Chemometric analysis of fatty acids profile of bream (Abramis brama), ruffe (Gymnocephalus cernua) and perch (Perca fluviatilis) meat from Lake Gopło and Włocławski Dam Reservoir

Analiza chemometryczna profili kwasów tłuszczowych mięsa leszcza (Abramis brama), jazgarza (Gymnocephalus cernua) i okonia (Perca fluviatilis) odłowionych z Jeziora Gopło i Zbiornika Włocławskiego

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ABSTRACT

The 18 fatty acid profiles have been determined in 63 samples of muscles from three freshwater fish species: bream, ruffe and perch by gas chromatography method. The fish were collected in natural condition from two reservoirs located in central Poland: Lake Gopło and Włocławski Reservoir. A chemometric study with the use of hierarchical cluster analysis (HCA), principal component (PCA) and stepwise linear discrimination analysis (LDA) was applied to characterize, classify and differentiate collected samples. The chemometric techniques by using fatty acids content as descriptors allow clearly distinguish 6 groups according to fish species and their geographical origin.

Keywords: fatty acids, freshwater fish, chemometrics

ABSTRAKT

Oznaczono profil 18 kwasów tłuszczowych w 63 próbach mięsa pochodzącego od trzech gatunków ryb słodkowodnych: leszcza, jazgarza i okonia. Ryby zostały odłowione z dwóch zbiorników zlokalizowanych w centralnej części Polski: z Jeziora Gopło i Zbiornika Włocławskiego. Do scharakteryzowania, klasyfikacji oraz rozróżnienia zebranych próbek zastosowano metody chemometryczne: hierarchiczną analizę skupień (HCA), metodę głównych składowych (PCA) oraz liniową analizę dyskryminacyjną (LDA). Metody chemometryczne oparte na zawartości kwasów tłuszczowych jako deskryptorów pozwoliły na wyraźne rozróżnienie 6 grup zgodnie z gatunkiem i pochodzeniem ryb.

Słowa kluczowe: kwasy tłuszczowe, ryby słodkowodne, chemometria

STRESZCZENIE SZCZEGÓŁOWE

Celem pracy było oznaczenie i porównanie profilu kwasów tłuszczowych zawartych w mięsie trzech gatunków ryb słodkowodnych. Materiał biologiczny stanowiły okonie (n=31), jazgarze (n=13) i leszcze (n=19) odłowione z Jeziora Gopło i Zbiornika Włocławskiego. Na każdym osobniku dokonano pomiarów długości ciała z dokładnością ± 0,1 cm, a masę ciała określano z dokładnością ± 0,01 g. Okonie odłowione ze zbiornika Włocławskiego ważyły średnio 100.56, a te z Gopła 96.49 g. Długość ciała ryb tego gatunku wynosiła średnio 18.6 cm dla osobników ze zbiornika i 17.22 cm dla ryb z Gopła. Jazgarze ze zbiornika Włocławskiego i Gopła ważyły odpowiednio 39.45 i 31.76 g a ich długość ciała wynosiła średnio 12.3 i 11.5 cm. Leszcze z Włocławka ważyły średnio 528.62 g i mierzyły 28.6 cm oraz 531.60 g i 30.3 cm (ryby z Gopła). Do badań pobrano nadosiową część mięśnia bocznego wielkiego ze środkowej części tułowia wraz ze skórą. Pobraną część mięsa zliofilizowano, a następnie oznaczono zawartość tłuszczu (% mokrej masy) i skład kwasów tłuszczowych (% oznaczanych kwasów tłuszczowych). Analizę kwasów tłuszczowych w postaci ich estrów metylowych wykonano metodą kapilarnej chromatografii gazowej z detektorem płomieniowo-jonizacyjnym. W grupie analizowanych kwasów tłuszczowych były kwasy nasycone (SFA) (C14:0, C15:0, C16:0, C17:0, C18:0, C24:0), jednonienasycone (MUFA) (C 14:1, C15:1, C16:1, C17:1, C18:1) i wielonienasycone (PUFA) (C16:2, C18:2 n-6, C18:3 n-6, C20:4 n-6, C20:5 n-3, C22:2, C22:6 n-3). Analizy wykazały, że spośród SFA w największych ilościach występował C16:0, C18:0, w grupie MUFA i PUFA kwasami dominującymi były odpowiednio C18:1 i C20:5 n-3. W grupie wszystkich oznaczonych kwasów najmniejszy udział miały głównie kwasy nasycone i jednonienasycone (C14:1, C15:0, C15:1, C17:0 i C17:1).

Do eksploracji zbioru danych (profili kwasów tłuszczowych) zastosowano metody chemometryczne: hierarchiczną analizę skupień (HCA), metodę głównych składowych (PCA) oraz liniową analizę dyskryminacyjną (LDA). Wykorzystanie zawartości oznaczonych kwasów tłuszczowych jako deskryptorów pozwoliło na jednoznaczne rozróżnienie sześciu grup ryb odpowiadających badanym trzem gatunkom z dwóch różnych miejsc odłowu.

INTRODUCTION

Fat and fatty acids are very important because of their effect on human health. Long chain n-3 polyunsaturated fatty acids (PUFA) cannot be synthesized by human bodies and should be supplied by the diet [9]. The most important source of long-chain PUFA with up to 5 to 6 double bonds (eicosapentaenoic acid - EPA and docosahexaenoic acid - DHA) are marine fish and seafood [24,25]. Freshwater fish contain more fatty acids of n-6 family (for example linoleic acid 18:2 n-6) than marine fish. The ratio of n-3 PUFAs to n-6 PUFAs in total lipids (the so-called essential fatty

acids ratio) is between 0,5 and 3,8 for freshwater fish and 4,7 to 14,4 for marine individuals [23].

PUFAs of n-3 family can reduce triacylogliceride (TAG), VLDL-, LDL-lipoprotein level in blood serum and may decrease blood pressure preventing coronary heart diseases and arteriosclerosis [15]. Moreover n-3 fatty acids decreasing risk of arrhythmia and thrombosis [8]. EPA and DHA prevent of human breast cancer growth, asthma, inflammatory disease and disorders of the immune system [6]. Fatty acids levels in the tissues of fish are determined by a variety of factors including environmental conditions, physiological state, species [2, 15,17], food type, fishing season [9, 16, 22], freshwater or marine origin and whether they are farmed or wild [8,10].

There are many possible techniques for classification of data. Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) are two commonly used techniques for data classification and dimensionality reduction. PCA, as well as Cluster Analysis (CA) have been successfully applied using fatty acid profile as variable data set for distinguish various meat processed products [3], identification of the farm origin of the salmon [18] or differentiation between the fattening diet system of goats [19]. LDA has been performed in order to obtain classification rules for differentiation between production systems and cattle breeds [5]. The results showed that the raw meat fatty acid profile is good descriptor for discrimination between breed with traditional and organic farming production.

The use of multivariate methods (HCA, PCA and LDA) afford a better understanding of the composition of meat samples according to the fish origin and species in contrast to direct comparison of each fatty acid content. PCA provides information on the most meaningful parameters which describe the whole data set interpretation and summarizes the statistical correlation among fatty acids.The aim of this study was to determine and compare of the fatty acid profile of muscles of three freshwater fish species collected from two different reservoirs. Differences in the composition of the fatty acid profile have been used to make discrimination between species of fish and their geographical origin.

MATERIALS AND METHODS

STUDY AREA

The Włocławski Reservoir is the largest reservoir in Poland (central part of country) with respect to area (ca 70-75 km^{-2}) [13]. The Włocławski Reservoir was created in the 1970s when a dam was constructed across the Vistula River at 675th km. The average depth is 5.5 m with the maximum of 15 m [1]. It is eutrophic, shallow and strongly rhedimnic reservoir (run-off-river reservoir). The dominant fish species in this reservoir is the common bream (*Abramis brama* L.) (60% in the weight of total commercial catch). Zoobenthos it is 30 taxa, including 10 forms of *Chironomidae*, 11 species of *Oligochatea* and 9 species of *Mollusca*. The Włocławski Reservoir is not a

river, not a lake, not even an effect of a simple combination between riverine and lacustrine features [14]. It is a specific superrheolimnic reservoir, more similar to a somewhat slowed down river rather than a typical dam reservoir [4]. Phytoplantcton of this reservoir has a typical river-like character, dominated by green algae and diatoms. The oxygen conditions in Włocławski reservoir are generally good.

The Lake Gopło is located in the southern part of kujawsko-pomorski province. The western part of this lake it's a strict nature reserve. The main morphometric indicators of Lake Gopło are as follows; surface area: 22 km^2, maximum depth:16 m, average depth ca 4.7 m and length of shoreline: 90 km [12]. Lake Gopło is eutrophic reservoir, based on the limnological classification and it is a zander type of lake, based on the fishing classification. Among the zooplankton of the Lake Gopło, there are identified 65 species of *Rotiferas*, 34 species of *Copepode* and 8 species of *Cladocera.* The main group of benthos it's *Chironomid* (dominated by *Chironomus plumosus*). The ichthyofauna is dominated by white bream (*Abramis bjoerkna* L.). Among the predatory fish, a significant part of the fishing are eel (*Anguilla anguilla* L.) and zander (*Sander lucioperca* L.). Analyses revealed that the waters are qualified as unclassified [20].

Fish samples

Three species of freshwater fish (bream, perch and ruffe) were collected from two reservoirs located in central Poland: Lake Gopło and Włocławski Reservoir. The fish were obtained in natural condition. After fish were caught, length and body weight measurements were recorded for each individual to the nearest 1mm and 0.01g respectively (Tab. 1.). A total of 63 samples were collected, including 31 perch and 19

Table 1. Mean values of biometric measurements of perch, ruffe and bream caught from Włocławski Resorvoir and Lake Gopło - total length (*Lt*), body length (*Lc*), body weight (BW) and age.
Tabela 1. Średnie wartości pomiarów biometrycznych dla okonia, jazgarza i leszcza odłowionego ze Zbiornika Włocławskiego i Jeziora Gopło – długość całkowita (*Lt*), długość ciała (*Lc*) i masa ciała (BW)

Species	Catch place	n	Total length (cm)	Body length (cm)	Body weight (g)	Age
Perch	Włocławski Reservoir	14	20,3	18,6	100,56	5+
	Lake Gopło	17	19,5	17,2	96,49	
Ruff	Włocławski Reservoir	6	15,0	12,3	39,45	4+
	Lake Gopło	7	13,5	11,5	31,76	
Bream	Włocławski Reservoir	9	35,4	28,6	528,62	6+
	Lake Gopło	10	38,0	30,3	531,60	

bream and 23 ruffe. There have been chosen males for analyses. Fish were collected in April (perch and ruffe) and in June (bream) from each reservoir.

The muscle samples for analysis were from the large side muscle of fish body above the lateral line. The muscle samples, including the skin were freeze dried. Due to relatively low amounts of muscle obtained from ruffe, the material from individuals of similar body length (about 5-6 pieces each) was combined.

Fat extraction

Extraction of total lipids was performer according to Folch et. al [7]. Two g of freeze dried fish muscle were accurately weighted, transferred to a 250 ml flask and 25 ml of chloroform and methanol mixture (2:1) were added. Next the flasks were put for 30 min into the ultrasonic bath to improving extraction efficiency. After extraction samples were filtered and evaporated to dryness in a rotating evaporator at 35ºC under the pressure.

Chromatographic analysis

Fat extracted from samples was methylated with solution of sodium methoxide (0.5 mol dm^{-3}) for 22 hours, at 37°C. The fatty acids profile was analysed in a gas chromatograph (Varian, USA, type 3800GC) equipped with flame-ionization detector and with capillary column Supelcowax10 30 m x0.32 mm x 0.25 µm. The temperature of the injector was 230°C, and that of the detector was 250°C. The volume of the injected sample was 1 µl (split 1:50). The carrier gas was helium at a flow rate of 1.5 cm^3 min^{-1}. The analyses were performed at a program temperature range of 90 to 225°C (11ºC min^{-1}), 225°C for 6 min, and then an increase from 225 to 240°C (6°C min^{-1}) and 240°C for 19 min. In order to extract methyl esters of fatty acids, isooctane was added. The group of the fatty acids analyzed included saturated acids (SFA) (C14, C15, C16, C17, C18, C24), monounsaturated acids (MUFA) (C14:1, C15:1, C16:1, C17:1, C18:1) and polyunsaturated acids (PUFA) (C16:2, C18:2n-6, C18:3n-3, C20:4n-6, C20:5n-3, C22:2, C22:6n-3). Methyl esters of fatty acids were identified according to Supelco PUFA-2 Animal Source and Supelco 37 component FAME Mix (Supelco, USA).

Statistical analysis

All data were statistically analysed by the t-test and one-way analysis of variance (ANOVA). Tuckey test was applied to compare the mean values of each fatty acids between different fish species (in the same reservoir). The normality and homogeneity of variance were studied using the Shapiro-Wilk test and the Levene test, respectively. Mean values and standard deviations (SD) are reported in Table 2. Differences were considered statistically significant if p£0.5.

The classification and discrimination of different fish species origin were achieved by three chemometric methods: cluster analysis (CA), linear discrimination analysis (LDA) and principal component analysis (PCA) using Statistica 8.0 (Statsoft, Inc®).

These methods were performed on the standardized dataset. Moreover, prior to performing PCA, the suitability of data for factor analysis was assessed by the Bartlett's test of spherity and Kaiser-Mayer-Olkin method (KMO) [21].

RESULTS AND DISCUSSION

Table 2 shows the proportions of 18 fatty acids (as percent of total fatty acids) identified in this study and significant statistical differences between fatty acids in the same fish species from different reservoirs and in different species from the same reservoir. Palmitic (C16:0), stearic (C18:0), oleic (C18:1) and eicosapentaenoic acid (C20:5n3) have the most contribution in total fatty acids. Palmitic acid C16:0 was the dominant saturated fatty acids (SFA) accounting 24.9% for bream and 33.8% for ruffe from Włocławski Reservoir. The lowest percentage of SFA was C15:0. Łuczyńska et al. [17] observed that in all analysed species of fish, palmitic acid (C16:0) was the dominant SFA. This fatty acid was the primary SFA in the meat of carp (*Cyprinus carpio* L.) in all seasons (14.0-16.6%) [9], for rapfen

(*Aspius aspius* L.), bream and pike (*Esox lucius* L.) [25] and for all analysed fish species by Kołakowska et al [15]. The same results were obtained by the authors of this paper for perch caught in different fishing seasons from Włocławski reservoir [22].

Of the MUFAs group, the highest percentage was of oleic acid (C18:1 n-9) was shoved the highest percentage while C14:1 and C15:1 were in the lowest percentage. The same results obtained Donmez [6] and Żmijewski et al. [25]. Oleic acid was identified as a primary MUFA in the meat of carp caught in all seasons (15.1-20.3%) and palmitoleic acid (16:1) was found the second most abundant MUFA (5.11-13.20%) [9]. Monoenoic fatty acids, mainly C18:1 comprised more than half of the proportion of fatty acids [15].

The analysis of PUFA-s indicated that linoleic acid and EPA (C20:5 n-3) shared the highest percentage in the fish species tested. Donmez [6] and Jankowska et al. [11] reported that EPA (C20:5 n-3) and DHA (C22:6 n-3) were the main fatty acids of the PUFAs group in common carp (*Cyprinus carpio* L.), chub (*Leusissus cephalus* L.), tench (*Tinca tinca* L.) and in perch. Analysis carried out by Łuczyńska et al. [17] indicated that arachidonic acid (C20:4 n-6) was dominant PUFAs of n-6 family, except in vendance (*Coregonus albula* L.) and in all species the n-3/n-6 ratio was higher than 1 and ranged from 1.5 to 4.40.

In the present work the ratio n-3/n-6 for perch and ruffe from both reservoirs was similar, about 0.8 and 0.7 respectively. However n-3/n-6 ratio for bream from Lake Gopło is almost twice greater than that for Włocławski Reservoir because the proportion of DHA in the fish collected from the later water was low. The percentages of PUFA, such as EPA and DHA in fish muscle are dependent on diet. Variations in fatty acids composition might be related to the changes in nutritional habits of the fish [9].

Table 2. Fatty acid compositions (percentage levels) of lipid extracts from muscles of fish collected from different waters

Tabela1. Skład kwasów tłuszczowych (zawartość procentowa) w ekstrakcie tłuszczu z mięśni ryb

Fatty acid Kwas tłuszczowy	Mean±SD Średnia ± odch. stand. Perch Okoń (*Perca fluviatilis*)		Ruffe Jazgarz (*Gymnocephalus cernua*)		Bream Leszcz *(Abramis brama)*	
	Lake Gopło (n=17)	Włoclawski Reservoir (n=14)	Lake Gopło (n=7)	Włoclawski Reservoir (n=6)	Lake Gopło (n=10)	Włoclawski Reservoir (n=9)
Myristic C14:0	1.1±0.1^{A,a}	2.7±0.5^{A}	3.1±0.1^{a}	2.3±0.2	2.7±0.4^{a}	2.7±0.3
Myristoleic C14:1	0.2±0.0	0.4±0.2	0.4±0.0	0.3±0.1	0.4±0.1	0.5±0.1
Pentadecanoic C:15:0	0.6±0.1^{a}	0.6±0.1	0.4±0.1^{a}	0.4±0.0	0.5±0.1^{a}	0.5±0.01
Pentadecadienoic C15:1	0.4±0.1	0.3±0.1	ND	ND	ND	ND
Palmitic C16:0	32.5±2.4^{a}	30.5±1.7^{b}	29.2±0.34^{a}	34.0±2.01^{c}	26.3±2.8	24.9±0.9^{b,c}
Palmitoleic C16:1	6.7±1.0^{A,a}	11.7±2.7^{a,b}	13.4±0.1^{A,B}	7.5±0.9^{B,C,b}	7.7±1.0	13.0±0.9^{c}
Heksadecadienoic C16:2	2.2±0.5^{A,a}	1.3±0.3^{A,b}	0.5±0.1^{a}	0.9±0.1	0.7±0.2^{a}	0.7±0.1^{b}
Heptadecanoic C17:0	0.9±0.1^{A,a}	0.6±0.1^{A}	0.5±0.1^{a,b}	0.5±0.0	1.1±0.2^{B,b}	0.7±0.2
cis-10-heptadecanoic C17:1	0.9±0.1^{a}	0.9±0.1^{b}	0.9±0.0^{A}	0.5±0.0^{A,b,c}	0.7±0.2^{B,a}	0.9±0.1^{B,c}
Stearic C18:0	6.9±0.6^{a}	6.41±1.17^{b}	6.0±0.2^{d}	7.4±0.2^{c}	9.6±0.5^{A,a,d}	14.4±0.6
Oleic C18:1	15.1±1.1^{A,a}	20.0±1.9^{A,b}	18.2±0.3	16.8±0.6^{C}	19.7±2.3^{B,a}	29.6±0.5^{B,b,c}
Linoleic C18:2n6	4.8±1.8^{a}	5.8±0.4	8.9±0.7^{A,a}	6.4±0.3^{A,b}	6.4±0.5^{B,a}	4.4±0.8^{B,b}
α-linolenic C18:3n3	1.2±0.1^{a,b}	1.7±0.4	5.1±0.5^{A,a}	1.1±0.3^{A}	4.7±0.6^{B,b}	1.4±0.2^{B}
Arachidonic C20:4 n6	7.9±1.2^{A,a,b}	4.2±1.3^{A,c}	4.7±0.6^{a}	5.3±0.7^{d}	5.4±0.7^{B,b}	1.6±0.4^{B,c,d}
Eicosapentaenoic (EPA) C20:5n3	8.3±1.0^{A,a}	5.7±1.0^{A,b}	4.5±0.2^{B,a,d}	7.0±0.7^{B,c}	8.5±1.2^{C,d}	2.8±0.5^{C,b,c}
Docosadienoic C22:2	1.8±0.3	1.5±0.3	ND	1.5±0.2	ND	ND
Docosahexaenoic (DHA) C22:6n3	4.2±1.0^{A,a,b}	2.4±0.7^{A,c}	1.4±0.1^{a}	2.5±0.5^{d}	2.3±0.3^{B,b}	0.7±0.3^{B,c,d}
Lignoceric C24:0	4.4±0.5^{a}	3.7±0.8^{b}	3.1±0.2^{A}	5.5±0.7^{A,b}	3.5±0.7^{B,a}	1.2±0.4^{B,b}
∑SFA	46.4±2.0^{A}	44.6±3.1^{A}	42.2±0.3^{B}	50.0±1.5^{B,a}	43.5±3.0^{a}	44.3±1.1^{a}
∑MUFA	23.3±1.9^{A,a,b}	33.3±4.5^{A,c}	32.8±0.5^{a}	25.3±1.1^{c}	28.5±3.2^{B,b}	44.1±0.8B,c
∑PUFA	30.3±2.6^{A}	22.1±3.0^{A,a}	25.0±0.2	24.7±1.9^{b}	27.9±1.8^{B}	11.5±1.2^{B,a,b}
n-3	13.7±1.6^{A,a}	9.8±1.6^{A,b}	10.9±0.2^{d}	10.7±1.1^{c}	15.5±1.4^{B,a,d}	4.9±0.8^{B,b,c}
n-6	16.9±1.5^{A,a}	11.9±1.7^{A,b}	14.9±0.3	14.1±1.0^{c}	14.0±1.0^{B,a}	6.6±0.7^{B,b,c}
n-3/n-6	0.8±0.1^{A,a}	0.8±0.1^{A}	0.7±0.0^{b}	0.8±0.0	1.1±0.1^{B,a,b}	0.7±0.1^{B}
EPA/DHA	2.0±0.0	2.4±0.1	3.2±0.1	2.8±0.1	3.7±0.2	4.1±0.1

Chemometric analysis

Cluster analysis and PCA

The Euclidean distance was used as a similarity measurement and the Ward's method as an amalgamation rule. The resulting dendrogram is depicted in Fig. 1 and shows six different clusters at $(D_{link}/D_{max})x100$ under 20. The clusters 1, 4 and 5 aggregate fish from Włocławski Reservoir bream (BW), ruffe (RW) and perch (PW), respectively. The fish from Lake Gopło: bream (BG), ruffe (RG) and perch (PG) are aggregated in cluster 2, 3 and 6.

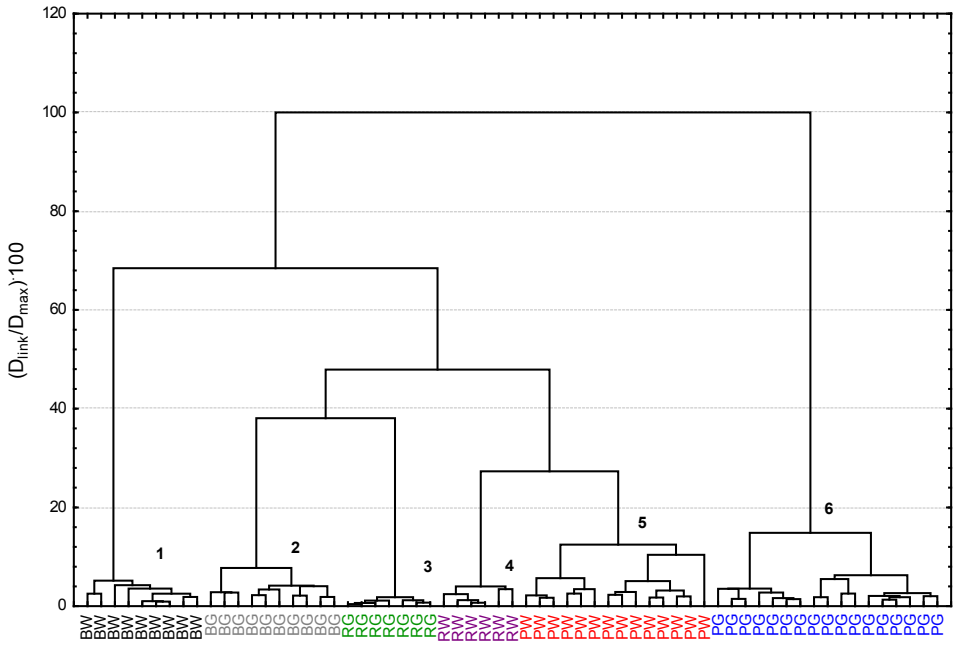

Figure. 1. The dendrogram produced by hierarchical cluster analysis (HCA) using Ward's method and squared Euclidean distances.
Rycina 1. Dendrogram otrzymany w hierachicznej analizie skupień (HCA) stosując metodę Warda i kwadrat odległości Euklidesowej.

The data matrix of variables (16 fatty acids) and scores (63 fish samples) was subjected to PCA in order to decrease the number of descriptors associated with the data set. PCA is mostly used for data compression and visualization. The main goal of this method is to explain the information contained in the data by a set of the so-called principal components (PCs).

Kaiser-Mayer-Olkin (KMO) measure of sampling adequacy was 0.796, which exceeded the recommended value 0.6. Bartlett's Test of sphericity yielded χ^2= 1462.99 (df=120. P=0.000). Table 3 shows the most significant principal components generated from fatty acid data matrix. The highest loading value for each variable (fatty acid) was highlighted in the boldface type. The first three PCs accounted for 80.22% of total variance explained by all the generated principal components. According to Kaiser's rule (eigenvalue >1) these PCs were used for further study. The first PC (PC1) has the highest eigenvalue (8.08) and describes 50.53% of total variance in the samples

Table 3. Loadings, eigenvalues and variances of the significant principal components (the most significant loadings are boldface)
Tabela 3. Ładunki czynnikowe, wartości własne i wariancje istotnych składowych głównych (najistotniejsze ładunki czynnikowe oznaczono pogrubionym drukiem)

Variables	PC1	PC2	PC3
C14:0	0.86	-0.21	0.18
C14:1	0.64	0.18	-0.12
C15:1	-0.72	0.49	0.28
C16:1	0.81	0.23	0.45
C16:2	-0.82	0.44	0.06
C17:0	-0.41	0.00	-0.76
C17:1	0.07	0.67	0.12
C18:0	0.54	0.34	-0.66
C18:1	0.83	0.43	-0.21
C18:2n6	0.30	-0.78	0.38
C18:3 n3	0.38	-0.72	-0.19
C20:4 n6	-0.91	-0.20	-0.15
C20:5 n3	-0.80	-0.40	-0.31
C22:2	-0.83	0.30	0.38
C22:6 n3	-0.95	0.05	-0.07
C24	-0.80	-0.38	0.21
Eigenvalue	8.08	2.84	1.90
Variance (%)	50.53	17.81	11.88
Cumulative(%)	50.53	68.33	80.22

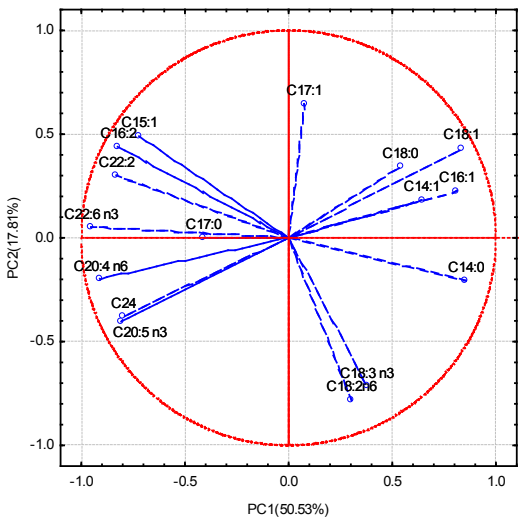

Figure. 2. Loading plot of PC1-PC2.
Rycina. 2 Wykres ładunków czynnikowych dla układu PC1-PC2.

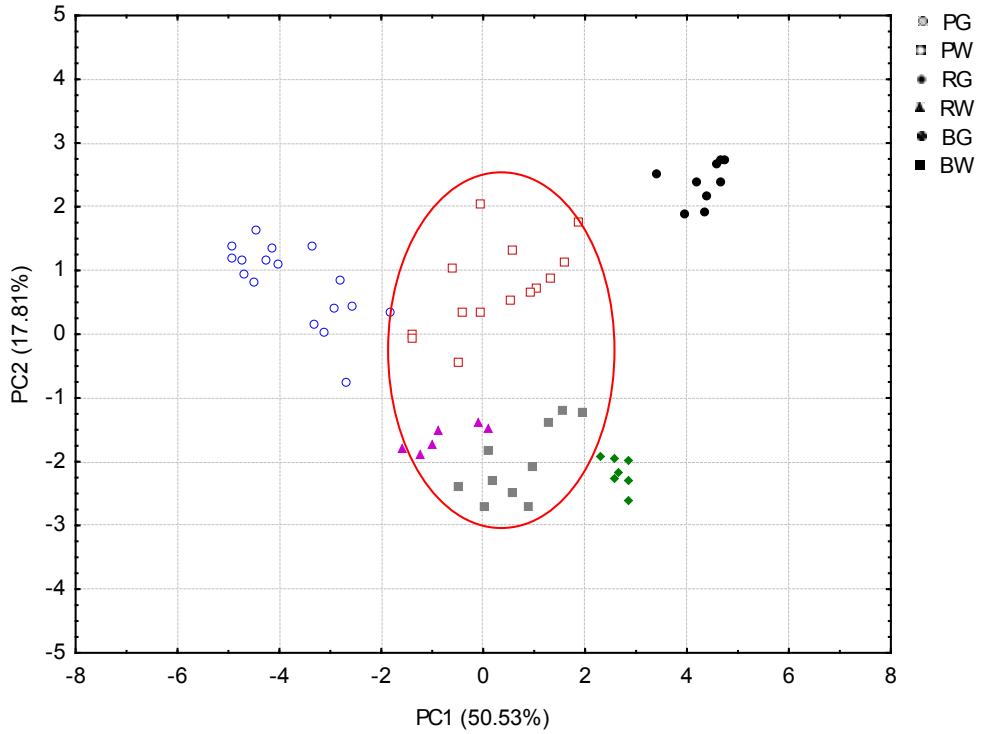

Figure 3. Score plot for PC1and PC2. Abbreviations: PG and PW – perch from Lake Gopło and Włocławski Reservoir, respectively. Similarly, BG, BW are bream and RG, RW are ruffe.
Rycina. 3. Wykres przypadków w układzie PC1 i PC2. Oznaczenia: PG i PW – odpowiednio okoń z jeziora Gopło i zbiornika Włocławskiego, analogicznie BG, BW dla leszcza i RG, RW dla jazgarza.

Fig. 2 illustrates the factor loading plot for the fatty acid data. Most variables correlate highly with first PC and have low correlation with PC2. The majority of fatty acids containing 18 or less carbon atoms have high positive loadings in PC1.as follows: myristic (0.86), myristoleic (0.64), palmitoleic (0.81) and oleic (0.83) (Table 3). All three very long fatty acids (VLFA), consisting of above 22 carbon atoms, and the long fatty acids such EPA and arachidonic acid gathered on the left side of PC1xPC2 area on Fig.3. and have negative loadings in PC1.

PC2 showed a high positive loading from cis-10-heptadecanoic acid (0.67) and high negative loadings from linoleic and α-linolenic acids. The loadings plot can explain correlations between variables. Variables closest to each other in two-dimensional space and far from the plot origin are positively correlated and variables opposite to other are negatively correlated. In Fig. 2 many high positive correlation can be observed e.g. between linoleic and α-linoleic acid, EPA and lignoceric acid and between C14:1 and C16:1. The last two acids are negatively correlated with arachidonic acid.

Data set can efficiently be compressed by PCA to three significant PCs, describing over 86% of the total data variance. The PC1 and PC2 scores allow a good separation of the three fish species and two geographical areas investigated (Fig.3).

PC1 contributes mainly to the discrimination between different reservoirs. Clusters forming for fish from Lake Gopło are very well separated but clusters for all fish species from Włocławski Reservoir are close to each other. As can be seen from Fig. 3 samples situated at the left side of the plot – PG (perch from Lake Gopło) exhibit the highest concentration of essential fatty acids such EPA and DHA, whereas samples BG situated at right side are characterized by higher content of shorter chain fatty acid (<20 carbon atoms).

Linear Discrimination Analysis

Discriminant function analysis is used to determine which variables discriminate between naturally occurring groups. Variable in LDA was selected by means of Wilks lambda statistic. For three variables: C16:2. C18:2n6 and C22:6n3 $p>0.05$ was achieved and these variables was rejected from model then using the 15 variables, an LDA was performed. First 5 discriminant functions (DF) have been were used to analysis. The calculated eigenvalues and canonical correlations as well as percent of total variance explained are shown in Table 4.

Table 4. Summary of canonical discriminant functions
Tabela 4. Podsumowanie kanonicznych funkcji dyskryminacyjnych

Function Funkcja	Eigenvalue Wartość własna	Canonical Correlation Korelacja kanoniczna	% of Variance % wariancji
1	154.60	0.997	58.29
2	79.55	0.994	29.99
3	15.56	0.969	5.86
4	10.66	0.956	4.02
5	4.86	0.911	1.83

The classification results of the fish samples are very satisfactory and allow 100% of cases to be correctly grouped. As shown in Fig. 4 the group separation is improved with respect to the PCA map. The best differentiation was obtained in two-dimensional space formed by second and third discrimination function. The all six groups have an excellent resolution without overlapping. Three clusters corresponding to fish species from Lake Gopło are located in upper part of plot and characterized by positive values of DF3 and negative values of DF2. Whereas clusters corresponding to fish samples from Włocławski Reservoir have positive values of second discrimination function and negative values of third DF.

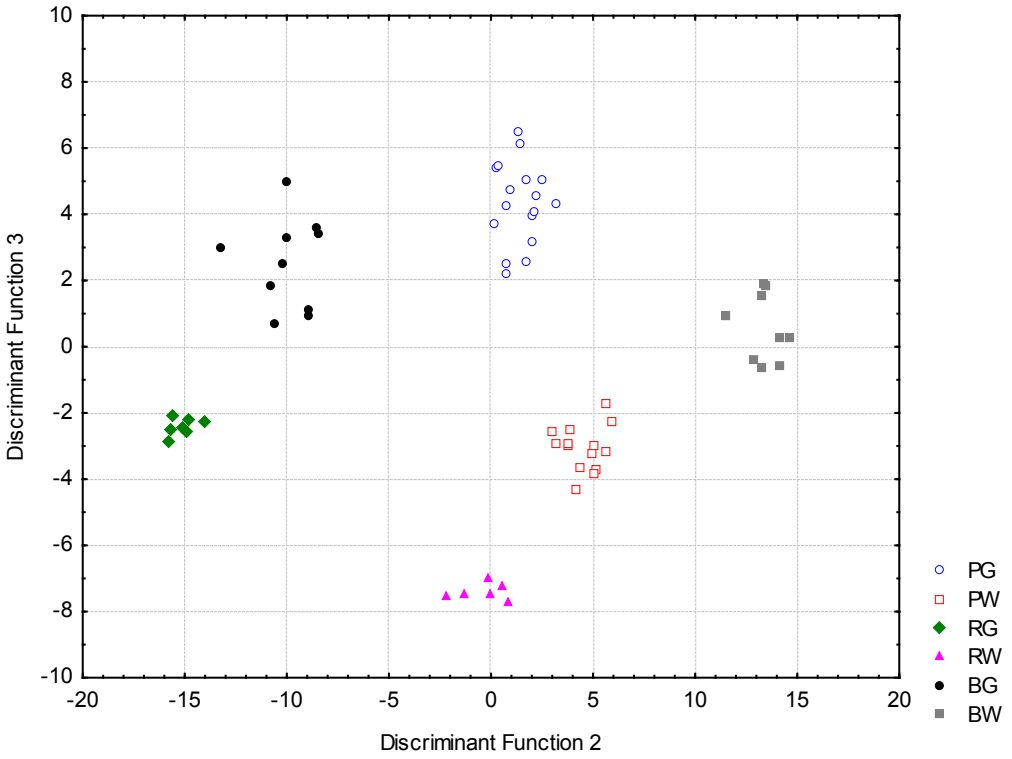

Figure 4. Projection of samples in two-dimensional space formed by second and third discriminant functions
Rycina 4. Projekcja przypadków na dwuwymiarową przestrzeń utworzoną przez drugą i trzecią funkcję dyskryminacyjną.

It should be kept in mind that, depending of the fish species and sex, diet composition and fishing season, different FA profiles are expected. The obtained results showed that it is possible to discriminate fish species from different reservoirs based on combination of fatty acid data (from GC analysis) along with chemometric approach (PCA, HCA and LDA). The differences observed between fatty acid profiles of the same fish species result from different food and oxygen conditions in Lake Gopło and Włocławski Reservoir. The latter reservoir instead to Lake Gopło is not a typical lake but has river-like character with specific hydrobiology.

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