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CHEMICAL COMPOSITION AND FATTY ACID PROFILE OF LIPIDS IN CARP (*CYPRINUS CARPIO* L.) MEAT AS AFFECTED BY COOKING METHODS

ANGELINA IVANOVA[1]*; MARIA ANGELOVA-ROMOVA[2]; ZHANA PETKOVA[2];
GINKA ANTOVA[2]; TANIA HUBENOVA[1]
[1]*Agricultural Academy, Institute of Fisheries and Aquaculture, 248 V. Levski Str., 4003 Plovdiv, Bulgaria*
[2]*University of Plovdiv "Paisii Hilendarski", Department of Chemical Technology, 24 Tzar Asen Str., 4000 Plovdiv, Bulgaria*

Abstract

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The aim of the present study is to analyze the chemical composition of the meat and fatty acid profile of carp lipids (Cyprinus carpio L.) before and after heat treatment (baking and frying). The water content (weight method), proteins (Kjeldahl procedure), fat (arbitrage method), ash (weight method) and fatty acid composition (GC) are determined for carp from aquaculture – pond – and cage reared and for free-living carp from Zhrebchevo dam lake. It has been established that the level of water in the baked and fried carp decreases as a result of the heat treatment. Increases in protein, fat and ash content in heat treated meat samples compared to fresh carp meat are reported. The fatty acid profile of baked and fried carp showed a slight decrease in the level of saturated and monounsaturated fatty acids. An increase in the percentage of polyunsaturated fatty acids as a result of heat treatment is reported. The omega-3 fatty acid group is characterized by minor changes and relatively stable levels in baked and fried carp.

The values of the biologically important ω-6: ω-3 ratio in the fresh carp meat from all tested rearing groups ranged from 1.69 to 2.63%. For baked and fried meat samples, higher values are reported, respectively 4.83-6.28% for baked and 4.28-7.60% for fried ones. As a result of the thermal treatment, the values increase in both processing methods and exceed the recommended 5:1 ratio. The fried carp meat are characterized by higher ω-6:ω-3 ratios compared to baked, indicating that carp lipids in baked carp are more balanced and biologically more complete and have a lower risk factor for human health.

Key words: Cyprinus carpio L., cooking method, chemical composition, lipids, fatty acid composition

Introduction

The carp (*Cyprinus carpio* L.) is one of the most cultivated fish species in the world and the major species of the freshwater aquaculture in Bulgaria, mainly due to the existing tradition of its consumption and its relatively constant demand on the market. This raises the issue of its quality and safety in both fresh and processed (after heat treatment) state in connection with the healthy human nutrition.

Techniques for the heat treatment of the meat are widely used to improve its quality and safety. Foods like meat and fish become edible and more acceptable after heat treatment. The various cooking methods affect the nutritive value of fish and especially vitamins, flavor compounds and polyunsaturated fatty acids (Marimuthu et al., 2012).The fish and fish products are thermally treated in various ways to improve their hygienic quality by inactivating pathogenic microorganisms and to emphasize their flavor and taste (Bognar 1998; Ghelichpour et al., 2012; Talab, 2014). During their processing chemical and physical reactions occur, resulting in improved

Corresponding author: astoeva@abv.bg

or lowered nutritive value. For example, the digestibility increases due to protein denaturation, but the content of thermolabile compounds, fat-soluble vitamins or polyunsaturated fatty acids often decreases (Garcia-Arias et al., 2003; Alizade et al., 2009). The cooking methods and the processing temperatures have a direct impact on the preservation of the food quality.

Most of the studies so far have been focused on determining the chemical composition and fatty acid profile of lipids in fresh carp meat. Data on the carp meat quality after heat treatment are fragmented. In this relation the aim of this work is to determine the chemical composition of carp meat and the fatty acid profile of its lipids before and after heat treatment (baking and frying).

Material and Methods

For the purpose of the study, a chemical characterization of the carp (*Cyprinus carpio* L.) meat and fatty acid profile of its lipid is made. The carp are raised in different fish farms in the country applying different rearing systems – semi-intensive polycultural rearing in ponds (Tri Voditsi Experimental base of IFA, Plovdiv) and intensive cage rearing in Zhrebchevo dam lake. Parallel study has been made also with free-living carp in the same dam lake (Table 1).

The carp in the ponds are fed with cold-pressed sunflower meal and wheat. The fish in the cages are fed with extruded feed (Bulmix, Sliven, BG) containing 24% protein and 4% fat in ratio 6:1. A representative number of fish weighing between 1511-2205 g (October, 2016) was selected for investigation at random choice.

For analysis individual samples are prepared from fish musculature (the lateral muscle) taken from one and the same position after removal of the skin and subsequent homogenization of the meat.

The carp cutlets are baked in a conventional oven according to a standardized method for the preparation of seafood (Association of Official Analytical chemists, 1980). The carp cutlets divided into two parts of roughly equal size are smeared with sunflower oil, placed in aluminum trays (individually for each sample) and baked for 40 minutes at 180°C. The fried samples are prepared from carp cutlets divided into two parts of roughly equal size and fried in sunflower oil at 180°C in a ceramic-coated pan.

The chemical composition of the fresh and thermal treated carp meat (n=5) was individually analyzed for each sample to determine: water content, % (through drying at 105°C, 24h; ISO 5984); protein content, % (by the method of Kjeldahl, ISO 5983, using a semi-automated DK 6 digester unit and UDK 132 distillation system, Velp Scientifica); fat content, % (by the method of Smidt-Boudzynski-Ratzlaff); mineral content, % (by burning in a muffle furnace at 550°C, ISO 6496).

The fatty acid composition of lipids is determined by applying gas chromatography (GC) after transmethylation of the respective sample with 2% H_2SO_4 in absolute CH_3OH at 50°C (ISO 5509: 2000). Fatty acid methyl esters (FAME) are purified by thin-layer chromatography (TLC) on 20x20 cm plates covered with 0.2 mm silica gel 60 G layer (Merck, Darmstadt, Germany) with mobile phase n-hexane: diethyl ether (97:3, v/v). GC is performed on a HP 5890 series II (Hewlett Packard GmbH, Vienna, Austria) gas chromatographer equipped with a 75 m x 0.25 mm (I.D.) x 18 μm (film thickness) capillary SP – 2560 column (Supelco, Sigma-Aldrich, St. Louis, MO, USA) and a flame ionization detector. The column temperature is programmed from 140°C (5 min), at 4°C/min to 240°C (7 min); injector and detector temperatures are kept at 250°C. Hydrogen is the carrier gas at a flow rate 0.8 ml/min; split was 50:1. Identification of fatty acids is performed by comparison of retention times with those of a standard mixture of fatty acids subjected to GC under identical experimental conditions (ISO 5508: 2004). The analytical standard of fatty acid methyl esters (SUPELCO F.A.M.E. Mix C4-C24, purity ~ 99%) is from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All solvents and reagents are of analytical grade from Merck (Darmstadt, Germany) and are used without additional purification.

The data are processed statistically, taking into account the average (x), its error (Sx) and the coefficient of variation (Cv, %) with the application of statistical program MSOffice 2010. The statistical significance of differences between two samples is determined by the Student's t-test at a level of $P<0.05$.

Results and Discussion

Chemical composition of carp meat before and after heat treatment

The values for water, protein, fat and ash content in fresh carp meat and after heat treatment are shown in Table 2. Considering the effect of the rearing system on the water content in the carp meat in the fresh samples, it is highest in the meat of the free living carp – 79.93%; followed by this from the semi-intensive rearing system (in ponds) – 76.25%, and the lowest – 75.28% is for the intensively reared carp (in cages).

This trend persists also after the thermal treatment of the meat. The water content in the baked and fried samples from the pond- and cage-reared fish is significantly lower ($P<0.01$,

Table 1. Characteristics of the studied carp rearing systems

Production systems	Pond-reared carp	Free-living carp	Cage-reared carp
Geographical	42°7′58.8″ N	42°37′16.6″ N	42°37′16.6″ N
coordinates	24°28′1.2″ E	25°51′45.9″ E	25°51′45.9″ E
Area, ha	1.3	2500	5
Depth, m	1.3	3.5	4.0
Average weight, g	1511	1947	2205
Degree of intensification	Semi-intensive		Intensive -
	(different grain feed and natural feed -zooplankton and benthos)	(natural feed - mostly zooplankton and benthos)	(only balanced pellets)

Table 2. Chemical composition of carp meat before and after heat treatments

Parameter	Heat treatment	Free-living carp	Pond-reared carp	Cage-reared carp
% Water	Fresh meat	79.93 ± 0.39	76.25 ± 0.88	75.28 ± 0.71
		(0.85)	(1.99)	(1.64)
	Oven baked fish steak	71.67 ± 0.07	67.61 ± 1.22	64.87 ± 1.42
		(0.17)	(3.12)	(3.78)
	Fish steak fried in oil	67.80 ± 1.56	66.40 ± 0.38	64.20 ± 0.07
		(3.96)	(1.00)	(0.20)
% Protein	Fresh meat	17.76 ± 0.38	17.08 ± 0.10	17.23 ± 0.36
		(3.75)	(0.98)	(3.61)
	Oven baked fish steak	24.78 ± 0.55	23.49 ± 0.03	24.60 ± 1.59
		(3.85)	(0.22)	11.21
	Fish steak fried in oil	24.12 ± 0.70	21.71 ± 0.39	21.64 ± 0.17
		(4.99)	(3.08)	(1.33)
% Fat	Fresh meat	1.48 ± 0.50	5.86 ± 0.98	6.68 ± 0.98
		(58.36)	(29.06)	(25.50)
	Oven baked fish steak	2.50 ± 0.55	7.91 ± 1.19	9.50 ± 0.30
		(38.13)	(26.07)	(5.54)
	Fish steak fried in oil	6.76 ± 0.92	10.91 ± 0.81	13.14 ± 0.17
		(23.63)	(12.92)	(2.19)
% Ash	Fresh meat	0.83 ± 0.01	0.82 ± 0.04	0.82 ± 0.02
		(2.68)	(7.66)	(3.65)
	Oven baked fish steak	1.05 ± 0.07	0.99 ± 0.01	1.03 ± 0.02
		(11.25)	(2.33)	(3.90)
	Fish steak fried in oil	1.13 ± 0.03	0.99 ± 0.05	1.02 ± 0.06
		(4.04)	(8.03)	(9.64)

*Mean values±Standard errors of means of 4 different determinations (coefficient of variation), (n=4)

P <0.001) than in the free-living carp. In the case of the baked fish it is 71.67% for the free-living carp and 64.87% for the cage-reared carp. In the fried carp, the water decreases even further and reaches values of 67.8% for free-living carp and 64.2% for the cage-reared carp. The water loss in the baked samples is 10-11%, while for the fried ones this percentage is higher and is in the range of 13-15%.

The data show a greater loss of water at baking for cage-reared fish (with higher fat content in the meat) compared to the pond-reared carp and to the free-living carp from the in Zhrebchevo dam lake. The baked carp meat is characterized by significantly lower water content compared to fresh carp meat (P<0.01, P<0.001). The heat treatment of carp meat influences the water content, as the quantity of water in baked and fried meat is significantly lower.

The protein content of the fresh meat in the free-living carp and in the carp from aquaculture is close – 17.76%-17.08%. After heat treatment (baking and frying) the protein content increases, respectively, in the baked samples to 24.78-23.49% and in the fried to 24.12-21.64%. A higher increase in the protein content is observed in the baked carp, with the highest values being from the free-living carp.

According to Kiosev and Dragoev (2009), frying mainly changes the proteins in fish meat. The frying causes complete destruction of tissue enzymes as well as destruction of microorganisms. Best results are obtained when a temperature between 160-180°C is used. At temperatures above 180°C, the meat is blackened, becomes bitter, dehydrated and very dry. Regardless of how it is baked - in a baking cabinet (radiant convection method), hot-baking (contact heating) and infra-roasting without the presence of fat or a minimum of fat, baking is done at 170-200°C until the temperature inside meat reaches 75-80°C.

The baked carp meat from all the studied rearing groups were characterized by significantly higher protein levels of 24.60 ± 1.59% (cage-reared carp) to 24.78 ± 0.55% (free-living carp), (P<0.01, P<0.001). The increased protein content in baked and fried fish compared to the fresh meat is at the expense of decreasing the amount of water in the heat treated meat. The results indicate that the level of the proteins in the heat treated samples is influenced by the type of the thermal treatment method used.

The heat treatment processes also affect the fat content in the carp meat. Considering the influence of the rearing system on the fat content in the fresh carp samples it is the lowest in the meat of the free-living carp - 1.48%; followed by the pond-reared carp – 5.86%, the highest being in intensive cage-reared carp – 6.68%. It is significantly lower than the fat content in the baked and fried samples. After thermal treatment, the fat content in carp from all three rearing systems increases to 2.50%, 7.91% and 9.5% respectively for the baked and to 6.76%, 10.91% and 13.14% respectively for the fried fish. Greater fat increase is found in the fried carp and lower in the baked carp; in the intensive cage-reared carp it is more pronounced (almost 50%). According to Ninan et al. (2008) and Rathod and Pagarkar (2013), the increased fat level is due to the reduction of water content in the heat-treated meat.

The thermal treatment of carp meat (baking and frying) also affects the ash content. The ash content of the carp muscle tissue in the thermal untreated samples is close to 0.82-0.83% for both the free-living and aquaculture-carp. The heating resulted in a significant increase of the ash level to 0.82-0.83% in the fresh meat; to 0.99-1.05% in the baked and to 0.99-1.13% in the fried carp (P<0.05, P<0.01).

Fatty acid profile in carp lipids in fresh meat and after heat treatment

The everage values for the fatty acid composition of the carp lipids in fresh state and after heat treatment are shown in Table 3.

The level of the saturated fatty acid group as a percentage of the total fatty acids in the fresh carp meat ranges from 27.4% (in the free-living carp) to 32.1% (in the cage-reared carp) of the total fatty acids; for the baked meat from 24.6% to 27.1% and for the fried carp respectively from 24.4% to 25.0%. A slight decrease in the level of saturated fatty acids is reported after administration of the processes of baking and frying. The values obtained for the saturated fatty acids in fresh carp meat are close to those obtained by Hadjinikolova (2008).

The level of the monounsaturated fatty acids group in the meat from the free-living carp and from aquaculture, regardless of its thermal treatment, is very close – 68.0-75.6%. The content of monounsaturated fatty acids for the fresh carp meat is from 57.7% (free-living carp) to 61.5% (pond-reared carp); for baked carp from 38.8% (free-living carp) to 56.9% (pond-reared carp); and for fried carp from 46.6% (free-living carp) to 56.6% (cage-reared carp).

The analysis of the results shows a decrease in the level of monounsaturated fatty acids resulting from the thermal treatment. They are most represented by the oleic acid (C18:1). For the fresh carp meat, the values for the oleic acid are in the range of 50.5% (cage-reared carp) to 54.1% (pond-reared carp) of the total fatty acid content. The second of the monounsaturated fatty acids is the palmitooleic (C16:1), with values of 5.3% (free-living carp) to 7.7% (cage-reared carp). Low concentrations of less than 0.5% are registered for the other MUFA. The results obtained show that the oleic acid determines the level of the monounsaturated fatty acids and its level is in line with the values for fresh water fish cited in the literature

Table 3. Fatty acid lipid composition of carp meat before and after heat treatments, n=3

Fatty acids, %	Free-living carp			Pond-reared carp			Cage-reared carp		
	Fresh meat	Oven baked fish steak	Fish steak fried in oil	Fresh meat	Oven baked fish steak	Fish steak fried in oil	Fresh meat	Oven baked fish steak	Fish steak fried in oil
C 8:0	0.1	-	-	-	-	0.1	-	-	0.1
C 10:0	-*	-	-	-	-	-	0.1	-	-
C 12:0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1
C 14:0	1.1	1.0	1.0	1.2	1.0	1.0	1.3	1.1	1.1
C 14:1	0.1	-	0.1	0.1	0.1	0.1	-	0.1	0.1
C 15:0	0.2	0.5	0.4	0.4	0.3	0.3	0.2	0.1	0.1
C 16:0	19.9	17.3	16.6	21.9	18.3	17.1	24.1	17.9	17.7
C 16:1	5.3	3.4	4.2	7.0	5.5	5.4	7.7	5.6	6.3
C 17:0	0.2	0.7	0.5	0.2	0.2	0.2	0.3	0.2	0.2
C 17:1	0.2	0.4	0.2	0.3	0.3	0.2	0.3	0.2	0.1
C 18:0	5.2	6.0	5.2	5.8	5.4	5.1	5.7	4.5	4.4
C 18:1	51.6	34.4	41.9	54.1	50.7	49.9	50.5	48.3	49.6
C 18:2 (ω-6)	9.2	26.4	23.4	4.4	13.1	16.3	5.1	15.9	14.2
C 18:3 (ω-6)	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.2
C 18:3 (ω-3)	3.9	3.8	3.1	2.9	2.4	2.2	3.4	3.1	3.2
C 20:0	0.2	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2
C 20:2 (ω-6)	0.3	0.6	0.4	0.2	0.2	0.2	0.2	0.3	0.2
C 20:3 (ω-6)	0.3	0.5	0.2	0.3	0.3	0.2	0.2	0.3	0.2
C 20:4 (ω-6)	0.5	1.5	0.6	0.5	0.6	0.4	0.4	0.6	0.3
C 20:5 (ω-3)	-	0.2	0.3	-	0.1	-	0.1	0.1	0.2
C 22:0	0.1	0.5	0.4	0.1	0.2	0.2	0.2	0.2	0.2
C 22:1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2
C 22:2 (ω-6)	0.3	0.9	0.2	-	0.1	0.1	0.2	0.1	0.2
C 24:0	0.3	0.8	0.6	0.4	0.4	0.4	0.2	0.5	0.5
C 24:1	0.3	0.5	0.1	0.2	0.3	0.2	0.2	0.4	0.4
C 22:6 (ω-3)	0.2	0.8	0.2	0.3	0.3	0.2	0.2	0.5	0.2
Saturated FA	27.4	27.1	25.0	30.0	25.9	24.4	32.1	24.6	24.4
Unsaturated FA	72.6	72.9	75.0	70.0	74.1	75.6	68.0	75.5	75.6
Monounsaturated FA	57.7	38.8	46.6	61.5	56.9	55.9	58.6	54.5	56.6
Poly unsaturated FA	14.9	34.2	28.4	8.6	17.2	19.7	9.4	21.0	19.0
∑ ω-6	10.8	29.5	24.8	5.4	14.4	17.3	5.9	17.4	15.4
∑ ω-3	4.1	4.7	3.6	3.2	2.8	2.4	3.5	3.6	3.6

Table 3. Continued

Ratio ∑ ω-6: ∑ ω-3	2.63	6.28	7.50	1.69	5.50	7.60	1.69	4.83	4.28

*- Not identified

(Hadjinikolova, 2004; Guler et al., 2008).

As a result of the thermal treatment, the level of polyunsaturated fatty acids increased. The baked carp meat is characterized by higher values in an interval of 17.2% (pond-reared carp) to 34.2% (free-living carp) of the total fatty acids content compared to the fried carp meat. For the fried carp, the values are 19.0% (cage-reared carp) to 28.4% (free-living carp). The highest rate of increase is reported for the free-living carp, where the values are approximately 19% higher for the baked samples and 14% for the fried ones. For the other two carp groups, the increase in the polyunsaturated fatty acids is 8-11%.

The changes in the polyunsaturated fatty acids are mainly due to an increase in the level of ω-6 fatty acids. Their average value in the baked carp are from 14.4% (pond-reared carp) to 29.5% (free-living carp), and in the fried carp from 15.4% (cage-reared carp) to 24.8% (free-living carp). ω-3 FA are characterized by minor changes and relative stabile level in the baked and fried meat samples.

There is a significant increase in the level of linoleic acid (C18:2 ω-6) in the baked and fried fish, which has a large share in the ω-6 group. This was observed in the carp of all three rearing groups studied. The higher values of the linoleic acid (C18:2 ω-6) and arachidonic (C20:4 ω-6) acids in the baked carp are a good indicator for better quality and nutritive value of the fish lipids in baked carp, compared to the fried ones.

The results show that the application of heat treatment leads to a decrease in the level of oleic acid (C18: 1) in the carp muscle tissue. According to Marichamy et al. (2009) the composition of fatty acids in fish muscle tissue changes after heat treatment depending on temperature, contact surface width, fish size and initial fat content. Our results show that this is most evident in samples of baked free-living carp from Zhrebchevo dam lake, which have the lowest fat level in the fresh fish fillet (1.48%).

The heat treatment also affects the level of the docosahexaenoic fatty acid (C22:6 ω-3). Its amount increases from 0.2% to 0.8% in baked free-living carp, and from 0.2% to 0.5% in baked cage-reared carp. Such influence is not taken into account for the pond-reared carp. The two essential fatty acids, eicosapentaenoic and docosahexaenoic, are considered as susceptible to oxidation as a result of thermal heating processes (Sant'Ana and Mancini-Filho, 2000).

The values of the biologically important ω-6:ω-3 ratio in the studied fresh carp meat ranged from 1.69-2.63. Lower values are observed in the carp from aquaculture – cage- and pond-reared. The application of both heat treatment methods leads to an increase in the ω-6:ω-3 ratio, respectively 4.83-6.28 for the baked and 4.28-7.60 for the fried carp. According to the World Health Organization guidelines (WHO, 2008) it is assumed that with a ω-6:ω-3 ratio of less than 5:1, raw materials and natural products have a low risk factor for human health.

According to Steffens and Wirth (2005) the ω-6:ω-3 ratio in carp ranges to a great extent (0.8 and 2.4) and is most affected by the type of feed used. Knowing the need to dietary inclusion of omega-3 essential fatty acids and their health-enhancing effects, nutritionists recommend a diet rich in omega-3 fatty acids as well as a decrease in ω-6: ω-3 ratio of 15-20: 1 to 1-4: 1 in the modern diet (Gebauer et al., 2006, Raes et al., 2004).

In our study, the fresh carp meat has a ratio of less than 5:1. The application of both heat treatment methods leads to an increase in the ω-6:ω-3 ratio, respectively to 6.28 for the baked and to 7.60 for the fried carp. Fried carp meat has higher values than those of baked ones. This gives reason to believe that carp lipids in baked carp are more balanced and biologically complete and have a lower risk factor for the human health.

Samples of the baked and fried carp reared in cages by using extruded pellets retain the biologically important ω-6:ω-3 ratio below the recommended level (5:1) as opposed to the free-living and pond-reared carp. This shows that the use of balanced pellets in the aquaculture leads to a product with a balanced fatty acid distribution and good nutritive value.

Piggot and Tucker (1990) in their studies indicate that the ω-6:ω-3 ratio can be used as an indicator for comparing the relative nutritive value of fish lipids. According to Arts et al. (2001) omega-3 fatty acids with C20 and C22 hydrocarbon atoms have more valuable nutritive properties than those with C18. In the cases of their dominance, especially with regard to eicosapentaenoic (C20:5 ω-3) and docosahexaenoic (C22:6 ω-3) acids, they are responsible for changes in the ω-6:ω-3 ratio. The same authors note that when using the omega-6:

ω-3 ratio in determining the nutritive value, the quantitative values of the individual polyunsaturated fatty acids should also be taken into account.

Conclusions

The method of the thermal treatment of carp meat has a significant effect on the content of the main components of its chemical composition. A greater change in the level of proteins and minerals is recorded in the baking process, while during the frying the fat content is more affected.

The fatty acid profile showed that the major group of fatty acids in the carp lipids in the fresh and processed meat is the monounsaturated fatty acid group. It was found that the application of the heat treatment led to a negligible decrease in the level of unsaturated fatty acids (UFA) and increase of the polyunsaturated fatty acids (PUFA). The increase of polyunsaturated fatty acids is a result of the thermal treatment and impacts to a greater extent the ω-6 fatty acids group compared to the ω-3 fatty acids group.

The heat treatment results in a significant increase in the level of linoleic (C18:2 ω-6), oleic (C18:1), palmitoleic (C16:1) and arachidonic (C20:4 ω-6) fatty acids in the baked carp meat which is a good indicator for better quality and nutritive value of fish lipids in baked carp meat compared to the fried ones.

It is established that the baked carp meat is characterized by a lower ω-6:ω-3 ratio, making it more balanced as food than the fried ones. It is recommended from both types of thermal treatment of carp meat the applying of the baking method more often than the fried, which preserves better the nutritive value of fish fat and the processed product has a lower risk factor for the human health.

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