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NOTE ON THE SPECIAL FILLET FATTY ACID COMPOSITION OF THE DWARF CARP (*CYPRINUS CARPIO CARPIO*) LIVING IN THERMAL LAKE HÉVÍZ, HUNGARY

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Abstract

Fatty acid (FA) composition of the fillet and the intestinal content of dwarf common carp (*Cyprinus carpio carpio*) living in Lake Hévíz was determined in wintertime collected samples and results were compared to widespread literature data on carp. Fillet FA profile of the thermally adapted (28 ^{o}C) Hévíz dwarf carps differed from profiles originated from divergent culture and feeding conditions in the overall level of saturation. Fillet myristic acid proportions largely exceeded all literature data in spite of poor dietary supply. Fillet fatty acid results indicate the effects of thermal adaptation (high saturation level) and the correlative effects of feed components rich

in omega-3 fatty acids, with special respect to docosahexaenoic acid. With the application of discriminant factor analysis the Hévíz sample was accurately differentiated from the literature data on carp fillet fatty acid profile, mostly based on C14:0, C18:1 n9, C18:2 n6, C20:1 n9 and C20:4 n6 FAs. In summary, fillet FA profile suggested thermal adaptation, location specificity and the ingestion of algal and bacterial material.

Keywords: carp – fillet – intestinal content – fatty acid – thermal habitat

Introduction

Polyunsaturated fatty acids (PUFA) play important roles in human diet because of their beneficial properties for prevention of several diseases [18]. Needs of PUFA are partly resolved by fish consumption in human diet. Therefore fatty acid (FA) composition of several fish species is well studied. It has been reported that the type and amount of fatty acids in fish tissues vary mainly with feeding of the fish, but other factors may also influence their FA composition. In addition, there are defined factors having profound effects on FA composition, for example: body size, age, origin, reproductive status and environmental temperature [7, 9, 12]. Secondly, after diet, also water temperature can largely modify the FA composition of fish. For example, FA of cold adapted and deep sea fishes are providing higher PUFA proportions in their FAs [4].

Common carp (*Cyprinus carpio* L.) is one of the most widely cultured freshwater fish species all over the world. Thus, the FA composition of common carp in context of divergent environmental factors, such as temperature, artificial feeding

or natural feed sources is extensively studied. The ability to adapt FA metabolism and composition to low winter temperature has as well been already investigated in common carp [7, 9]. The body traits and muscle chemical composition of common carp reared under different temperatures [12], as well the seasonal variations of the fatty acid composition were examined [14, 16, 22] but it still remains unknown how an extreme thermal environment and the specifically adapted natural feed source influences the FA composition of common carp, in a special, isolated population.

Thus, our investigation had two aims: first to determine the fatty acid profile of the Hévíz carp fillets and intestinal contents, being descriptive of a still less explored population, and as second to evaluate (classify) the data by taking available literature results on carp fillet into consideration.

Materials and methods

Lake Hévíz is the largest thermal lake in Europe. It is a geological and a balneological unique. The extent of the lake is 4.4 hectares, the bottom soil is peaty sludge. Compared to other natural waters (10-12 ^{o}C mean annual water temperature), Lake Hévíz varies between 24 ^{o}C and 38 ^{o}C (annual mean: 30.7 ^{o}C). Mean summer and winter temperatures are 33-35 ºC and 24-28 ºC, respectively. Dominant minerals in the water are Ca^{++}, Mg^{++}, $HCO3^{--}$, S, Ra and organic compounds.

The vegetation of the lake consists of water lily species (*Nymphaea rubra, Nymphaea alba*). The fauna of Lake Hévíz was described by several authors [21]. The sediments are rich in bacterial communities [19], periphyton is harboring Nematoda species [2], and the main fish species (*Gambusia affinis*) of the lake is well examined [24].

Fish (origin and sampling)

Carp population of Lake Hévíz has a dwarfish habit, it is isolated from the nearby populations. Low growth rate and small adult size (maximum weight of 400-450 g in 8-9 years old fish; unpublished observations) may be consequences of the adaptation to the extreme environment.

For this analysis fish (n=10, adult males, 344.2 ± 63.9 g) were caught by gill-net in December 2010, at 28 ^{o}C water temperature. Fish were dissected to sample the intestinal contents (being characteristic for the feed) and the fillet. Samples were stored at -70 ^{o}C until analysis.

Fatty acid analysis

Fillet (n=10) and intestinal tract contents (n= 1, pooled from 10) were analyzed for fatty acid composition. Samples were homogenized in ceramic mortars in a 20-fold volume of chloroform:methanol (2:1 vol:vol) and total lipid content was extracted [11]. Solvents were ultrapure-grade (Sigma-Aldrich, Schnelldorf, Germany) and 0.01 % w:v butylated hydroxytoluene was added to prevent fatty acid oxidation. Solvents were evaporated under nitrogen stream and complex lipids were transmethylated with a base-catalysed $NaOCH_3$ method [5] and were stored in hexane until analysis.

Gas liquid chromatography was performed on a Shimadzu 2100 apparatus, equipped with a SP-2380 (Supelco, Bellefonte, USA) type capillary column (30 m x 0.25 mm internal diameter, 0.20 μm film) and flame ionisation detector (FID $2x10^{-11}$). Characteristic operating conditions were: injector temperature: 270 ^{o}C, detector

temperature: 300 ^{o}C, helium flow: 28 cm/sec. The oven temperature was graded: from 80 to 205 ^{o}C: 2.5 ^{o}C/min, 5 min at 205 ^{o}C, from 205 to 250 ^{o}C 10 ^{o}C/min and 5 min at 250 ^{o}C. To identify individual FA, a standard (Mixture Me100, Larodan Fine Chemicals, Malmö, Sweden) was used. Results are given as weight % of the total fatty acids.

From the primary FA results, unsaturation index (UI) was calculated as: (1 x Σ monoenoic FA) + (2 x Σ dienoic FA) ... etc. The average FA chain length was calculated from the multiplication of the chain length values and the respective proportion of each fatty acid.

Statistics

To seek for differences between the Hévíz and published data (i.e. pairwise comparison), the Mann-Whitney U test was used [25]. To explore the classification pattern of the fillet FA profile among literature data and those of the Hévíz population, Discriminant Factor Analysis (DFA) was used [1]. This latter software provides an opportunity to choose the best variables for the classification models. The selection of variables is based on their discrimination power which is calculated for each variable individually. Depending on the pattern of samples, the variables can be selected to reduce variability within a group and to increase the intergroup distance. This function is used to automatically select the best variables for the application or user can perform the selection manually after reviewing the values of discrimination power for each variable.

Results

Characteristics of the FA profile of the Hévíz population

The FA profile of the **intestinal content** (Table 1) provided uncommonly high arachidonic (AA) and docosahexaenoic (DHA) acid proportions, latter factor leading to a total of 20% n3 FA proportion in its lipids. The unsaturation index (UI) of intestinal content largely exceeded that of the fillet (170 vs 126.7). It revealed by the dissection, that every fish had a well-developed testis and abundant visceral fat. Substantial amounts of intestinal content were found, precluding starvation.

When compared our FA data to those of other authors (Table 2, Mann-Whitney U test based comparison) nearly all individual FA proportion values were significantly different. Tendentious similarity was found with data obtained from warm climate areas and natural feeding [14, 17, 22], while moderate climatic circumstances and grain feeding [3, 15] led to more expressed differences.

Comparison to literature data

Comparing our **fillet FA** data to those in the litearature from carps fed natural feed and additional grains, nearly all individual FA proportion values were significantly different.

Using the automatic classification method, AlphaSoft 12.3 selected C14:0, C18:1 n9, C18:2 n6, C20:1 n9 and C20:4 n6 to perform DFA classification (Figures 1 and 2). (It has to be added that only those FAs were used from which all literature sources provided detectable amounts.) In Figure 1 groups nr. 7, 8 and 9 [3, 15, 26] are similar, while nr. 1., i.e. the Hévíz sample is strongly different, with spatial separation. The DFA approach gave very good results, where 93% of cross-validated grouped

cases were correctly classified. The proportional classification contribution of the above-mentioned single fatty acids is shown in Figure 2.

Discussion

Characteristics of the FA profile of the Hévíz population

Concerning the body and fillet (and intestinal content) fatty acid composition of the Hévíz, isolated dwarf carp population, as far as the authors are aware, this is the first study.

The marked presence of arachidonic and docosahexaenoic acids in the FA profile of the intestinal content refers to animal or algal feed components [20]. Since recognized animal remains were rare in the intestine (personal observation), it is likely that in Lake Hévíz fish ingest and utilize the microflora growing on the decomposing macrophyte remains in the lake sediment (based on the experienced crystalline material in the mortars during extraction). In addition, the UI value of the intestinal content exceeded that of the fillet total lipids. This refers to a specific type of thermal adaptation; namely high or increasing proportions of arachidonic and docosahexaenoic acids in the lipids of carp (hepatic phospholipids) refer to cold acclimation [8]. Herein, interestingly, we experienced the opposite, a limited or restricted uptake of ingested dietary polyunsaturated FAs into muscle lipids.

It is as well an interesting finding that the level of saturated FAs in the Hévíz carp fillet lipids was 5-10% higher, as compared to all literature data (incl. warm environments as well). We hypothesize again a warm adaptation process behind this result [8], since the difference between the Hévíz and some of the cited water

temperatures [3, 15] was ca. 20 ^{o}C, adding that the relatively high dietary unsaturation was as well not mirrored (i.e. not fully taken up or transported to the skeletal muscle) by the fillet lipids. In addition, it is well known [6, 23] that cold adaptation markedly increases lipid unsaturation in the fillet polar and total lipids in marine fish and in carp [10]. Thus, it seems that besides relatively rich dietary PUFA supply, warm environment did no necessitate an expressed recruitment of these fatty acids in the fillet lipids.

DFA classification and comparison to literature data

During FA-profile based DFA classification of the samples originated from different regions, the group of Héviz carp showed obvious isolation (Figure 1). Samples of same region and described by the same authors (group 4 and 5, both from Madagascar) are linked together and form one single group. Basically, all samples seem to be closely related, only group 2 (Turkish) and group 1 (Hévíz) is very much different. The classification was tested by cross-validation when each sample was identified once as independent. The ratio of correctly classified cross-validated samples was 93%.

The basis of the marked classification was interestingly a restricted bunch of FAs, namely, C14:0, C18:1 n9, C18:2 n6, C20:1 n9 and C20:4 n6. It has to be added that as well C17:1 gained a relatively high classification score. Figure 2 shows the impact of the mentioned five individual FAs on the discriminating factors (DFs). It can be seen by investigating the zero-vectors (position of the FAs) that C14:0, C18:1 n9 and C18:2 n6 have great impact on both the first and second DF. C20:1 n9 has low impact on DF2 and a little more on DF1, while C20:4 n6 affects largely DF2 but has

less influence on DF1. First DF described the 69.2% of the total variance, while DF2, DF3, DF4, DF5 had 23.5%, 5.7%, 1.2%, 0.4%, respectively. Since DF1 dominates the classification, the FA that has more impact on this factor is the most considerable.

Investigating the possible origin and role of the above mentioned FAs, the first (myristic acid) may be of both endogenous and exogenous origin. It has to be however emphasized that the proportion of this FA was 2.5-5 times higher in the Hévíz population, as compared to the literature data, with a surprisingly minor presence in the diet (Table 1; 0,09%).

Oleic acid (C18:1 n9) is a desaturation product of stearic acid, thus its origin is, similarly to myristic acid, double, meanwhile its (and its precursors) dietary occurrence was high (18.1). Linoleic acid (C18:2 n6) is essential for vertebrates and its dietary provision was very similar to its tissue presence. In case of its endogenously further elongated and desaturated product, arachidonic acid (C20:4 n6), the diet seemed to be rather rich, leading to a percentage contribution of over 4% in the fillet lipids. This level was only comparable to that measured in other natural and warm ponds in Turkey by Guler et al. in 2008 [14] and Kalyoncu et al. in 2010 [17]. Interestingly, Hungarian fishpond data were also not statistically different [26] from the Hévíz data for this acid, most probably due to the feeding of linoleic acid rich components (sunflower seed). (This was supported by the relatively high fillet linoleic acid proportion in the fillet of those carps.)

Conclusions

The intestinal content of the Hévíz drawf carp population provided evidence for a dominantly benthic feed basis of this isolated population. However, the relatively high supply of arachidonic and docosahexaenoic acids did ultimately not lead to extraordinary high tissue proportion of these fatty acids, instead, the fillet lipids were strongly saturated. This refers to a specific thermal adaptation. The classification based merely on the fillet fatty acid profile was successful, and provided reliable separation of the Hévíz population from widespread data published, with tendentious similarity to data obtained under warm climatic conditions and natural feeding.

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Table 1. Fatty acid composition of the intestinal content

Fatty acid	
C14:0	3.77
C14:1 n5	0.13
C15:0	0.63
C15:1 n7	n.d.
C16:0	23.4
C16:1 n7	4.55
C17:0	1.67
C17:1 n9	n.d.
C18:0	11.5
C18:1 n9	18.1
C18:2 n6	5.24
C18:3 n6	0.54
C18:3 n3	2.13
C20:0	0.18
C20:1 n9	1.20
C20:2 n6	0.73
C20:3 n3	0.55
C20:3 n6	n.d.
C20:4 n6	6.55
C20:5 n3	3.21
C22:0	0.06
C22:5 n3	1.95
C22:6 n3	12.1
Σ saturated	41.3
Σ unsaturated	57.0
Σ monoenoic	24.0
Σ PUFA	33.0
Σ n3	20.0
Σ n6	13.1
Σ n9	19.3
n6 / n3	0.65
unsaturation index	170.4
average FA chain length	16.7

n.d.: not detectable

Table 2. Fillet fatty acid composition of the Hévíz carps and its comparison to literature data

	Hévíz	**[26]**	**Sig.**	**[14]**	**Sig.**	**[17]**	**Sig.**	**[22]**	**Sig.**	**[3]**	**Sig.**	**[15]**	**Sig.**	**[16]**
Culture	thermal lake	fishpond		natural pond		artificial pond		natural ponds		fishpond		fishpond		Indus River
Feed	natural	mixed grain + natural		natural		natural		natural		wheat + benthic		grain+oilseeds		Natural
Country	Hungary	Hungary		Turkey		Turkey		Madagascar		Czech Rep.		Poland		Pakistan
C14:0	5.74 ± 0.82	0.99 ± 0.37	0.002	1.90 ± 0.33	0.005	1.81 ± 0.32	0.0001	1.63 ± 0.12	0.0001	1.13 ± 0.09	0.005	1.36 ± 0.57	0.0001	3.28
C14:1	0.29 ± 0.05	0.15 ± 0.05	0.005	0.78 ± 0.29	0.005	0.36 ± 0.1	n.s.	0.28 ± 0.04	n.s.	0.00 ± 0.00	n.s.	0.54 ± 0.26	0.002	0.74
C15:0	0.69 ± 0.16	0.19 ± 0.13	0.005	0.96 ± 0.15	0.02	0.81 ± 0.08	n.s.	1.53 ± 0.11	0.0001	n.a.		n.a.		1.65
C15:1	0.02 ± 0.00	n.a.		1.24 ± 0.42	0.06	1.71 ± 0.68	0.0001	0.25 ± 0.05	0.0001	n.a.		n.a.		0.82
C16:0	21.5 ± 1.21	16.6 ± 2.27	0.005	15.68 ± 0.82	0.005	18.7 ± 0.43	0.001	15.1 ± 1.17	0.0001	18.8 ± 0.16	0.005	21.94 ± 3.63	n.s.	33.0
C16:1 n7	6.29 ± 0.48	9.29 ± 1.92	0.002	9.51 ± 3.50	0.16	12.5 ± 1.15	0.001	5.17 ± 0.47	n.s.	9.11 ± 0.24	0.005	5.34 ± 1.94	0.07	6.08
C17:0	1.04 ± 0.20	0.51 ± 0.12	0.002	1.64 ± 0.29	0.005	0.79 ± 0.2	0.0500	3.02 ± 1.7	0.002	n.a.		n.a.		3.34
C17:1	1.22 ± 0.21	n.a.		1.56 ± 0.15	0.02	1.57 ± 0.31	0.03	1.72 ± 0.24	0.0001	n.a.		n.a.		8.89
C18:0	4.43 ± 0.92	4.80 ± 0.63	n.s.	4.42 ± 0.54	n.s.	4.87 ± 0.61	n.s.	5.16 ± 0.29	0.03	1.41 ± 0.13	0.005	4.51 ± 1.22	n.s.	11.2
C18:1 n9	32.75 ± 2.58	39.56 ± 9.12	n.s.	2.94 ± 1.24	0.005	25.8 ± 2.81	0.001	19.57 ± 1.71	0.0001	55.32 ± 0.31	0.005	42.02 ± 3.43	0.0003	23.5
C18:2 n6	7.00 ± 2.92	8.41 ± 1.73	n.s.	7.57 ± 2.87	n.s.	5.45 ± 1.55	n.s.	11.95 ± 1.07	0.0001	6.74 ± 0.18	n.s.	20.47 ± 3.95	0.0002	6.41
C18:3 n6	0.34 ± 0.13	n.a.		3.03 ± 1.20	0.005	0.07 ± 0.04	0.001	0.84 ± 0.09	0.0001	0.17 ± 0.01	0.01	n.a.		1.03
C18:3 n3	3.83 ± 0.46	1.75 ± 1.08	0.005	0.70 ± 0.29	0.005	4.24 ± 0.9	n.s.	1.94 ± 0.24	0.0001	0.82 ± 0.04	0.005	1.97 ± 1.84	0.05	1.22
C20:0	0.12 ± 0.01	n.a.		1.38 ± 1.13	0.01	0.18 ± 0.03	0.001	0.23 ± 0.05	0.0001	0.14 ± 0.06	n.s.	0.75 ± 0.37	0.001	0.17
C20:1 n9	2.07 ± 0.23	3.09 ± 1.07	0.05	1.87 ± 0.89	n.s.	1.23 ± 0.35	0.0001	1.84 ± 0.23	0.0001	3.81 ± 0.28	0.005	2.93 ± 0.64	0.04	0.52
C20:2 n6	0.63 ± 0.14	n.a.		0.19 ± 0.06	0.03	0.86 ± 0.1	n.s.	0.57 ± 0.05	n.s.	n.a.		0.45 ± 0.07	n.s.	0.23
C20:3 n6	0.58 ± 0.11	n.a.		1.02 ± 0.26	0.01	0.04 ± 0.02	0.0001	0.70 ± 0.08	n.s.	n.a.		0.45 ± 0.08	n.s.	0.61
C20:3 n3	0.42 ± 0.05	n.a.		0.54 ± 0.13	n.s.	0.02 ± 0.01	0.0001	0.49 ± 0.07	0.016	n.a.		n.a.		0.3
C20:4 n6	4.53 ± 1.42	2.59 ± 2.36	n.s.	5.58 ± 1.08	n.s.	4.69 ± 1.47	n.s.	1.80 ± 0.54	0.0001	0.09 ± 0.01	0.005	0.85 ± 0.49	0.03	0.42
C20:5 n3	1.74 ± 0.41	1.53 ± 1.50	n.s.	4.83 ± 0.65	0.005	6.09 ± 0.27	n.s.	2.58 ± 0.48	0.0001	0.65 ± 0.05	0.005	n.a.		0.34
C22:0	0.05 ± 0.02	n.a.		0.27 ± 0.12	0.01	0.35 ± 0.09	0.0001	n.a.		n.a.		0.97 ± 0.01	0.01	0.31
C22:5 n3	1.07 ± 0.28	0.82 ± 0.51	n.s.	0.95 ± 0.61	n.s.	2.34 ± 0.23	0.0001	2.75 ± 0.95	n.s.	0.07 ± 0.01	n.s.	n.a.		0.16
C22:6 n3	3.52 ± 0.85	1.52 ± 1.21	0.01	6.67 ± 3.02	0.01	4.15 ± 1.1	n.s.	3.97 ± 1.32	n.s.	n.a.		n.a.		0.36
Σ saturated	33.71 ± 2.04	23.10 ± 2.04	0.002	25.95 ± 1.33	0.005	27.66 ± 0.91	0.001	27.0 ± 2.52	0.0001	21.48 ± 0.16	0.005	28.74 ± 3.88	0.002	53.5

Σ unsaturated	66.29 ± 2.04	68.68 ± 1.63	0.05	48.9 ±2.52	0.005	71.11 ± 1.26	0.0011	56.4 ± 0.57	n.s.	76.68 ± 0.27	0.005	70.83 ± 3.89	0.003	51.6
Σ monoenoic	42.63 ± 2.77	52.10 ± 8.17	0.01	17.9 ± 3.89	0.005	43.17 ± 3.81	n.s.	28.8 ± 2.28	0.0001	68.24 ± 0.44	0.005	48.19 ± 3.15	0.003	40.5
Σ polyenoic	23.66 ± 3.4	16.62 ± 7.73	n.s.	30.1 ± 5.17	0.03	27.94 ± 2.55	0.044	27.59 ± 2.26	0.007	8.44 ± 0.25	0.005	22.64 ± 3.82	n.s.	11.1
Σ n3	10.57 ± 1.69	5.62 ± 3.98	0.02	13.69 ± 3.39	0.03	16.84 ± 2.46	0.0001	11.7 ± 2.73	n.s.	1.54 ± 0.09	0.005	2.13 ± 1.86	0.0002	2.38
Σ n6	12.74 ± 2.85	11.0 ± 3.66	n.s.	17.3 ± 4.44	n.s.	11.1 ± 1.45	n.s.	15.86 ± 0.53	0.003	6.91 ± 0.18	0.005	20.51 ± 4.23	0.001	8.7
Σ n9	34.82 ± 2.71	42.65 ± 9.22	n.s.	4.8 ± 0.82	0.005	27.04 ± 3.07	0.001	21.41 ± 1.87	0.0001	59.13 ± 0.58	0.005	42.61 ± 3.95	0.001	24.0
n6/n3	1.23 ± 0.39	2.88 ± 1.84	0.01	1.34 ± 0.56	n.s.	0.67 ± 0.15	0.017	1.42 ± 0.32	n.s.	4.51 ± 0.23	0.005	32.71 ± 31.8	0.0002	3.66
unsaturation index	126.68 ± 10.58	105.35 ± 21.2	0.05	140.32 ± 19.63	n.s.	154.7 ± 10.5	n.s.	123.44 ± 12.6	n.s.	88.26 ± 0.46	0.005	96.03 ± 6.97	0.0002	69.6
average FA chain length	17.63 ± 0.09	16.38 ± 0.25	0.002	13.87 ± 0.73	0.005	17.43 ± 0.36	0.001	15.92 ± 0.67	0.0001	18.36 ± 0.03	0.005	20.96 ± 0.8	0.0002	17.2

sig.: P<0,05 as compared to the data obtained at Hévíz
n.a.: not available; n.s.: P>0.05

Figure 1. The discriminant factor analysis based classification of the Hévíz carp fillets and those found in literature (group numbering: 1: Hévíz; 2: [26]; 3: [17]; 4, 5: [22]; 7: [3]; 8: [15]; 9: [16])

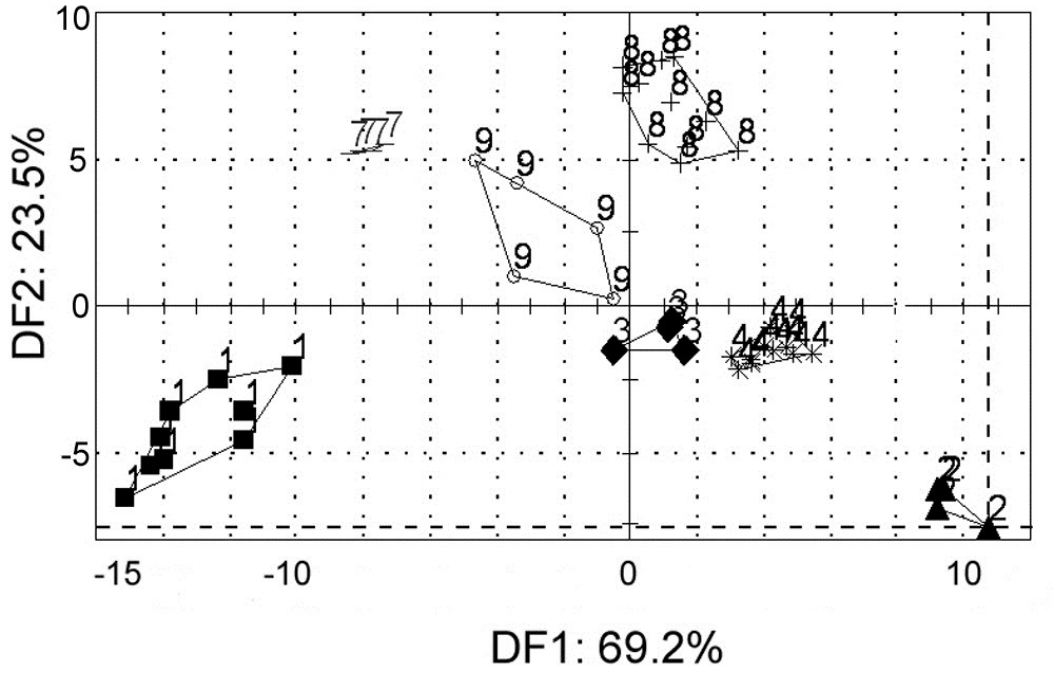

Figure 2. Results of the DFA classification with indication of impact of the involved FAs, individually