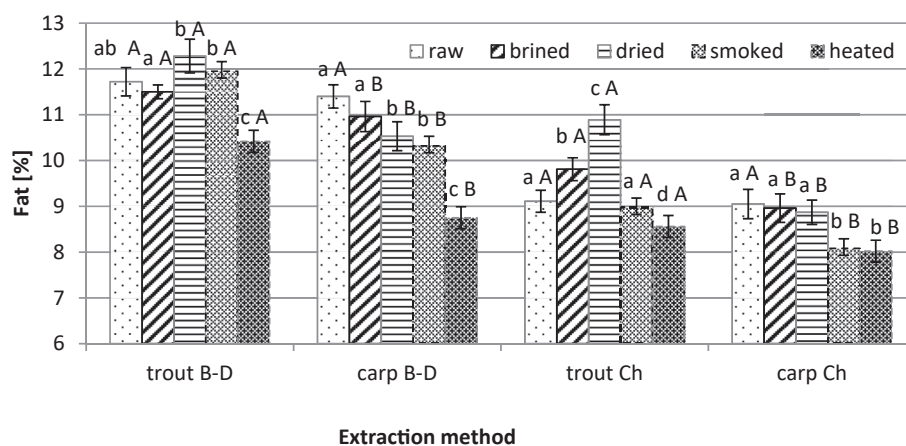
process resulted in a severe denaturation of proteins, their syneresis, which led to release of lipids bound with proteins in the tissue. This is indirectly proven by increase in the product water activity after this stage and a very small decrease in the amount of water in the tissue, in comparison to the previous stage (Figure 1, Table 1). As a result of the entire hot smoking process, the final contents of lipids were about 18% lower in the carp samples and about 10% lower in the trout samples.

#### 3.2. Changes of qualitative lipid parameters at particular smoking stages

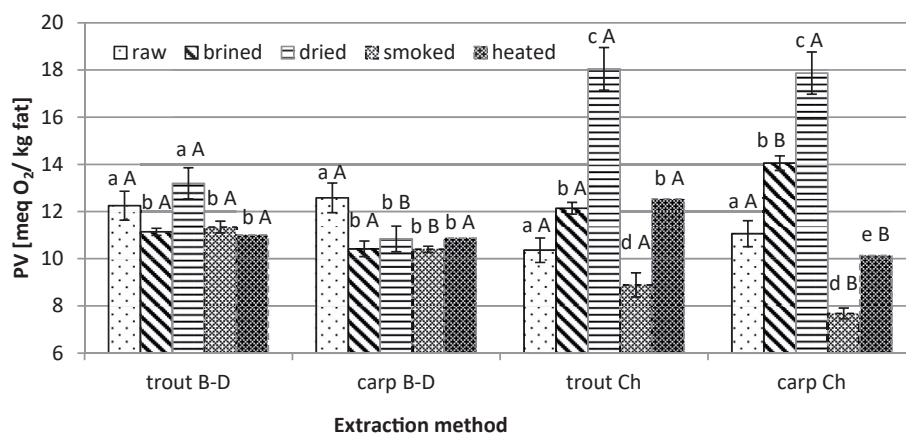
In order to characterize qualitative changes in the lipid fraction of the studied fish species, the amount of peroxides (PV), secondary oxidation products (AsV) and the degree of hydrolysis of lipids (AV) was determined during the smoking process. The studies were carried out both in the fraction of lipids extracted using chloroform-based method (free lipids), as well as the fraction extracted by Bligh and Dyer method of the 1:1 chloroform: methanol ratio (bound lipids).

##### 3.2.1. Changes in primary oxidation products

Changes in the primary oxidation products of lipids were expressed by determination of peroxide value. Figure 2 shows the changes of peroxide value (PV) in the process of hot smoking. The level of fish oxidation after brining, depending on the species and type of extraction, ranged from 10 to less than 14 meqO<sub>2</sub>/kg of lipids. Comparing these data to the initial values determined in the raw material, a significant increase in peroxides was observed only in Ch-lipids. B-D-lipids of the raw material as well as after brining had a comparable level of oxidation



**Figure 1.** Comparison of changes in fat content extracted with two methods (Ch - chloroform extraction, B-D - chloroform methanol extraction) at individual stages of heat smoking of carp and trout. a, b, c, d, e - significant differences (n = 3) between individual stage of smoking within one species and one method of extraction. A, B - significant differences (n = 3) between species for the same stage of smoking and the same extraction method.



**Figure 2.** Comparison of changes in peroxide value (PV) of lipids extracted in two ways, at each stage of heat smoking of carp and trout. a, b, c, d, e - significant differences ( $n = 3$ ) between individual stage of smoking within one species and one method of extraction. A, B - significant differences ( $n = 3$ ) between species for the same stage of smoking and the same extraction method.

(Figure 2). The increase in the oxidation level in the Ch-fraction may be the effect of prooxidant activity of sodium chloride. This theory confirms the studies carried out on smoked salmon, which was brined in various methods, where we could also notice an increase in the oxidation level in salmon lipids (Espea et al., 2002). Moreover rate and degree of lipid degradation in fish is dependent on the presence of pro-oxidant substances, metal ions ( $\text{Fe}^{2+}$ , hemin,  $\text{Cu}^{2+}$ , and  $\text{Fe}^{3+}$ ), salt used, fish species and muscle type (dark or white) (Wawreb et al., 2019). In the sample from drying process, another significant increase in the oxidation level was particularly noticeable in the fraction of lipids extracted using chloroform method. The amount of peroxides expressed in PV reached 18  $\text{meqO}_2/\text{kg}$  of lipids at this stage of the process and was twice as high as in the raw material. Such a dynamic growth of peroxides was caused by increasing of temperature up to 40 °C and, above all, by increased absorption of oxygen by adipose tissue as a result of forced air movement in the smoking chamber. These physical factors are catalysts that initiate auto-oxidation processes of particularly sensitive fish lipids (Kolakowska, 2010; Haman et al., 2019).

The subsequent stages of hot smoking processes trigger very dynamic changes, especially in free lipid fraction (Ch-lipids). The smoking stage decreases drastically the amount of peroxides down to the level from the beginning of the process. Such a decrease of primary oxidation products may be caused by saturation of lipids with smoke components, which shows strong antioxidant properties (Tenyang et al., 2013; Albishi et al., 2019). The next stage of heating up to the temperature of 80 °C without use of smoke, causes a subsequent, slight increase in the oxidation of free lipid fractions (Ch-lipids), reaching the level at the beginning of the process. As reported by Domiszewski (2013) who investigated the influence of heating temperature on qualitative parameters of herring, sprat and trout muscle tissue lipids changes, the temperature of 60–80 °C can still catalyse the process of auto-oxidation. However, the course of this process depends on the composition of lipids and the presence of other protein-originated tissue components that inhibit the formation of primary oxidation products. Analysing the described case of heat smoking process we see the compatibility of this theory comparing the dynamics of free lipid oxidation (Ch-lipids) and the lipids extracted using B-D method, which can be protected by proteins in the tissue and react with the products of their changes. Having characterized the trend of lipid changes (B-D-lipids) we observe a similar trend as it was the case of free lipids (Ch-lipids), but the dynamics of these changes is much smaller. One of the main factors of this difference is a different distribution of fish fat and its different susceptibility to oxidation (Bienkiewicz et al., 2013) as well as easier access and interaction of lipids (B-D-lipids) with antioxidant protein components. The study carried out by Serpen et al. (2012) on sea bream fillets showed that heating of tissue for 20 min

increases antioxidant activity of tissues measured by ABTS and FRAP methods by about 15 and 40% (Figure 2).

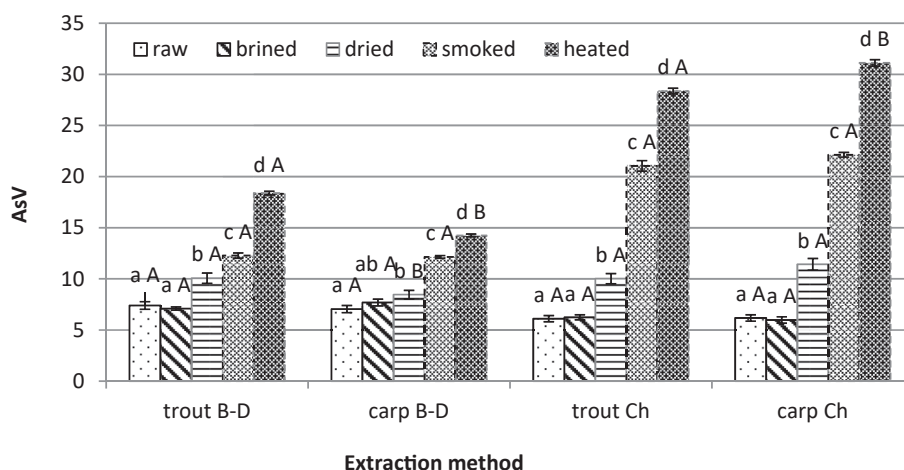
### 3.2.2. Changes in secondary oxidation products

The determination of anisidine value was used to characterize changes in the secondary oxidation products of carp and trout lipids at individual stages of the smoking process. There was no significant difference in the anisidine value between two species, both for free lipids (Ch-lipids) and compound lipids (B-D-lipids). Free lipids (Ch-lipids) had about 15% lower values in comparison to compound lipids (B-D-lipids) (Figure 3). No significant differences were found in the raw material following brining process. It was only after the drying process that the AsV increased slightly in both fish species and in both lipid fractions. The subsequent stages of the smoking process resulted in a further increase in the evaluated index, but in the case of lipids (Ch-lipids), the increase was much more dynamic in comparison to lipids (B-D-lipids). After the smoking stage, anisidine value amounted to 21 and went up to 31 after heating. As far as B-D lipids are concerned, the value was 12 for both species and 18 for trout and 14 for carp respectively in the final stage. This value did not show any significant differences between the studied species, except for the last stage of smoking. Having analysed literature data we observe that TBARS method was applied for determination of secondary oxidation products in fish lipids (Espea et al., 2002; Grigorakis, 2017). On the other hand, the relationships observed in time confirm the same trends for anisidine value. The formation of aldehydes in fish can be attributed to enzymatic reactions. However, in the case of a heat smoking process, this can be attributed first and foremost to thermal degradation, Maillard's reaction and lipid alterations. These reactions take place during thermal processes such as cooking, baking, smoking (Ames, 1998; Guillén et al., 2006; Gang et al., 2019).

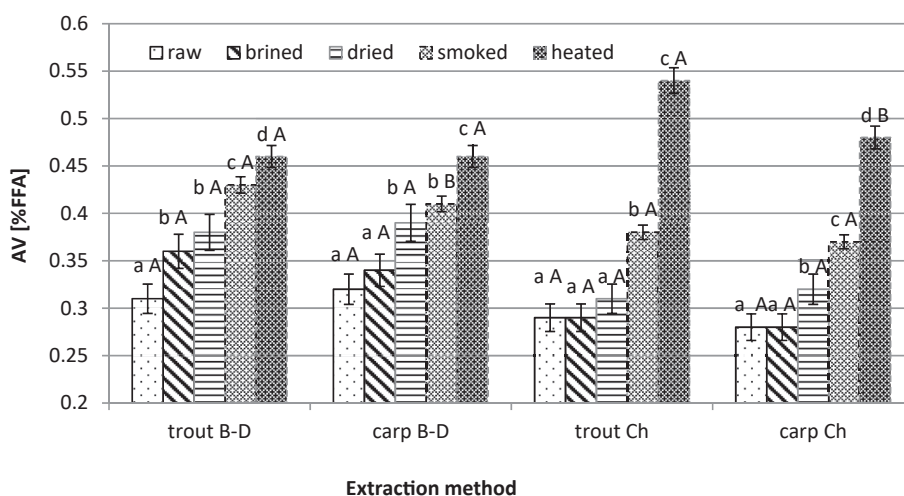
### 3.2.3. Changes of lipid hydrolysis level

As was the case with the evaluation of secondary oxidation products in the smoking process, no statistically significant differences between the studied species were found also in the case of lipid hydrolysis level. During the entire smoking process an amount of free fatty acids increased. The same phenomenon was observed by Tenyang et al. (2018) during smoking of herring. This trend concerned both lipid fractions. However, the amount of FFA (Ch-lipids), which initially amounted to 0.3% in the raw material, in terms of oleic acid after the whole process, an amount of FFA was almost twice as high. The most dynamic growth was observed at the drying stage, where FFA value increased from 0.4% FFA to 0.5% FFA for both fish (Figure 4). The amount of FFA in the smoking process increased periodically together with the rise in temperature during the process. This translated into an increased level of





**Figure 3.** Comparison of changes in anisidine value (AsV) of lipids extracted in two ways, at each stage of heat smoking of carp and trout. a, b, c, d, e - significant differences (n = 3) between individual stage of smoking within one species and one method of extraction. A, B - significant differences (n = 3) between species for the same stage of smoking and the same extraction method.



**Figure 4.** Comparison of changes in acid value (AV) of lipids extracted in two ways, at each stage of heat smoking of carp and trout. a, b, c, d, e - significant differences (n = 3) between individual stage of smoking within one species and one method of extraction. A, B - significant differences (n = 3) between species for the same stage of smoking and the same extraction method.

protein denaturation and water release, which together with temperature, catalyzed hydrolytic processes. This assumption confirms an increased water activity of the product after the last stage of smoking (Table 2). This perception is indirectly confirmed by Esmailnia (2015) studies who investigated changes in water activity and oxidation processes during storage of smoked trout. These studies show that water activity in trout flesh depended on smoking parameters and this activity increased during storage, as was the case with lipid hydrolysis level. On the other hand, there was no significant protective or catalyzing effect of

smoke in the lipid hydrolysis. This relation was confirmed by studies of NMR spectra carried out on smoked sea bass lipids using smoke products (Nieva-Echevarría et al., 2017). Technological process parameters are much more important than the smoke itself due to the fact they have an influence on lipolytic activity, which is confirmed by studies conducted by Zhou and Zhao (2007) on raw meat.

### 3.3. Changes in contents of EPA and DHA in the smoking process

In terms of nutrition, Omega-3 fatty acids, are particularly valuable as a food ingredient. All dietary recommendations show the need to increase Omega-3 long-chain fatty acids (LC n-3 PUFA) in the diet (Leventakou et al., 2014). Depending on the species and method of farmed, the fish is one of the best sources of EPA and DHA. However, high level of unsaturation and lack of natural antioxidant substances in the fish tissue (Kołakowska et al., 2006) may lead to oxidation and occurrence of natural quantitative losses as a result of numerous technological processes, as well as during storage of the finished product (Bilgin and Degirmenci, 2019). Therefore, it is important to optimize the process of fish treatment in order to protect fish lipids against factors that catalyze their oxidation as best as possible. Table 2 shows changes in the total EPA and DHA

**Table 2.** Changes of the total content of EPA and DHA in lipids extracted in two ways, at individual stages of heat smoking of carp and trout.

EPA + DHA [mg/1g of lipids]					
	raw	brined	dried	smoked	heated
trout B-D	107 ± 2 <sup>a</sup>	109 ± 1 <sup>a</sup>	107 ± 2 <sup>a</sup>	82 ± 3 <sup>b</sup>	81 ± 1 <sup>c</sup>
trout Ch	97 ± 2 <sup>a</sup>	96 ± 4 <sup>a</sup>	90 ± 5 <sup>b</sup>	74 ± 4 <sup>b</sup>	68 ± 3 <sup>c</sup>
carp B-D	49 ± 2 <sup>a</sup>	48 ± 3 <sup>a</sup>	45 ± 2 <sup>a</sup>	45 ± 4 <sup>a</sup>	42 ± 2 <sup>b</sup>
carp Ch	44 ± 3 <sup>a</sup>	41 ± 3 <sup>a</sup>	34 ± 3 <sup>b</sup>	24 ± 3 <sup>c</sup>	22 ± 3 <sup>c</sup>

a b c d e - significant differences in rows.

content during the smoking process. The initial content of these acids in total was 107 mg/g of lipids for trout and 49 mg/g of lipids for carp. These quantities are consistent with literature data, but it needs to be emphasized they differ depending on the season and type of feeding (Kaur and Sehgal, 2016). The content of these acids also differed depending on the method of extraction. The amount of EPA and DHA in the chloroform (Ch) extracts was about 10% lower for trout and about 20% lower for carp. The differences in the amount of extracted fat and fatty acids using selective extraction with application of different solvents were described by Bienkiewicz and Kołakowska (2004). They studied the interaction between fish lipids and starch in model systems, showing greater and stronger binding of Omega-3 fatty acids. This relation may also work in muscle tissue to a very similar extent. Similar relationships between protein and lipid interactions during fish processing, lipid conformation and their protective effect during protein denaturation during smoking and drying capelin are reported by Odoil et al. (2019).

During the smoking process, the amount of EPA and DHA decreased for all studied samples. At the stage of drying, statistically significant losses of these acids were found in both fish species, but only when it comes to Ch-lipids. In this case, the amount of these acids was reduced by 10% for trout and 15% for carp. These statistically significant losses of fatty acids correlate with a strong growth of primary oxidation products in this lipid fraction (Figure 2) at this stage of technological process. Reduced amount of lipid as a result of dripping, increased oxidation and loss of Omega - 3 fatty acids was observed during various technological treatments conducted by Kołakowska et al. (2006), who carried out studies on trout, García-Arias et al. (2003), who baked an anchovy or Larsen et al. (2010) during salmon heat treatment as well as Schneedorferová et al. (2015) during investigation of changes in the acid ratio of n-3 to n-6 while baking and frying sea and freshwater fish, including carp. Subsequent stages of smoking process resulted in even greater statistically significant losses of EPA and DHA in both fish species and in both types of lipids, i.e. B-D lipids and Ch lipids. After the final stage of smoking, i.e. heating. the losses of these acids were about 25% in the B-D lipid fraction for both fish species in order to obtain the level of temperature that guarantees microbiological safety (Løvdal, 2015). On the other hand, when it comes to Ch-lipid fraction, these losses were even bigger and amounted to 32% for trout and 40% for carp (Table 2). The study carried out by Domiszewski (2013) showed that heating for as long as 60 min resulted in a decrease of EPA and DHA by about 10 in percentage terms. Subsequent heating for as long as 120 min at the temperature of 100 °C intensified a decrease to around 20–25%.

#### 4. Conclusion

Two species of freshwater farmed fish, i.e. carp and trout were compared in terms of changes in the quality of lipids at particular stages of heat smoking. The subject of evaluation were two lipid extraction methods, which allowed the lipids of the fish to be differentiated between free lipids (Ch-lipids) and intra-tissue lipids known as B-D lipids. The conducted study indicates a difference in changes in lipids qualitative parameters, depending on the method of extraction. This is particularly clear to be seen in the oxidation processes measured by peroxide value (PV) and the evaluation of secondary oxidation products (AsV). However, no significant difference was observed in this respect between the two fish species, compared with each other. Hydrolytic changes during the smoking process also indicate significant differences between the types of lipids (Ch-lipids and B-D-lipids) but show no differences between the species. It was found that heat smoking results in loss of fat during the process, especially at the last two stages of the smoking process. Physical factors (temperature) as well as oxidative processes in lipids cause losses in the amount of Omega-3 fatty acids. Therefore, it is important for the technological processes of heat smoking to minimize the time, during which the temperatures are reached to guarantee microbiological safety of the product.

Presented results of lipid analysis were supported by the parameters describing raw material freshness (TVB - N). Determination of water activity, water and salt content as well as the parameters relevant for the description of the technological process was also performed. This made it possible to explain the relations and correlations between these determinations and the changes in qualitative parameters of lipids such as acid value (AV).

#### Declarations

##### Author contribution statement

Grzegorz Bienkiewicz: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Grzegorz Tokarczyk: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Barbara Czerniajewska-Surma: Conceived and designed the experiments; Wrote the paper.

Jacek Suryn: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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##### Additional information

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#### References

- Albishi, T., Banoub, J.H., de Camargo, A.C., Shahidi, F., 2019. Date palm wood as a new source of phenolic antioxidants and in preparation of smoked salmon. *J. Food Biochem.* 43, e12760.
- Ames, J.M., 1998. Applications of the Maillard reaction in the food industry. *Food Chem.* 62 (4), 431–439.
- Anonymous, 2005. Commission Regulation (EC) No 2074/2005 of 5 December 2005 Laying Down Implementing Measures for Certain Products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the Organization of Official Controls under Regulation (EC) No 854/2004 of the European Parliament and of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, Derogating from Regulation (EC) No 852/2004 of the European Parliament and of the Council and Amending Regulations (EC) No 853/2004 and (EC) No 854/2004. *Official Journal of the European Union.* L 338/27.
- AOAC, 2002. Association of official analytical chemists international (AOAC). *Official methods of analysis*, seventeenth ed. of AOAC international. Method 950.46, Method 940.25, Method 938.08.
- AOCS, 1993. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, fourth ed. AOCS, Champaign. cd 18-19, 1–2.
- Barbosa, R.G., Trigo, M., Prego, R., Fetti, R., Aubourg, S.P., 2017. The chemical composition of different edible locations (central and edge muscles) of flat fish (*Lepidorhombus whiffiagonis*). *Int. J. Food Sci. Technol.* 53 (2), 271–281.
- Bienkiewicz, G., Kołakowska, A., 2004. Effects of thermal treatment on fish lipids-amylose interaction. *Eur. J. Lipid Sci. Technol.* 106 (6), 376–381.
- Bienkiewicz, G., Domiszewski, Z., Tokarczyk, G., Plust, D., 2013. Distribution of lipids and oxidative changes therein in particularized parts of rainbow trouts filets. *Food. Sci. Technol. Qual.* 1 (86), 70–80.
- Bilgin, Ş., Değirmenci, A., 2019. Quality changes in reared, hot-smoked meagre (*Argyrosomus regius* Asso, 1801) during chill storage at 4 ± 1 °C. *Food Sci. Technol.* 2 (39).
- Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37 (8), 911–917.
- Chirwa, E.R., Kassam, D., Jere, W.L., Mtethiwa, A., 2017. A review of the farming of common carp (*Cyprinus carpio* L.) in Malawi: policy research directions for aquaculture development in Malawi. *Int. J. Fish. Aquac.* 9 (5), 42–51.
- Conway, E.J., Byrne, A., 1933. Micro-diffusion analysis of TVN. *Biochem. J.* 27, 419–429.
- Domiszewski, Z., 2013. Effect of heating fatty fish: baltic herring (*Clupea harengus* membras), European sprat (*Sprattus sprattus*) and rainbow trout (*Oncorhynchus*

- mykiss) on lipid oxidation and contents of eicosapentaenoic and docosahexaenoic acids. *Int. J. Food Sci. Technol.* 48, 786–793.
- Domiszewski, Z., Bienkiewicz, G., 2010. Determining fish fatty acid composition: a comparison of preparation fatty acid methyl esters direct and AOAC methods. *Folia Pomeranae Universitatis Technologiae Stetinensis* 281, 19–30.
- Duman, M., Karaton Kuzgun, N., 2018. Quality changes of nugget prepared from fresh and smoked rainbow trout during chilled storage. *Br. Food J.* 9 (120), 2080–2087.
- Dursun, S., Erkan, N., 2014. The effect of edible containing on the quality of smoked fish. *Ital. J. Food Sci.* 26 (4), 370–382.
- Esmailnia, R., 2015. Measuring the amount of water activity (aw) in hot smoked rainbow trout (*Oncorhynchus mykiss*) in various salinity percentages. *J. Appl. Environ. Biol. Sci.* 4 (11S), 103–111.
- Espea, M., Nortvedta, R., Liea, Ø., Hafsteinsson, H., 2002. Atlantic salmon (*Salmo salar*, L.) as raw material for the smoking industry. II: effect of different smoking methods on losses of nutrients and on the oxidation of lipids. *Food Chem.* 77 (1), 41–46.
- Gang, K.Q., Wu, Z.X., Zhou, D.Y., Zhao, Q., Zhou, X., Lv, D.D., Rakariyatham, K., Liu, X.Y., Shahidi, F., 2019. Effects of hot air drying process on lipid quality of whelks *Neptunea arthritica cumingi* Crosse and *Neverita didyma*. *J. Food Sci. Technol.* 56 (9), 4166–4176.
- García-Arias, M.T., Álvarez-Pontes, E., García-Linares, M.C., García-Fernández, M.C., Sánchez-Muniz, F.J., 2003. Cooking–freezing–reheating (CFR) of sardine (*Sardina pilchardus*) fillets. Effect of different cooking and reheating procedures on the proximate and fatty acid compositions. *Food Chem.* 83 (3), 349–356.
- Grigorakis, K., 2017. Fillet proximate composition, lipid quality, yields, and organoleptic quality of Mediterranean farmed marine fish: a review with emphasis on new species. *Crit. Rev. Food Sci. Nutr.* 57 (14), 2956–2969.
- Grigorakis, K., Kogiannou, D., Genevieve, C., Pérez-Sánchez, J., Agnes, A., Zsuzsanna, J.Sándor, 2018. Impact of diets containing plant raw materials as fish meal and fish oil replacement on rainbow trout (*Oncorhynchus mykiss*), gilthead sea bream (*Sparus aurata*), and common carp (*Cyprinus carpio*) freshness. *J. Food Qual.* (18), 1–14.
- Guillén, M.D., Errecalde, M.C., Salmerón, J., Casas, C., 2006. Headspace volatile components of smoked swordfish (*Xiphias gladius*) and cod (*Gadus morhua*) detected by means of solid phase microextraction and gas chromatography–mass spectrometry. *Food Chem.* 94 (1), 151–156.
- Haman, N., Bodner, M., Ferrentino, G., Scampicchio, M., 2019. Lipid autooxidation of fish, lard, corn and linseed oils by isothermal calorimetry. *Ital. J. Food Sci.* 2 (31), 323–331.
- Hryszko, K., Lirski, A., 2017. Wielkość produkcji ryb karpiowatych w akwakulturze europejskiej. *Komunikaty Rybackie* 1, 27–31.
- Husain, S., Patang, P., 2018. Analysis of the use of various types of fuel and smoking room temperature value of nutrition and organoleptic smoke carp (*Cyprinus carpio* sp.). *Int. J. Adv. Chem. Technol. Res.* 11 (5), 414–420.
- Kaur, M., Sehgal, G., 2016. Studies on the availability of EPA and DHA of different cultured carp species during different seasons. *Int. J. Sci. Res.* 5 (11), 1026–1029. November 2016.
- Kotakowska, A., 2010. Lipid oxidation in food systems. In: Sikorski, Z.E., Kolakowska, A. (Eds.), *Chemical, Biological and Functional Aspects of Food Lipids*. CRC Press, New York, pp. 163–184.
- Kotakowska, A., Domiszewski, Z., Bienkiewicz, G., 2006. Effect of biological and technological factors on the utility of fish as a source of n-3 PUFA. In: Teale, M.C. (Ed.), *Omega 3 Fatty Acid Research*. Nova Science Publishers, New York, pp. 83–108.
- Labuza, T.P., Acott, K., Tatini, S.R., Lee, R.Y., Flink, J., McCall, W., 1976. Water activity determination: a collaborative study of different methods. *J. Food Sci.* 41, 910–917.
- Larsen, D., Quek, S.Y., Eyres, L., 2010. Effect of cooking method on the fatty acid profile of New Zealand King Salmon (*Oncorhynchus tshawytscha*). *Food Chem.* 119 (2), 785–790.
- Leventakou, V., Roumeliotaki, T., Martinez, D., Barros, H., Casas, M., Chatzi, L., 2014. Fish intake during pregnancy, fetal growth and gestational length in 19 European birth cohort studies. *Am. J. Clin. Nutr.* 99 (3), 506–516.
- Løvdaal, T., 2015. The microbiology of cold smoked salmon. *Food Control* 54, 360–373.
- Mahmoud, M.A.A., Buettner, A., 2017. Characterisation of aroma-active and off-odour compounds in German rainbow trout (*Oncorhynchus mykiss*). Part II: case of fish meat and skin from earthen-ponds farming. *Food Chem.* 232, 841–849.
- Malesa-Ciećwierz, M., Szulecka, O., Adamczyk, M., 2019. Polycyclic aromatic hydrocarbon contamination of Polish smoked fish: assessment of dietary exposure. *J. Food Process. Preserv.* 43, e13962.
- Migdał, W., Dudek, R., Kapinos, R., Kluska, W., 2016. Wędzenie tradycyjne. In: Migdał, W. (Ed.), *Wędzenie Tradycyjne. Punkty Krytyczne*. Kraków – Pilzno: Polskie Stowarzyszenie Producentów Wyrobów Wędzonych Tradycyjnie, pp. 10–14.
- Neira, L.M., Agustinelli, S.P., Ruseckaite, R.A., Martucci, J.F., 2019. Shelf life extension of refrigerated breaded hake medallions packed into active edible fish gelatin films. *Packag. Technol. Sci.* 32, 471–480.
- Nieva-Echevarría, B., Goicoechea, E., Guillén, M.D., 2017. Effect of liquid smoking on lipid hydrolysis and oxidation reactions during in vitro gastrointestinal digestion of European sea bass. *Food Chem.* 97, 51–61.
- Odoli, C.O., Oduor-Odote, P., Arason, S., 2019. The influence of lipid content and pretreatment methods on protein conformation in fish (capelin, *Mallotus villosus*) during smoking and drying. *Food Sci. Nutr.* 7, 1446–1454.
- Pietrzyk, C., 1958. Spectrophotometric determination of lipid peroxides by thiocyanate technique. *Ann. Natl. Inst. Hyg.* 9, 75–84.
- Pieńkowska, B., Hryszko, K., 2016. Rynek ryb. Stan i perspektywy. In: Seremak-Bulge, J. (Ed.), *Spożycie Ryb i Owoców Morza Oraz Ich Przetworów*. IERIGZ-PIB, Warszawa, pp. 30–34.
- Pokorny, J., Janicek, G., Dawidek, J., 1975. Determination of the interaction products of proteins with lipids. *Zesz. Probl. Postepow. Nauk. Rol.* 167 (12), 155–170.
- Pokorny, J., Kolakowska, A., Bienkiewicz, G., 2010. Lipid - protein and lipid - saccharide interaction. In: E Sikorski, Z., Kolakowska, A. (Eds.), *Chemical and Functional Properties of Lipids*. CRC Press, New York, pp. 455–472.
- Polish Standards Method, 2009. PN-EN ISO 660:2009. Vegetable and Animal Oils and Fats - Determination of the Acid Value and Acidity.
- Schneedorferová, I., Tomčala, A., Valterová, I., 2015. Effect of heat treatment on the n-3/n-6 ratio and content of polyunsaturated fatty acids in fish tissues. *Food Chem.* 176, 205–211.
- Serpen, A., Gokmen, V., Fogilano, V., 2012. Total antioxidant capacities of raw and cooked meats. *Meat Sci.* 90 (1), 60–65.
- Skalecki, P., Florek, M., Litwińczuk, A., Staszowska, A., Kaliniak, A., 2013. The nutritional value and chemical composition of muscle tissue of carp (*Cyprinus carpio*, L.) and rainbow trout (*Oncorhynchus mykiss*, Walb.) obtained from fish farms in the Lublin. *Sci. Ann. Pol. Soc. Anim. Prod.* 9 (2), 57–62.
- Tenyang, N., Womeni, H.M., Tiencheu, B., Takugan, F.N.H., Tchouanguep, M.F., Villeneuve, P., Linder, M., 2013. Lipid oxidation of catfish (*Arius maculatus*) after cooking and smoking by different methods applied in Cameroon. *Food Nutr. Sci.* 4 (9A), 176–187.
- Tenyang, N., Tiencheu, B., Womeni, H.M., 2018. Effect of smoking and refrigeration on lipid oxidation of *Clupea harengus*: a fish commonly consumed in Cameroon. *Food Sci. Nutr.* 6, 464–473.
- Tokarczyk, G., Szymczak, B., Szymczak, M., Domiszewski, Z., 2011. Changes in selected chemical and microbiological indicators during warm smoking process of thawed Whitefish (*Coregonis clupeaformis*). *Food Sci. Technol. Qual.* 5 (78), 119–131.
- Wawireb, M., Tsighe, N., Mahmuda, A., Abrahama, B., Wainainab, I., Karimib, S., Abdulkerima, Z., 2019. Effect of salting and pressing on quality characteristics of spotted sardine (*Amblygaster sirm*) during different storage conditions. *J. Food Compos. Anal.* 79, 47–54.
- Zachara, A., Gałkowska, D., Juszcak, L., 2017. Contamination of smoked meat and fish products from Polish market with polycyclic aromatic hydrocarbons. *Food Control* 80, 45–51.
- Zhou, G.H., Zhao, G.M., 2007. Biochemical changes during processing of traditional Jinhua ham. *Meat Sci.* 77, 114–120.