ESTIMATION OF FAT CONTENT AND FATTY ACIDS PROFILE IN THE MEAT OF SPINY-CHEEK CRAYFISH (ORCONECTES LIMOSUS RAF.) FROM THE BRDA RIVER AND THE LAKE GOPŁO

OCENA ZAWARTOŚCI TŁUSZCZU I PROFILU KWASÓW TŁUSZCZOWYCH W MIĘSIE RAKA PRĘGOWATEGO (ORCONECTES LIMOSUS RAF.) Z RZEKI BRDY I JEZIORA GOPŁO

Magdalena STANEK[1], Bogumiła KUPCEWICZ[1,2], Janusz DĄBROWSKI[3], Bogdan JANICKI[1]

[1]University of Technology and Life Sciences, Faculty of Animal Breeding and Biology, Department of Small Ruminant Biology and Environmental Biochemistry, Mazowiecka Street 28, 85-084 Bydgoszcz, Poland. Tel. (22) 374 9739, e-mail: winiarska@utp.edu.pl

[2]Department of Inorganic and Analytical Chemistry, Faculty of Pharmacy, Collegium Medicum in Bydgoszcz, UMK Torun, M. Curie-Skłodowskiej Street 9, 85-094 Bydgoszcz, Poland.

[3]University of Technology and Life Sciences, Faculty of Animal Breeding and Biology, Department of Ecology, Kordeckiego Street 20, 85-225 Bydgoszcz, Poland

ABSTRACT

The aim of the present paper was to compare the content of fat and the profile of fatty acids in the meat of the abdomen section of spiny-cheek crayfish caught from the Brda River with individuals obtained from the Lake Gopło. The meat of spiny-cheek crayfish from two reservoirs didn't differ significantly as regards the fat content, however significant differences were report for most fatty acids ($p<0,05$). Reports show that the content of fat in crayfish meat is similar to the meat of low-fat fish which contain up to 2% of fat. In the group of SFA, the highest percentage share was recorded for C16:0, both in the meat of crayfish caught in the Brda River (21.33% of total acids) and in Lake Gopło (15.36% of total acids). In the MUFA group as the most abundant fatty acid was reported C18:1 n-9t, both in the meat of river crayfish (18.73% of total acids), and lake crayfish (25.52% of total acids). The PUFA analyses showed that the highest percentage share was reported for C20:5 n-3c in both groups.

Key words: spiny-cheek crayfish, fatty acids, fat

STRESZCZENIE

Celem pracy było porównanie zawartości procentowej tłuszczu oraz profilu kwasów tłuszczowych w mięsie z odcinka odwłokowego raków pręgowatych odłowionych z rzeki Brdy z osobnikami pozyskanymi z jeziora Gopło. Mięso raka pręgowatego odłowionego z dwóch różnych środowisk nie różniło się pod względem zawartości tłuszczu, jednak znaczące różnice odnotowano dla większości kwasów tłuszczowych ($p<0,05$). Badania wykazują, że zawartość tłuszczu w mięsie raka pręgowatego jest zbliżona do zawartości tłuszczu w mięsie chudych ryb, które zawierają do 2% tłuszczu. W grupie SFA najwyższe zawartości odnotowano dla kwasu C16:0, zarówno w mięsie raków odłowionych z Brdy (21.33% sumy kwasów), jak i Gopła (15.36% sumy kwasów). W grupie MUFA w największych ilościach oznaczono C18:1 n9t, zarówno w mięsie raków rzecznych (18.73% sumy kwasów), i jeziornych (25,52 % sumy kwasów). Kwas C20:5 n-3c występował w największych ilościach w grupie PUFA w mięsie obu grup raków.

Słowa kluczowe: rak pręgowaty, kwasy tłuszczowe, tłuszcz

STRESZCZENIE SZCZEGÓŁOWE

Obiektem badań był rak pręgowaty (Orconectes limosus Raf.) odłowiony w marcu i kwietniu 2008 roku z rzeki Brdy oraz z jeziora Gopło. Łącznie do badań pobrano 65 samców. Były to osobniki w przedziale długości całkowitej od 8,0 cm do 11,5 cm. Pobraną część mięsa z odcinka odwłokowego zliofilizowano, a następnie oznaczono skład kwasów tłuszczowych (% oznaczanych kwasów tłuszczowych) i zawartość tłuszczu (% mokrej masy). W grupie analizowanych kwasów tłuszczowych były kwasy nasycone (SFA) (C14:0, C15:0, C16:0, C17:0, C18:0, C24:0), jednonienasycone (MUFA) (C16:1 n-7c, C17:1 n-7c, C18:1 n-9c, C18:1 n-9t, C20:1 n-9c, C24:1 n-9c i wielonienasycone (PUFA) (C18:2 n-6c, C18:3 n-6c, C20:2 n-6c, C20:4 n-6c, C20:5 n-3c, C22:6 n-3c). Rak pręgowaty pod względem smaku i jakości mięsa nie odbiega od raków rodzimych [6, 17]. We Francji wartość kulinarna raka pręgowatego stawiana jest na równi z pochodzącym z Turcji rakiem błotnym (Astacus leptodactylus) oraz z luizjańskim (Procambarus clarkii) [6]. Rak ten jest niedoceniany pomimo, że przewyższa rodzime raki zawartością białka właściwego w tkance mięśniowej [14]. Przyrządzanie raków jest powszechnie uznawane jako sprawdzian najwyższego kunsztu kulinarnego, a dania te cieszą się powodzeniem u smakoszy na całym świecie [11]. Badania wykazały, że mięso raków zawierało średnio 0,43 i 0,44 % tłuszczu odpowiednio u osobników rzecznych i jeziornych i wartości te nie różniły się istotnie statystycznie. Średnia zawartość procentowa wody była o 4,59% wyższa u osobników pozyskanych z Gopła, niż u tych z Brdy. W grupie analizowanych SFA największy procent stanowił C16:0, zarówno w mięsie raków złowionych w Brdzie (21,33%), jak w Gople (15,36%). W obu grupach najmniejszy procent kwasów stanowił C15:0 i C24:0. Największy procentowy udział w grupie MUFA miał C18:1 n-9t zarówno w mięsie raków rzecznych (18,73%), jak jeziornych (25,52%). Najmniejsze ilości spośród MUFA stanowił C24:1 w obu grupach raków. Analiza PUFA wykazała, że w obu grupach największy procentowy udział stanowił C20:5. U raków pozyskanych z Brdy wartość ta wynosiła 13,22%, a u osobników z Gopła 15,41%. Najmniejsze ilości w obu grupach stanowił kwas C22:6. Zastosowanie analizy głównych składowych (PCA) i analizy skupień (CA) pozwoliło na rozróżnienie mięsa raków pochodzących z rzeki i jeziora na podstawie profilu kwasów tłuszczowych.

INTRODUCTION

Spiny-cheek crayfish (Orconectes limosus Raf.) were brought to Europe at the end of the 19th century to the place called Baranówek in the vicinity of Dębno Lubuskie [15]. The aim of the acclimatization was to replace the native species of European crayfish genus (Astacus astacus L.) which was dying out in huge numbers from a disease referred to as crayfish plague. Initially, the crayfish were put into a small pond and then penetrated into open waters. The species, having no considerable environmental requirements and demonstrating a considerable biological vigour as well as resistance to crayfish plague started spreading fast throughout our country.

The characteristic crayfish body structure makes their meat yield relatively low [16]. It ranges, depending on the individual and the size of the species, from about 12 to 18% [9]. The highest yield is reported for signal crayfish, with the biggest claws, then European, Danube crayfish and, finally, spiny-cheek crayfish. A lowered meat yield is reported for crayfish caught from reservoirs of lower water quality, with toxic algal blooms [4] and oxygen-deficit hypolimnion [17]. Spiny-cheek crayfish does not reach considerable body size since it has a short life span; about 5 years [10]. Most frequently, the catch includes individuals below 10 cm of the total length [2, 5]. Small claws of those crayfish include little meat and are not interesting for culinary purposes. For that reason, in practise, the main edible part of spiny-cheek crayfish is meat obtained from the abdomen.

To encourage the water users to catch spiny-cheek crayfish, they should be adequately promoted, possibly, as a product considered to be 'ecological food'. The taste and quality of meat of spiny-cheek crayfish is not inferior to native crayfish [6, 17]. In France, the culinary value of spiny-cheek crayfish is equal to pond crayfish (Astacus leptodactylus) of Turkish origin and with red swamp crayfish (Procambarus clarkii) [6]. Spiny-cheek crayfish is underestimated even though it is superior to native crayfish in its content of true protein in the muscle tissue [14]. The crayfish meat is juicy, low-fat, low-in-calories and rich in protein. Many consumers consider it to be a delicacy comparable to caviar [4].

In our country, there exist very abundant resources of spiny-cheek crayfish. In the waters of the former Suwałki Province, Krzywosz et al. [8] defined the total biomass of the catch population of those crayfish as about 800 tonnes. The species dominates throughout Poland, except for the south-eastern part of the country [7] and so it should be regularly caught at a large scale.

The aim of the present paper was to compare the content of fat and the profile of fatty acids in the meat of the abdomen section of spiny-cheek crayfish caught from the Brda River with the individuals obtained from the Lake Gopło. Besides, the obtained results were compared with

Table 1. Content of fat and water (%) in the meat of crayfish from the Brda River and Lake Gopło
Tabela1. Zawartość procentowa tłuszczu i wody w mięsie raków z Rzeki Brdy i Jeziora Gopło

Catch place Miejsce odłowu	n	Fat Tłuszcz	Water Woda
		mean value ± SD średnia ± odch. standardowe	mean value ± SD średnia ± odch. standardowe
Brda River Rzeka Brda	4	0.43 ± 0.01^{a}	79.05 ± 0.49^{a}
Lake Gopło Jezioro Gopło	5	0.44 ± 0.05^{a}	83.64 ± 1.38^{b}

Values in columns with different letters differ significantly (t-test, $p<0.05$)
Wartości w kolumnach oznaczone różnymi literami różnią istotnie statystycznie (t-test , $p<0,05$)

the meat quality of other crayfish species.

MATERIAL AND METHODS

Spiny-cheek crayfish was caught using the pond tools in March and in April 2008 in the Brda River mouth, below the old lock, and from the Lake Gopło (fig.1). In total 65 males were collected for research. Those were individuals of the total length from 8.0 cm to 11.5 cm. Due to relatively low amounts of meat obtained from the abdomen part of individual crayfish, the material from individuals of similar body length (about 5-7 pieces each) was combined. As a result, there were obtained in total 9 meat samples, which were freeze dried and the composition of fatty acids (% of the fatty acids determined) and the content of fat (% of wet weight) were determined. The analyzed meat samples were freeze dried in Lyovac GT2 freeze-drier by Finn-Aqua (Finland). Terms of freeze-drying run were as follows: temperature -40°C, pressure 6 10^{-2} mbar and process time 22-24 hours).

The percentage content of fat in the crayfish meat was determined with the modified method by Folch et al. [3]. Therefore about 2g of freeze-dried tissue was weighted. The total fat was extracted from crayfish muscle using 30 ml of the mixture of the composition: chloroform-methanol (2:1). After shaking, filtering and evaporation of the solvent to dryness under nitrogen atmosphere, the percentage content of fat in the tissue was determined (% content of wet weight).

The fatty acids profile was determined with the gas chromatograph 3800GC with the flame-ionization detector provided by Varian (USA). The temperature of the injector was 230ºC, and that of the detector was 250ºC. The volume of the injected sample was 1 µl (split 1:50). The analysis involved the use of column Supelcowax10 30 m × 0.32 mm × 0.25 µm. 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 225ºC (11ºC min^{-1}), 225ºC for 6 min, and then an increase from 225 to 240ºC (6ºC min^{-1}) and 240ºC for 19 min. Once fat was extracted with the method by Folch et al. [3], methylation with the solution of sodium methoxide (0.5 mol dm^{-3}) was performed for 22 hours, at the temperature of 37ºC. In order to extract methyl esters of fatty acids, isooctane was added. The group of the fatty acids analyzed included saturated acids (SFA) (C14:0, C15:0, C16:0, C17:0, C18:0, C24:0), monounsaturated acids (MUFA) (C16:1 n-7c, C17:1 n-7c, C18:1 n-9t, C18:1 n-9c, C20:1 n-9c, C24:1 n-9c) and polyunsaturated acids (PUFA) (C18:2 n-6c, C18:3 n-6c, C20:2 n-6c, C20:4 n-6c, C20:5 n-3c, C22:6 n-3c). Methyl esters of fatty acids were identified applying model Supelco PUFA-2 Animal Source and Supelco 37 component FAME Mix (Supelco, USA).

Statistical Analysis

Data analyses were performed by using the Statistica 8.0 software (StatSoft, USA). All data (SFA, MUFA, PUFA, n-3, n-6 and n-3/n-6) were statistically analysed with the t-test (to compare the mean values). The normality of the data was tested using the Shapiro-Wilk's test and the homogeneity of variance by means of Levene's test. Moreover chemometric methods were used: principal components analysis (PCA) and cluster analysis (CA). The data were standardized by substracting each value from reference value (sample mean) and dividing by the standard deviation of sample.

RESULTS AND DISCUSSION

The average contents of fat in the crayfish meat in the individuals from the Brda River and the Lake Gopło were respectively 0,43 and 0,44% (tab.1). There were no statistically significant differences between those values. The fat contents in the meat of the different crayfish species are similar and do not exceed 1% [4], and according to Walkowiak [16], the amount of fat in

Table 2. Fatty acids content (% of total acids; mean ± SD) in the meat of crayfish from the Brda River and Lake Gopło.
Tabela 2. Zawartość kwasów tłuszczowych (% sumy kwasów; średnia ± odchylenie standardowe) w mięsie raków z Rzeki Brdy i Jeziora Gopło.

Fatty acids Kwasy tłuszczowe	Catch place Miejsce odłowu		P
	Brda (n=4)	Gopło (n=5)	
	Mean ± SD	Mean ± SD	
SFA			
C14:0 (Tetradecanoic acid)	0.78 ± 0.04	0.69 ± 0.05	0.02
C15:0 (Pentadecanoic acid)	0.16 ± 0.02	0.50 ± 0.05	< 0,001
C16:0 (Hexadecanoic acid)	21.33 ± 0.33	15.36 ± 0.44	< 0,001
C17:0 (Heptadecanoic acid)	4.60 ± 0.25	2.02 ± 0.11	< 0,001
C18:0 (Octadecanoic acid)	4.63 ± 0.12	6.18 ± 0.05	< 0,001
C24:0 (Tetracosanoic acid)	0.40 ± 0.01	0.09 ± 0.20	0.018
Σ SFA	32.89 ± 0.47	24.76 ± 0.53	< 0,001
MUFA			
C16:1 n7 (cis-9-heksadecenoic acid)	8.56 ± 0.06	4.59 ± 0.52	< 0,001
C17:1 n7 (cis-10-heptadecenoic acid)	1.41 ± 0.01	0.88 ± 0.09	< 0,001
C18:1 n9 (trans-9-octadecenoic acid)	18.73 ± 0.18	25.52 ± 0.66	< 0,001
C18:1 n9 (cis-9-octadecanoic acid)	3.26 ± 0.03	3.50 ± 0.46	0.343
C20:1 n9 (cis-11-eicosenoic acid)	1.60 ± 0.02	1.14 ± 0.04	< 0,001
C24:1 n9 (cis-15-tetracosenoic acid)	0.77 ± 0.02	0.39 ± 0.03	< 0,001
Σ MUFA	34.35 ± 0.20	36.02 ± 1.50	0.065
PUFA			
C18:2 n6 (all-cis-9,12-octadecadienoic acid)	5.77 ± 0.09	7.74 ± 0.92	0.004
C18:3 n6 (all-cis-6,9,12-octadecatrienoic acid)	2.42 ± 0.04	2.31 ± 0.54	0.711
C20:2 n6 (all-cis-11,14-eicosadienoic acid)	2.27 ± 0.02	2.35 ± 0.06	0.049
C20:4 n6 (all-cis-5,8,11,14-eicosatetraenoic acid)	8.61 ± 0.15	10.58 ± 1.11	0.010
C20:5 n3 (all-cis-5,8,11,14,17-eicosapentaenoic acid)	13.22 ± 0.40	15.41 ± 0.91	0.003
C22:6 n3 (all-cis-4,7,10,13,16,19-docosahexaenoic acid)	0.48 ± 0.03	0.78 ± 0.15	0.006
Σ PUFA	32.76 ±0.66	39.18 ± 0.97	< 0,001
Σn-3	13.70 ±0.41	16.19 ± 1.03	0.003
Σn-6	19.07 ± 0.25	22.99 ± 0.48	< 0,001
n-3/n-6	0.72 ± 0.01	0.70 ± 0.05	0.638

Table 3. Loadings and eigenvalues for the first two PCs
Tabela 3. Ładunki zmiennych oraz wartości własne dla dwóch pierwszych PC

Component	PC1	PC2
SFA	0.997389	0.050561
MUFA	-0.707234	0.633425
PUFA	-0.946631	-0.315391
n-3	-0.801826	-0.595428
n-6	-0.991863	-0.071982
n-3/n-6	0.581947	-0.772983
Eigenvalue	4.35645	1.46047
% of total variance	72.61	24.34
Cumulative variance	72.61	96.95

a) Map of Poland b) Brda River mouth

Figure 1. Place of crayfish catch.
Rysunek 1. Miejsce odłowu raków.

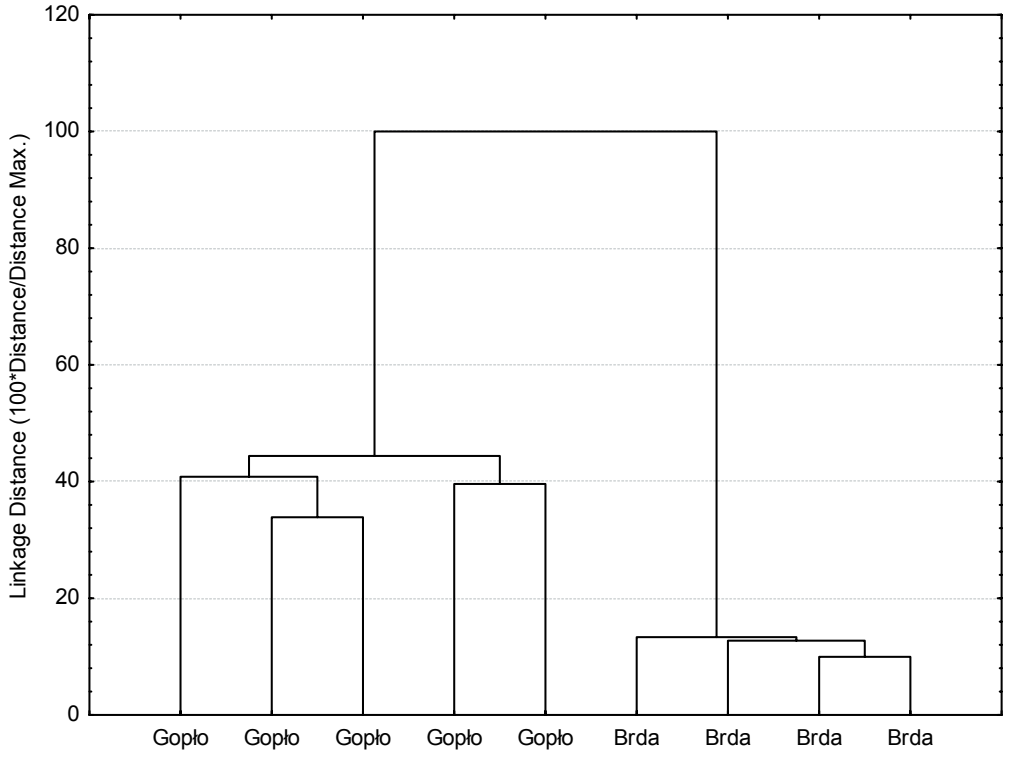

Figure 2. Dendrogram for crayfish meat samples from Brda River and Lake Gopło
Rysunek 2. Dendrogram dla próbek mięsa raków z rzeki Brdy i jeziora Gopło

crayfish necks ranges from 0.4 to 0.9%. Those values show that the content of fat in crayfish meat is similar to the meat of low-fat fish which contain up to 2% of fat [12]. In the body of crustaceans the great amounts of fat is mainly accumulated in autumn [20]. Reports by Własow et al. [17] demonstrated that the percentage content of fat in the meat of crayfish caught from the Mazuria Lakeland ranged from 0.15 to 0.30%, and the content of water from 80.50 to 81.68%. In the meat of spiny-cheek crayfish caught from Lakes Dgał, Harsz and Pobłędzie fat accounted for 0.24 – 0.30%, and water – from 81.43 to 81.68% [18]. The content of water in the meat of spiny-cheek crayfish from Lake Wdzydze was 81.07% in females and 80.12% in males [1]. The average percentage content of water in the meat of spiny-cheek crayfish was 4.59% higher in the individuals from Lake Gopło than in the river individuals (tab.1).

As the analyses indicate, in the group of SFA, the highest percentage share was recorded for C16:0, both in the meat of crayfish caught in the Brda River (21.33% of total acids) and in the Lake Gopło (15.36% of total acids) (tab.2). In both groups the lowest percentage of acids was reported for C15:0 and C24:0. The average percentage contents of all saturated acids differed statistically significant between the samples collected from the crayfish caught from two environments ($p<0,05$). In the MUFA group C18:1 n-9t was reported as the most abundant fatty acid both in the meat of river crayfish (18.73% of total acids) and lake crayfish (25.52% of total acids). And those values differed significantly ($p<0,05$). The lowest amounts among MUFA were reported for C24:1 n-9c in both crayfish groups. The average percentage contents of monounsaturated acids (except for C18:1 n-9c) in the meat samples differed significantly between the individuals caught from various places. The PUFA analyses showed that in both groups the highest percentage share was reported for C20:5 n-3c. In the crayfish from the Brda River, the value was 13.22% of

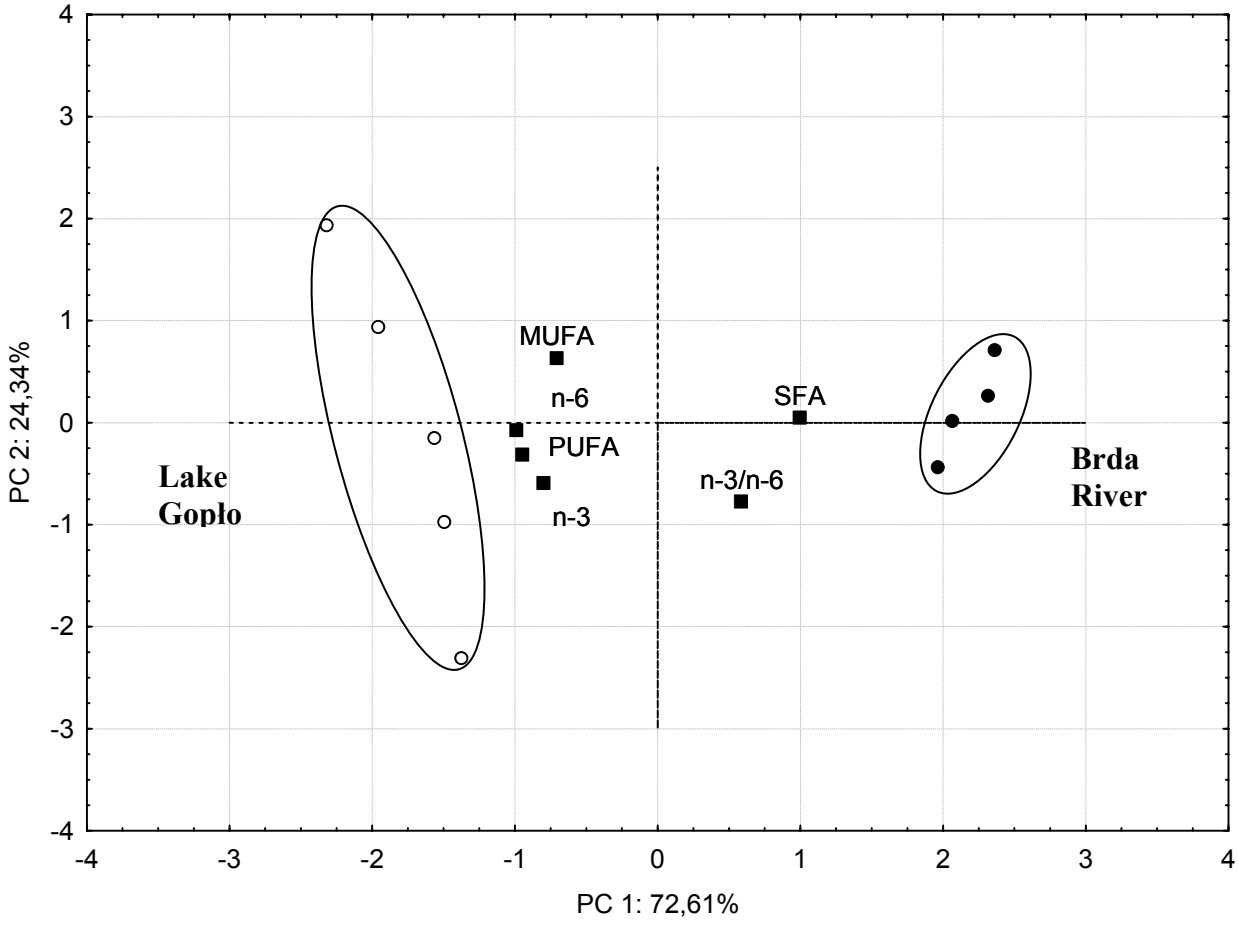

Figure 3. Distribution of crayfish meat samples and variables in the PC1- PC2 coordinates system.
Rysunek 3. Rozmieszczenie próbek mięsa raków i zmiennych w układzie współrzędnych PC1-PC2

total acids, and in the individuals from the Lake Gopło – 15.41% of total acids. The lowest amounts were reported for C22:6 n-3c in both groups. The analysis indicated that there were statistically significant differences in the average percentage contents of all PUFA between two crayfish groups ($p<0,05$) (except for C18:3 n-6c). The ratio of n3/n6 PUFA was 0.72 in the meat of crayfish from river and 0.70 in samples of individuals caught from a lake. This coefficient fell within the range for freshwater fish (0.5-3.8) [13].

Analyses carried out by Walkowiak [16] demonstrated that in the extract from crayfish crust, of all the SFA, C16:0 was most abundant, while in the MUFA group, the highest share was reported for C18:1 n-9, and the sum of PUFA was 34.7%. The PUFA sum in the meat of spiny-cheek crayfish from the Brda River was 32,76%, and in the tissue of individuals caught in the Lake Gopło the value was 39.18%.

The analyses concerned of the quality of the fat extracted from rusty crayfish (Orconectes rusticus) meat indicated that in the SFA group the highest amounts were reported for C16:0 (19.7 mol%). Acid C18:1 was analysed in largest amounts in the MUFA group (21.5 mol%). Within the PUFA group C18:3 was determined as predominant (9.2 mol%) [19].

The data set of six parameters obtained from the fatty acid profile (sum of SFA, MUFA, PUFA, n-3, n-6 and n-3/n-6 ratio) was subjected to cluster analysis (CA) and principal component analysis (PCA).

The CA with a single linkage and the Euclidean distance measure results is presented in the form of dendrogram (fig. 2). In this plot, there are two definite clusters for similarity 3.0 according to the data groups the Brda and the Gopło.

The analysis of PCA yielded two principal components with the eigenvalue exceeding 1 which explains up to 96.95% of the total variance. Table 3 contains loadings for the principal component (PC1 and PC2) with their eigenvalues and variances. The first PC explains 72.61% of total variance and presents high positive loadings for SFA and negative loadings for MUFA, PUFA, n-3 and n-6 variables. PC2 includes 24.34% of the variance in the data set and has a significant contribution from n-3/n-6 ratio. To visualize the data the loadings and scores are shown in the space of the two first principal component (fig. 3). The PCA and CA results obtained for the data show that the fatty acids profile of the crayfish meat differs between crayfish from the Brda River and the Lake Gopło. According to principal component analysis, the most useful variables for discrimination were the content of saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA).

The crayfish meat quality may determine many factors, for example: sex, season of the harvest [1,18]. In addition, as many studies show the level of n-3 and n-6 PUFA is only dependent upon the type of diet, because those fatty acids are synthesized de novo only by plants. Differences in fatty acid profile between crayfish caught from the Brda River and the Lake Gopło may be caused by other environmental conditions in which individuals lived. Various environmental conditions and the different diet affect the chemical composition of animal body, including their fatty acids profile. Spiny-cheek from the Lake Łabap (northern part of the Lake Mamry complex) had high body mass and high meat yields. This resulted from the fact that this lake is rich in nutrients and is surrounded with agricultural fields [18].

CONCLUSIONS

1. The meat of spiny-cheek crayfish caught from the Brda River and Lake Gopło did not differ significantly as regards the content of fat, however significant differences were reported for most fatty acids ($p<0,05$).

2. Results of principal component analysis (PCA) and cluster analysis (CA) of the fatty acid profile made for two group of crayfish can be used to distinguish the origin of crayfish meat.

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