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Address of Editorial office:
Agricultural Science and Technology
Faculty of Agriculture, Trakia University
Student's campus, 6000 Stara Zagora
Bulgaria
Telephone: +359 42 699488
+359 42 699446
www.agriscitech.eu

Technical Assistance:
Nely Tsvetanova
Telephone: +359 42 699446
E-mail: editoffice@agriscitech.eu

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Product Quality and Safety

Proximate composition, lipid quality and heavy metals content in the muscle of two carp species

A. Merdzhanova*, V. Panayotova, D.A. Dobreva, K. Peycheva

Department of Chemistry, Faculty of Pharmacy, Medical University, 9000 Varna, Bulgaria

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Abstract. *The aim of the presented study was to characterize the quality of edible tissue of freshwater common carp (Cyprinus carpio) and bighead carp (Aristichthys nobilis), based on their proximate and lipid composition (lipid classes, fatty acid profile, fat soluble vitamins, carotenoids and cholesterol). Health risk assessment was evaluated based on the analysis of some toxic elements (As, Cd, Ni, Pb and total Hg). Proximate composition (moisture, crude protein and total lipid) was determined using standard procedures. Lipids were subsequently separated into neutral (NL) and polar lipids: Phospho- (PL) and Glycolipids (GL) by means of column and thin-layer chromatography. Lipid classes were derivatized into fatty acid methyl esters (FAMEs) which were analysed by gas chromatography–mass spectrometry (GC-MS). Vitamins A, D_3 and E, beta-carotene, astaxanthin and cholesterol were analysed simultaneously using high performance liquid chromatography (HPLC). Heavy metals (As, Pb, Cd, Hg and Ni) were determined by optical emission spectrometry with inductively coupled plasma (ICP-OES) following a microwave digestion procedure. Protein content was higher in bighead carp (18.5%) and lower for common carp (15.5%), whereas lipid content showed opposite trend. Similarities in lipid classes distribution were observed for both species: NL>GL>PL. Neutral lipids constituted approximately 70% of TL in both species, as FAs profile was dominated by monounsaturated fatty acids (MUFA), whereas polyunsaturated FAs (PUFA) prevailed in polar fractions. Omega-3 PUFAs were higher in all lipid classes compared to omega-6 PUFAs. Cholesterol content was low (17-24 mg.100^{-1}g ww). Astaxanthin was detected only in bighead carp, whereas beta-carotene, vitamin D_3 and vitamin A showed similar concentrations in both samples. Vitamin E content was higher in bighead carp (10.4 mg.100 g^{-1} w.w.). Trace elements content was higher in bighead carp showing a maximum value of As (0.312 mg.kg^{-1} w.w). All determined toxic elements were found below the recommended value in carp muscle. The results of the present study confirmed the high quality and safety of common carp and bighead carp meat. These freshwater species are valuable sources of essential nutrients such as proteins, vitamin D_3 and long chain omega-3 PUFAs. Together with the nutrients, the information for low concentrations of toxic elements makes them valuable components of a healthy human diet.*

Keywords: common carp, bighead carp, lipid composition, nutrition quality, health risk assessment

Abbreviations:

FA-fatty acid; SFA-saturated fatty acid; MUFA-monounsaturated fatty acid; PUFA-polyunsaturated fatty acid; n-3 – omega 3 PUFA; n-6 – omega-6 PUFA; NL-neutral lipids; PL-Phospholipids, GL-glycolipids

Introduction

The official statistics information of the National Agency for Fisheries and Aquaculture, reported 7557.14 tons the total productions from Bulgarian aquaculture fish farms for 2012. The traditionally large interest of the consumers and the appropriate climatic and hydrological environmental factors in Bulgaria have made the carp species as the most cultivated in inland waters. Among carp species, the production of common carp (*Cyprinus carpio*) and bighead carp (*Aristichthys nobilis*) prevail. These fish are important components in polyculture and usually are used in the biological control of the pond water quality (Multiannual National Strategic Plan for Aquaculture in Bulgaria, 2014-2020). In recent years interest in investigations of nutrition quality of carp species based on proximate composition, biologically active lipids and heavy metals content has increased. Several studies reported high quality of common and bighead carps as food sources for human. Most of them focus mainly on fatty acid composition of total lipids (Cirkovich et al., 2012; Mraz et al, 2012; Bohm et al., 2014). A few studies were conducted on lipid class composition on carp species such as common carp and Indian carp (Mraz and Pickova, 2009; Day et al., 2015). Most of them investigated the polar and the neutral lipid fractions due to their high functional potential, but comparable data for glycolipid fraction of carp tissue especially for bighead carp, was not found in literature. Moreover, in freshwater fish tissues FAs of both lipid fractions occur as neutral or storage lipid FAs (NLFAs), polar lipids FAs (PLFAs) and glycolipids FA (GLFAs). Both polar phospholipids (PL) and glycolipids (GL) fractions are main integral component of cell membranes and play essential functions in cell physiology in animal tissues. Furthermore, polar lipids are usually rich in PUFAs, especially of omega-3 series as eicosapentaenoic (C20:5 n-3, EPA) and docosahexaenoic (C22:6 n-3, DHA) long-chain acids, that support cell membrane fluidity and permeability at lower water temperatures. In addition, neutral lipids are energy long-term stored sources in fish tissues and their FA composition are strongly affected by content of diet FAs, whereas the PLFA profile in cell membranes is regulated to meet taxa-depending requirements (Bohm et al., 2014) and is more sustainable of seasonal changes. Phospholipids and glycolipids may positively influence the digestibility of some fat soluble vitamins as alpha-tocopherol and carotenoids and triacylglycerol (TAG) by promoting chylomicron

* e-mail: a.merdzhanova@gmail.com

production. PL are very good emulsifiers and provide more bioavailability and absorption of high bioactive EPA and DHA, compared to non-emulsified form of these omega-3 FAs (Berger, 2014). Other valuable and important components of total lipid fraction are fat soluble vitamins (A, E and D_3), cholesterol and carotenoids. There were limited data in scientific literature for carp species fat soluble vitamins content (A, E and D_3) farmed in Bulgaria (Dobreva et al., 2014). No comparable results were found for carotenoid and cholesterol content in edible tissue of these carp species in our country.

In addition to the assessment of nutritional quality, fish may also be affected by environmental pollution. Exposure to some contaminants could imply health risks, especially for the more vulnerable consumer groups, such as pregnant women and children (Jacobs et al., 2014). Heavy metals can be classified as potentially toxic (arsenic, cadmium, lead, mercury, nickel, etc.), probably essential (vanadium, cobalt) and essential (copper, zinc, iron, manganese, selenium) (Munoz-Olivas and Camara, 2001). The essential elements can also produce toxic effects when the metal intake is excessively escalated. When it comes to estimate the potential risks to human health of heavy metals consumption from fish, several ways have been adopted such as calculating the carcinogenic effect and non-carcinogenic effect. Risk assessment is one of fastest methods needed to evaluate the impact of the hazards on human health and also needed to determine the level of treatment which is meant to solve the environmental problem that occurs in everyday life (Zhang et al., 2012). These methods are typically based on the Target Hazard Quotients (THQ) and Hazard Index (HI) (Peycheva et al., 2017).

The aims of the present study are to determine the lipid classes, FA composition, carotenoids, cholesterol and fat soluble vitamins content, and concentration of some heavy metals (As, Cd, Ni, Hg and Pb) in edible tissues of two commonly consumed carp species in Bulgaria.

Material and methods

Sample collection and preparations

Samples of common carp (*Cyprinus carpio*) and bighead carp (*Aristichthys nobilis*) were purchased from a fish farm near Plovdiv in October 2017. Fish specimens were transported to the laboratory in iceboxes. The specimens biometric characteristics were determined (Table 1).

Table 1. Biometric characteristics of fish samples (mean±SD)

Parameters	Bighead carp (n=6)	Common carp (n=6)
Mean weight (g)	940.0±1.50	285.5±0.50
Mean length (cm)	38.5±0.50	25.0±0.50
Habitat	Pelagic	Demersal
Food habits	Herbivorous	Omnivorous

*n - number of specimens; SD - standard deviation

Six specimens of each species were chosen randomly to determine the sample mean. Average samples of edible carp tissue were used for a proximate, fatty acids, fat-soluble vitamins, cholesterol, carotenoids and heavy metal analysis. All fish tissue, without skin, were homogenized at 700 rpm, using a Molineux blender.

Proximate compositions analysis

The homogenized fish tissue (2.000±0.010g) was dried in air oven at (105±2°C) for 16-18 hours to a constant weight. The moisture was calculated according to AOAC 950.46. The crude protein content was determined by the Kjeldahl method (Bulgarian State Standard /BSS/ 9374:1982).

Extraction of total lipids (TL)

Three replicate samples of raw meat homogenates (5.000±0.001g) were extracted by the Bligh and Dyer method (1959). Lipid content was defined gravimetrically and the results were expressed as g per 100g wet weight ($g.100g^{-1}$ ww).

Separation of lipid classes

Total lipids were separated into different classes by column chromatography. Neutral lipids (NL) phospholipids (PL) and glycolipids (GL) were obtained using a glass column (10mm dia×20cm) packed with a slurry of activated silicic acid (70 to 230 mesh; Merck, Darmstadt, Germany) in chloroform. The fraction containing NL was eluted with chloroform, PL fraction was eluted with methanol, while GL fraction was eluted with acetone: methanol (9:1v/v). The amount of lipid classes obtained was determined by gravimetry. The purity of each fraction was tested by thin-layer chromatography, using Silica gel F254 plates (thickness = 0.25mm; Merck, Darmstadt, Germany).

Preparation of fatty acids methyl esters (FAME) and GS-MS analysis

The dry residues of each fraction were methylated using 2% H_2SO_4 in methanol and n-hexane (BSS EN ISO 12966-2:2017). Combined extracts were filtered through anhydrous Na_2SO_4 and evaporated under gentle steam of nitrogen. FAMEs were separated by Gas Chromatograph Thermo Scientific FOCUS with TR-5 MS capillary column (30m, 0.25mm i.d.) and MS detector (Polaris Q). For peaks identification two parameters were used: mass spectra of FAME mix standard (SUPELCO 37 F.A.M.E. Mix C4-C24) and internal Data Base (Thermo Sciences Mass Library, USA). Additionally, docosapentaenoic acid (C22:5n3, DPA) was identified and quantified by PUFA No. 3 from Menhaden oil (Sigma-Aldrich, Merck). Results were expressed as a relative percentage of each FA with respect to the total FAs. (BSS EN ISO 12966-4:2015)

Saponification and extraction of vitamins, pigments and cholesterol

Alkaline saponification was used to analyze β-carotene, astaxanthin, cholesterol, all-trans-retinol, alpha-tocopherol, ergocalciferol, and cholecalciferol content. Sample preparation procedure was performed following the method of Dobreva et al. (2017). Six replicates of each group were prepared and subjected to saponification at 50°C for 30min. After cooling the analytes were extracted twice with n-hexane:dichloromethane = 2:1 (v/v) solution. The combined extracts were evaporated under nitrogen and redissolved in methanol:dichloromethane solution, filtrated (0.45μm syringe filter) and injected (20μl) into the HPLC system.

HPLC analysis

The chromatographic separation was performed by HPLC system (Thermo Scientific Spectra SYSTEM) equipped with UV2000 and FL3000 detectors. All-trans-retinol, ergocalciferol,

cholecalciferol, alpha-tocopherol, β-carotene, astaxanthin and cholesterol were determined simultaneously using reversed-phase analytical column (Synergi 4μ Hydro-RP 80A pore 250x4.6mm). The mobile phase consisted of acetonitrile: methanol: 2-propanol = 75:20:5 with a flow rate of 1.1 mL/min (Dobreva et al., 2017). Ultraviolet detection was used for cholesterol (208nm), ergocalciferol (265nm), cholecalciferol (265nm), β-carotene (450nm) and astaxanthin (474nm). Concentrations of all-trans-retinol (at λex = 334nm and λem = 460nm) and α-tocopherol (at λex = 288nm and λem=332nm) were measured by fluorescence detection.

Nutrition quality indices (NQI)

Nutrition quality was evaluated based on several indices and ratios: the indices of atherogenicity (AI), thrombogenicity (TI), cholesterolemic index (h/H); n-6/n-3 and PUFA/SFA ratios, according to Simopolous (2013). Ulbricht and Southgate (1991) suggest two indices that may well describe the atherogenic (AI) and thrombogenic (TI) properties of fatty acids. The functional effects of individual PUFAs on cholesterol metabolism (hypo- and hyper-cholesterolemic effect) was estimated by h/H index (Santos-Silva et al., 2002). The cholesterol/SFA index (CSI) was calculated according to Connor et al. (1986) to assess the potential effect of dietary lipids on serum cholesterol.

Heavy metal analysis

Approximately 1.0g of homogenized muscle tissue sample was dissolved with 10cm^3 HNO_3 (ultra-pure Merck ® Darmstadt, Germany) in a digestion system and diluted to final volume of 25cm^3 with double deionized water. MARS 6 Microwave Sample Preparation System (CEM Corporation, USA) delivering a maximum power and temperature of 800W and 200°C, respectively, and internal temperature control, was used to assist the acid digestion process. One reagent blank for every digestion was included as a representative standard reference, homogeneity and process efficacy in sample replicated. The digested sample was transferred into a flask and left to cool down.

All fish samples were analyzed for Cd, As, Ni, Hg and Pb using an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The ICP-OES model used in this study was Optima 8000 (Perking Elmer, USA). The instrument working parameters were as follows: plasma gas flow - 10 L/min, auxiliary gas flow - 0.4 L/min, nebulizer gas flow - 0.6 L/min, peristatic pump flow rate - 1.5 ml/min, spay chamber - cyclonic glass, nebulizer - concentric glass, MEINHARD® Type C. Results were justified using a calibration curve generated from the responses obtained from multiple dilutions of a prepared multi-element calibration standard (Optima Family Multi-Element Standard, Matrix per Volume: 2% HNO_3). Analytical quality control included analysis of a 2% ultrapure HNO_3 blank and a sample duplicate from the microwave digestion. For the determination of Hg was used CF-HG-ICP-OES with reducing agent 0.2% $NaBH_4$. For calibration verification standard a DORM-2 (NRCC, Ottawa) certified dogfish tissue was used with recoveries between 90% and 108%.

The determination of the As, Cd, Hg, Ni and Pb levels on edible tissue of the analysed carp species and comparing the obtained results with the recommended levels set by the European Union (EU) are important for evaluation of fish quality and safety. Moreover, the edible tissue was focused in this study due to its the major contribution to human diet.

Assessment of Human Health Risk

The human health risk assessment of heavy metals from fish consumption was assessed using the following formulas:

1) The target hazard quotient (THQ) of heavy metals estimates a possible alert regarding adverse health effects that may be caused by an individual metal. The THQ formula (USEPA, 2000) is:

$$THQ = \frac{(M_C \,.\, I_R \,.\, 10^{-3} \,.\, EF \,.\, ED)}{(RfD \,.\, BWa \,.\, ATn)},$$

Where:

Mc - heavy metal concentration in fish species (mg/kg ww);

EF – exposure frequency (365 days/year);

IR – rate of consumption;

ED – exposure duration (72.5 years), equivalent to the average lifetime in Bulgaria;

RfD – oral reference dose provided by the USEPA(USEPA, 2004);

BWa – average body weight (60kg for adults);

ATn – averaging exposure time for non-carcinogens (365 days/year × ED);

10^{-3} - unit conversion factor.

The THQ values, developed by the USEPA (1989), have been recognized as useful parameters for human health risk assessment of metals associated with the intake of fish products. The THQ is also a non-carcinogenic risk assessment which is a ratio between the estimated dose of metal exposure and the oral reference dose. A THQ>1 signifies that the level of exposure is higher than the oral reference dose, which assumes that a daily exposure at this level is likely to cause negative health effects during a lifetime in a human population (Bogdanovic et al., 2014).

2) *The hazard index (HI)* of multiple heavy metals evaluates possible health effects that can be caused by the combination of all metals studied. The HI was calculated by adding all the calculated THQi values for the analyzed elements (Jovíc and Stankovic, 2014), by using the following equation:

$$\sum_{i=1}^{n} \mathrm{THQ_i},$$

Where: THQi is the targeted hazard quotient of an individual metal and n in the present study is 5.

3.) *Target cancer risk (TR)* indicates carcinogenic risks. The model for estimating TR was shown as follows:

$$\mathrm{TR} = \frac{(\mathrm{M_C} \,.\, \mathrm{I_R} \,.\, 10^{-3} \,.\, \mathrm{CPSo.EF} \,.\, \mathrm{ED})}{(\mathrm{BWa} \,.\, \mathrm{ATc})},$$

Where:

Mc – metal concentration in the samples (mg/kg ww);

CPSo – the carcinogenic potency slope, oral;

IR – rate of consumption;

EF – exposure frequency (365 days/year);

ED – exposure duration (72.5 years), equivalent to the average lifetime in Bulgaria;

BWa – average body weight (60 kg for adults);

ATc – the averaging time, carcinogens (day/years), calculated by multiplying exposure frequency in exposure duration over lifetime.

TR value for intake of As, Ni and Pb was calculated to indicate the carcinogenic risk since Cu, Hg and Zn for example do not cause any carcinogenic effects.

Statistical analysis

The results were analyzed by Graph Pad Prism 6.0 software. Unpaired t-test was applied to estimate the differences between the analyzed samples. The differences were considered significant at $p<0.05$. The data for the heavy metal were subjected to a statistical analysis. Metal concentrations for all the species were calculated by application of Excel 2007 (Microsoft Inc., USA). Student-T test was employed to estimate the significance of values.

Results and discussion

Proximate composition

Water, proteins, and lipids make up about 98% of the total mass in fish tissue. The chemical composition of fish significantly varies due to geographical locations, stages of maturity, sizes or seasons. Moreover, proportions of these macronutrients are species-specific and the main differences in proximate composition occur in lipid content between species (FAO, 2010). In this study bighead carp contained significantly higher protein content (18.50%) compared to common carp (15.60%, $p<0.001$). The opposite trend was observed for total lipids (TL), where only 1.50 g.100g^{-1}ww was found in bighead carp tissue, while common carp showed 3.6 g.100g^{-1}ww edible tissue. These results, enable to classify both carp species as fish with low fat (<5 g.100g^{-1}ww) and high protein content (15-20%) in accordance with Commission Regulation (EC) No. 116/2010. Guler et al. (2008) and Swapna et al. (2010) reported similar results for TL content of common carp muscle tissue from Beysehir Lake, Turkey (4.45g.100g^{-1}ww, winter season) and from Indian local markets (3.80g.100g^{-1}ww). Ljubojevic et al. (2013) showed similar data for protein content of common carp (15.64%) and bighead carp (18.0%) from Novi Sad fish markets, the Republic of Serbia. Hong et al. (2015) reported similar results for proximate composition: muscle protein (17.9%) and TL (0.55g.100g^{-1}ww) contents in farmed bighead carp from Beijing region, China. The presented results indicated that both analysed carp species contain appreciable amount of proteins and low lipid quantity, thus these species could be included in diets to supplement our daily nutrient requirements.

Lipid classes distribution and fatty acid composition

The total lipids of both carp species were separated into neutral (NL), glyco- (GL) and phospholipids (PL). Similarity in lipid classes distribution was observed for both species: NL>GL>PL. Results for lipid classes and FA compositions of total lipids and NL, GL and PL fractions of bighead and common carp species edible tissues are presented in Tables 2 and 3. Neutral lipids constituted approximately 70% of TL in all studied specimens. GL content ranged between 17.6% of TL (common carp) up to 19.5% of TL (bighead carp), whereas PL levels were the lowest: from 9.3% (common carp) to 11.2% (bighead carp).

Table 2. Lipid classes, fatty acids profile (FA, % of total FA) of lipid fractions, FA ratios and Nutrition quality indices of bighead carp edible tissue (mean±SD) (n=6)

	TL	NL	PL	GL
% of TL		70.30±2.60	0.111.20±1.50	18.50±1.80
g.100-1g w. w.	1.50±0.08	1.05±0.05	0.17±0.03	0.272±0.055
Fatty Acid	TL	NL	PL	GL
C 10:0	0.01±0.01	0.03±0.01	0.01±0.01	0.04±0.01
C 12:0	0.02±0.01	0.04±0.01	0.15±0.02	0.20±0.03
C 14:0	0.68±0.02	1.35±0.06	0.46±0.03	1.05±0.10
C 15:0	0.11±0.01	0.13±0.02	Nd	0.12±0.02
C 16:0	18.63±1.05	24.68±2.14	18.10±1.23	17.98±1.16
C 17:0	0.13±0.01	0.14±0.02	0.14±0.02	0.05±0.01
C 18:0	3.85±0.80	3.93±0.07	8.74±0.50 [a]	5.73±0.35
C 20:0	0.06±0.01	0.06±0.01	0.06±0.01	0.05±0.01
C 21:0	0.03±0.01	0.03±0.01	Nd	Nd
C 22:0	0.10±0.01	0.05±0.02	0.08±0.02	0.04±0.01
C 23:0	0.03±0.01	0.01±0.01	Nd	Nd
C 24:0	0.20±0.02	0.22±0.03	0.08±0.01	0.10±0.02
SFA	23.86	30.69[a]	27.82	25.36
C 14:1n-5	0.06±0.02	0.08±0.02	0.22±0.03	0.10±0.01
C 16:1n-7	8.77±0.34	13.45±0.84	3.82±0.27	5.68±0.45
C 18:1n-9	15.84±1.06	20.79±1.68	7.47±0.55 [a]	10.58±0.80
C 17:1	0.17±0.02	0.06±0.01	0.02±0.01	0.15±0.01
C 20:1n-9	1.43±0.06	1.56±0.05	0.68±0.03	0.80±0.03
C 22:1n-9	0.02±0.01	0.03±0.01	0.02±0.01	0.02±0.01
C 24:1n-9	0.12±0.02	0.08±0.02	0.07±0.01	0.05±0.01

MUFA	26.41	36.05[a]	12.30[a]	17.38
C 18:3 n-6	Nd	Nd	Nd	0.11±0.01
C 18:2 n-6	3.66±0.37	4.50±0.48	2.07±0.30	3.43±0.21
C 18:3 n-3	5.04±0.45	3.87±0.32	2.09±0.32[a]	3.93±0.35
C 20:3 n-3	0.61±0.03	0.45±0.03	0.47±0.02	0.70±0.03
C 20:3 n-6	1.33±0.07	0.96±0.04	1.79±0.08	1.02±0.04
C 20:2 n-6	1.06±0.10	0.53±0.02	0.89±0.03	0.95±0.02
C 20:4 n-6	1.42±0.12	1.23±0.15	2.15±0.40	1.68±0.20
C 20:5 n-3	11.60±0.80	7.38±0.68	14.89±0.85[a]	15.38±0.55[a]
C 22:6 n-3	21.17±1.76	12.19±0.90	32.52±2.06[a]	26.42±1.70[a]
C 22:2 n-9	0.45±0.02	1.01±0.05	0.25±0.01	0.38±0.04
C 22:5 n-3	3.70±0.42	1.19±0.04	2.78±0.24	3.35±0.42
PUFA	49.74	33.31[a]	59.90[a]	57.35[a]
FA ratios, omega-3 PUFA concentrations and Nutrition quality indices				
Σ n-3	42.12	25.08	52.75	49.78
Σ n-6	7.47	7.22	6.90	7.19
n-6/n-3	0.18	0.29	0.13	0.14
PUFA/SFA	2.08	1.08	2.15	2.26
EPA ($g.100^{-1}gww$)	0.160	0.082	0.025	0.053
DHA($g.100^{-1}gww$)	0.296	0.135	0.060[a]	0.095
AI	0.28	0.44	0.28	0.30
TI	0.12	0.26	0.11	0.11
h/H	3.36	1.76	3.17	3.90
CSI	1.24	1.20	0.93	0.95

*AI= [(C12:0+ (4×C14:0) +C16:0)]/ (n6PUFA +n3PUFA+ MUFA);
TI =(C14:0+C16:0+C18:0) /[(0.5MUFA) + (0.5n6PUFA) + (3n3PUFA) + (n3PUFA/n6PUFA)];
h/H= (C18:1n9+C18:2n6 +C18:3n3+C20:4n6+C20:5n3+ C22:6n3)/ (C14:0) +C16:0);
CSI= (1.01x SFAg.100-1g w. w.) + (0.05 x cholesterol mg.100-1g w. w.); a p<0.001, b p<0.01, c p<0.05

Different results are reported for lipid class distribution in freshwater fish species in literature. Generally, the major lipid fraction of muscle TL are NL. The main variation between lipid classes were found in polar PL and GL fractions. Swapna et al. (2010) analysed five freshwater fish species from Indian local markets and presented similar lipid class pattern (NL>GL>PL) for common carp edible tissue. The same author found higher PL and lower GL content of mrigal (*Cirrhinus mrigala*) and tilapia (*Oreochomis mossambicus).* De Oliveira Sales and Maia (2012) reported higher PL (23.1%) and significantly lower GL (1.5%) amounts for tilapia do Nilo (*Oreochomis niliticus*) from Brazil. Comparable information for bighead carp lipid class distribution was not found in literature. The observed variations in lipid classes distribution can be explained by the fact that GL and PL fractions also can be degraded and utilised as fuel during period of starvation, maturations or specific farming conditions (controlling overload of fish growing) (Godavarthy and Kumari, 2014).

It is known that fatty acids are derived from two major sources: fish diet and biosynthesis (Swapna et al., 2010). In the presented study, TL was characterized by the dominance of unsaturated FAs, but significant differences in FA distributions between carp species were observed. Bighead carp TL showed the following FA pattern: PUFA>MUFA>SFA, whereas common carp presented MUFA>PUFA>SFA alignment. In both species MUFAs prevailed in NL fraction, but bighead carp contained more unsaturated FAs (MUFA>PUFA>SFA) compared to common carp (MUFA>SFA>PUFA). The obtained results for polar fractions (PL and GL) showed similar FA distribution: PUFA> SFA> MUFA for both carp fish. Earlier studies on lipids of different fish species (Petursdottir et al., 2008) have shown that the FA profile of NL fractions (and TL, respectively) of muscle lipids reflects the diet. The analyzed carp species differ in food habits and this is well illustrated by the FA profile of their NL fractions.

Herbivorous Bighead carp showed well balanced FA pattern in its NL fractions, with insignificantly prevailing MUFA, compared to PUFAs, whereas omnivorous common carp contained mainly MUFA (approx. 46% of NL) and two times lower PUFA levels. The highest SFA levels were found in the NL fractions in both species. Among SFA, C16:0 (palmitic acid) and C18:0 (stearic acid) prevailed in all lipid fractions. NL serve as energy sources in fish tissues, consequently they contain higher levels of long chain SFAs (such as C16:0, C18:0, C20:0 and C22:0) which catabolism is preferred (Tocher, 2003).

Polar fraction (PL+GL) as membrane constituents have similar SFA profile, which is homeostatically regulated independently of dietary intake (Abbot et al., 2012). In addition, membrane physical properties such as fluidity are influenced by FA chain length, as short chain FAs reduced the possibility for membrane adaptations at lower temperature compared to long chain SFAs (Carta et al., 2017).

Table 3. Lipid classes, fatty acids profile (FA, % of total FA) of lipid fractions, FA ratios and Nutrition quality indices of common carp edible fish tissue (mean±SD)

	TL	NL	PL	GL
% of TL		69.90±2.80	9.85±0.85	19.95±1.75
g.100-1g w. w.	3.60±0.15	2.52±0.12	0.36±0.06	0.72±0.04
Fatty Acid	TL	NL	PL	GL
C 10:0	0.01±0.01	0.04±0.01	0.02±0.01	0.02±0.01
C 12:0	0.02±0.01	0.02±0.01	0.10±0.02	0.30±0.02
C 14:0	0.40±0.03	0.50±0.04	0.95±0.05	1.05±0.24
C 15:0	0.05±0.01	0.10±0.02	0.01±0.01	Nd
C 16:0	16.23±1.05	23.20±2.15	17.51±1.55[a]	19.21±1.67
C 17:0	0.07±0.01	0.07±0.01	0.05±0.01	0.04±0.01
C 18:0	4.85±0.27	7.42±0.30	7.78±0.80	5.91±0.40
C 20:0	0.07±0.01	0.07±0.01	0.21±0.02	0.12±0.02
C 21:0	0.02±0.01	0.02±0.01	Nd	0.02±0.01
C 22:0	0.14±0.02	0.51±0.03	0.65±0.03	0.20±0.03
C 23:0	0.04±0.01	0.01±0.01	Nd	Nd
C 24:0	0.13±0.01	0.60±0.03	0.38±0.01	0.22±0.01
SFA	22.02	32.59[a]	27.71	27.07
C 14:1n-5	0.05±0.01	0.07±0.01	0.45±0.05	0.06±0.01
C 16:1n-7	5.18±0.40	6.62±0.50	4.51±0.20[a]	4.76±0.32
C 18:1n-9	28.72±2.60	38.08±2.55	8.67±0.60[a]	16.68±1.24
C 17:1	Nd	Nd	Nd	Nd
C 20:1n-9	6.52±0.55	1.10±0.08	1.37±0.08	2.39±0.45
C 22:1n-9	0.01±0.01	0.11±0.02	0.20±0.03	0.21±0.03
C 24:1n-9	0.08±0.02	0.06±0.01	0.25±0.02	0.27±0.01
MUFA	40.55	45.98[a]	15.44[a]	24.39
C 18:3 n-6	0.15±0.04	0.13±0.01	Nd	0.14±0.02
C 18:2 n-6	9.56±0.50	9.59±0.35	2.82±0.30[a]	8.57±0.85
C 18:3 n-3	3.15±0.20	3.62±0.14	0.21±0.03	1.25±0.14
C 20:3 n-3	0.16±0.02	0.10±0.01	0.08±0.01	0.05±0.01
C 20:3 n-6	4.32±0.15	0.85±0.05	1.73±0.25	1.20±0.16
C 20:2 n-6	1.69±0.21	0.82±0.07	0.65±0.03	0.66±0.02
C 20:4 n-6	0.80±0.05	1.50±0.10	2.56±0.53	1.67±0.19
C 20:5 n-3	8.78±0.85	2.77±0.27	23.78±2.56[a]	17.56±0.85[a]
C 22:6 n-3	6.84±0.43	1.12±0.15	20.61±1.84[a]	13.88±0.47[a]
C 22:2 n-9	0.40±0.03	0.26±0.02	0.95±0.10	1.00±0.08
C 22:5 n-3	1.58±0.04	3.15±0.34	3.19±0.40	2.56±0.15
PUFA	37.43	21.43[a]	56.58[a]	48.54
FA ratios, omega-3 PUFA concentrations and Nutrition quality indices				
Σ n-3	20.51	10.79	47.87	35.30
Σ n-6	16.52	11.39	7.76	12.24
n-6/n-3	0.82	1.06	0.16	0.35
PUFA/SFA	1.70	0.65	2.04	1.80
EPA (g.100^{-1}gww)	0.290	0.072	0.086	0.111
DHA(g.100^{-1}gww)	0.226	0.040	0.080	0.100

AI	0.25	0.37	0.30	0.33
TI	0.23	0.50	0.16	0.16
h/H	3.98	2.51	3.53	3.20
CSI	1.68	1.71	0.98	1.08

*AI= [(C12:0+ (4×C14:0) +C16:0)]/ (n6PUFA +n3PUFA+ MUFA); TI =(C14:0+C16:0+C18:0) /[(0.5MUFA) + (0.5n6PUFA) + (3n3PUFA) + (n3PUFA/n6PUFA)]; h/H= (C18:1n9+C18:2n6 +C18:3n3+C20:4n6+C20:5n3+ C22:6n3)/ (C14:0) +C16:0); CSI= (1.01x SFAg.100^{-1}g w. w.) + (0.05 x cholesterol mg.100^{-1}g w. w.); [a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$

Due to this fact, in our study we found similarly higher levels of C 16:0 and C18:0 in PL and GL fractions of both winter harvested (October 2017) species. Low amounts of hypercholesterolaemic SFAs such as C12:0 and C14:0 (under 1.35% of TL) were determined in both species and in all lipid fractions. Based on these findings, we can classify carps muscle lipids as beneficial and appropriate for regular consumptions.

Within the MUFA group, large differences were found for oleic (C18:1n9, OA) and palmitoleic (C16:1n7) acid levels between lipid classes in the analyzed carp species, but in all cases C18:1n9> C16:1n7. The highest levels of OA were found for NL fractions followed by TL of both species, but common carp NLs and TLs contain significantly high OA (38% of total NL) compared to bighead carp (20.79%). Generally, OA is one of the major MUFAs present in neutral lipids of both animal and vegetal origin (FAO, 2010). In contrast, polar lipids incorporate significantly lower OA, especially in PL fractions (under 9%), which leads to lower total MUFA levels in these fractions in both carp species. Similar results for high OA levels in triacylglycerol (TAG) fractions (up to 30% of TAG) compared to PL were reported for farmed common carp from Czech Republic (Mraz and Pickova, 2009) and for freshwater catla (*C. catla*) and mrigal (*C. mrigala*) from India (Prabhakara Rao et al., 2013).

It is known that freshwater fish contains lower PUFA levels compared to marine fish, especially long chain FAs such as EPA and DHA omega-3 (n-3). Variations in the PUFA profile of freshwater and seawater fish are influenced by their diet (omnivorous or herbivorous species) and the habitat of the species (Steffens and Wirth, 2005). The observed results illustrate well the diet and habitat related differences between the analyzed bighead and common carps. PUFA levels were significantly higher ($p<0.001$) in bighead carp TL compared to common carp TLs and the lowest in common carp NL fraction. Opposite trends were reported for polar fractions (PL+GL), where PUFAs have the highest amounts ranging between 48.54% (common carp GL) and 59.91% (bighead carp PL). Specific differences were found among individual PUFAs, such as $C_{18} < C_{20-22}$ PUFAs in all lipid fractions. Distributions of essential C_{18} PUFAs such as linoleic acid (C18:2n6, LA) and alpha-linolenic acid (C18:3n3, ALA) are strongly correlated with quantities of their long chain metabolites ARA, EPA and DHA. Bighead carp feed mainly on phytoplankton, macrophytes and zooplankton which are rich in long chain n-3 PUFAs (LC n-3PUFAs) and consequently DHA prevailed in all lipid fractions (DHA>EPA). In contrast, common carp as omnivorous species contained significantly lower LC n-3PUFAs in TL and NL fraction, as the amount of EPA was higher than DHA in all lipid fractions (EPA>DHA). Freshwater fish are able to convert C_{18} PUFAs to long chain C_{20-22} PUFAs, which could be one possible reason for the highest levels of these n-3 PUFAs. The following tendency was found in this study: higher C_{18} PUFAs – lower C_{20-22} PUFAs in TL and NL fractions in both carps. An opposite trend was observed for polar fractions, especially in PL: lower C_{18} PUFAs – seven to ten times higher C_{20-22} PUFAs levels. Membrane permeability is positively influenced by high unsaturated FAs such as EPA, ARA and DHA. Common carp contained significantly lower EPA, DHA and ARA amounts and two possible reasons for this are low activity of specific enzymes (desaturases and elongases) or deficiencies of some essential elements such as Zn, Mn and Ca (Steffens, 2006; Buchtova et al., 2008). Generally, both species contained the lowest levels of n-3PUFAs in their neutral lipids and higher levels in polar lipids, especially in PL: from two (bighead carp) to four times (common carp). The total n-3 and n-6 PUFA values are often reported when assessing the health benefits of fish lipids. The levels of n-3PUFAs were higher compared to n-6 PUFAs in both species and in all lipid fractions.

Two important FA ratios: PUFA/SFA and n-3/n-6 are mostly used to assess the nutritional quality of lipid fractions. According to several nutritional recommendations (HMSO, 2001; FAO, 2010), the PUFA/SFA ratio in human diets should be above 0.45 and the n-6/n-3 ratio should not exceed 4.0. The obtained result (Tables 2 and 3) for n-6/n-3 ranged between 0.13 (bighead carp PL) and 1.06 (common carp NL); for PUFA/SFA: from 0.65 (common carp NL) to 2.26 (bighead carp GL) which illustrates well the high lipid quality of the analysed carp species in agreement with recommendations. To assess accurately the functional properties of lipid fractions, the absolute amounts of n-3 LCPUFA (EPA and DHA) were reported (in g.100^{-1} g w.w.). Based on The European Food Safety Authority (EFSA, 2012) recommendation for 0.500g EPA+DHA (as daily intake), the edible portion of 150g fish filets can supply between 140% (bighead carp TLs) and 155% (common carp TLs) of the recommended daily intake (RDI). The type of lipids (FA, respectively) consumed may promote the development of CVD. Two basic processes: atherosclerosis and thrombosis, provoke heart diseases, but some FAs (such as SFAs) have greater role in these negative health processes. In attempt to assess the effect of different FAs, as major constituents of the analysed carp lipids, the AI, TI and h/H indices were determined (Table 2 and 3). Levels higher than 1.0 (for AI and TI) are unfavourable to human health, whereas higher h/H levels (>1.0) are being recommended. AI and TI were calculated as the highest in NL and the lowest in PL; h/H was the highest in polar lipids and the lowest in NL compared to other fractions. In additions, CSI index, showed similar trends: highest levels in polar fractions – lowest levels in NL fractions. Based on these results we can summarise that bighead carp lipids, especially polar fractions have very good hypo-cholesterolemic (high h/H values), anti-atherogenic (low levels of AI), and anti-thrombogenic (low levels of TI) potential, compared to common carp lipids. Polar lipid fractions (GL and PL) of the studied carp species are appropriate for pharmaceutical uses and can be preferred for a well-balanced and healthier diet.

Fat soluble vitamins, carotenoids and cholesterol

Fat soluble vitamins, cholesterol and carotenoids contents are presented in Table 4. Vitamin A, D_3, astaxanthin and beta-carotene are expressed as microgram per 100g wet weight (µg.100^{-1}g w.w.), vitamin E and cholesterol are expressed as milligram per 100g wet weight (mg·100 g^{-1} w.w.)

Table 4. Fat soluble vitamins, carotenoids and cholesterol contents (mean±SD)

Fat soluble nutrients	Bighead carp (n=6)	Common carp (n=6)
Beta-carotene (µg.100-1g w.w.)	180.00±5.50	188.00±6.54[c]
Astaxanthin (µg.100-1g w.w.)	16.00±0.20	Nd
Vitamin A (µg.100-1g w.w.)	44.50±1.50	41.25±0.50[b]
Vitamin E (mg.100 g-1 w.w.)	10.40±0.50	5.52±0.50[a]
Vitamin D3 (µg.100-1g w.w.)	18.00±0.25	17.30±0.30[c]
Cholesterol (mg.100 g-1 w.w.)	17.60±0.23	24.47±0.45[a]

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$

Among the important components, the ones with high antioxidant activity are vit.E, astaxanthin and beta-carotene. Carotenoids are vital components, which affect normal growth, metabolism and reproductivity of fish. Cyprinidae species, as well as carps can synthesize astaxanthin from beta-carotene by oxidative metabolic pathway and accumulate this component in their tissues. An earlier study found that fans carp can convert xenophiles as astaxanthin into retinoid (vit.A) through reductive metabolic pathway (Yuangsoi et al., 2010). Based on the observed results we can assume that high levels of beta-carotene are related to low metabolic activities, influenced by low water temperature and preparation and adaptation of fish organism for over-wintering (all samples were from October 2017).

In both species fat soluble vitamins followed the distributions: vit.A> vit.D_3> vit.E. Significant differences between species were observed mainly for vit. E. Common carp contained two times lower vit. E levels due to differences of feeding regime or feed type, compared to herbivorous bighead carp. Vitamin D_3 content was similar in both species and presented three times higher amounts than the recommended daily intake of this vitamin in Bulgaria (Regulation No. 1/22.01.2018). Thus, the analysed bighead and common carp species can be classified as excellent sources of vit.D_3, which increase their nutritional value. In addition, a correlation for bighead carp lipids was observed: higher n-3LCPUFA – higher vit.E content, compared to common carp (lower n-3LCPUFA – lower amount of vit.E). An earlier investigation of farmed common carp from Bulgaria reported a similar distribution of fat-soluble vitamins (vit.E> vit.A> vit.D_3) in muscle TL, but significantly lower content of all three vitamins: from two times (vit. D_3) to six times (vit.E) (Dobreva et al., 2014).

Significantly higher levels of cholesterol were found in common carp TLs ($P<0.001$) compared to bighead carp (Table 4). Presently, assessment of food quality and nutrition conception in the developed countries, based on cholesterol content, recommends approx. 140mg /day as moderate and safe levels for the human health (Stanek et al., 2011). Additionally, the dietary cholesterol can affect blood LDL-cholesterol levels, and patients who are undergoing secondary CVD prevention are not recommended to consume >180mg/day of cholesterol (Scherr et al., 2015). Based on the presented results, we can classify both carp species as beneficial and suitable for healthy diet. Ljubojevic et al., (2013) reported different results for cholesterol content of farmed bighead carp (65.29mg.100^{-1}g w.w.) and common carp (56.38 mg.100^{-1}g w.w.) from Novi Sad, Republic of Serbia. Bighead carp lipids contain higher levels of bioactive compound with antioxidant properties (astaxanthin, beta-carotene, vitamins A and E) compared to common carp. Nevertheless, both carp species contained high quality lipids, rich in compounds beneficial for the human health.

Heavy metal level in the analysed fish species

The concentration levels of the studied heavy metals (As, Cd, Hg, Ni and Pb) detected in crap samples are illustrated in Table 5. Arsenic levels in all of the samples were up to 0.132 mg.kg^{-1} w.w. Compared with the literature data, As pollution in the collected samples was lower than that found by Hosseini et al. (2015) in the south central shoreline of the Caspian Sea, Iran and almost within the heavy metal range for *Rutilus frisii kutum* in Noor and Babolsar coast, Iran (Dadar et al., 2016). The As level in common crap from Topolnitsa reservoir, Bulgaria did not vary significantly depending on the season in which the fish were caught - from 60 µg/kg up to 70 µg/kg (Yancheva et al., 2014). According to Bulgarian standard (Regulation No. 31/29.07.2004), the maximum permissible level set for As in fish is 1mg/kg which indicated that results from this study are within the permissible limits.

The cadmium levels found in this study were between 0.014 mg.kg^{-1} w.w and 0.039 mg.kg^{-1}. Cd levels in the literature varied between 0.005 mg/kg w.w in carp and pikeperch and 0.01 mg/kg w.w in catfish from Serbian part of Danube River (Subotić et al., 2013); between 0.003 mg.kg^{-1} w.w and 0.005 mg.kg^{-1} w.w in fish muscle in the rivers of Lithuania (Virbickas et al., 2006); and around 0.03 mg.kg^{-1} w.w for common carp from two ponds in Slovak Republic (Andrej et al., 2006). In a previous study about four freshwater fish species from Mandra Lake Cd levels were found between 0.020 mg.kg^{-1} w.w in freshwater beam up to 0.046 mg.kg^{-1} w.w in common roach (Peycheva et al., 2017). The limit of 0.05 mg.kg^{-1} wet weight for fish species set by the Codex Alimentarius (Anonymous, 2004) was not exceeded.

Table 5. Mean concentration and standard deviations of metals in carp species (mg.kg^{-1} wet weight)

Carp species and standards	As	Cd	Hg	Ni	Pb
Bighead carp	0.019±0.046	0.014±0.001	0.236±0.011	0.023±0.003	0.094±0.017
Common carp	0.312±0.013[a]	0.039±0.009[b]	0.321±0.021[b]	0.067±0.006[a]	0.169±0.020[a]
EC (2001)	6.0	0.50	0.5-1	40.0	1.0
WHO (2000)	1.0	0.50	0.50	30.0	1.0
BFC (Regulation No 31, 2004)	5.0	0.05	0.50	0.5	0.2

[a] $p<0.001$, [b] $p<0.01$

A higher level of Hg in fish is known to induce health risks to fish as well as to fish consumers (Sweet and Zelikoff, 2001; Moallem et al., 2010). Levels more than 0.5 mg.kg^{-1} of Hg in fish are related to certain effects in fish, such as loss of appetite, reduced coordination, etc. (Bernhoft, 2012). In the present work, the maximum Hg level in the collected samples was lower than 0.321 mg/kg w.w. In a study conducted on wild and farmed carp (*Cyprinus carpio*) and Caspian kutum (*Rutilus frisii kutum*) in Iran the results were between 0.003 mg.kg^{-1} and 0.065 mg.g^{-1} indicating that Hg perhaps does not pose a threat to the fish themselves (Heshmati et al., 2017). According to European Commission Regulation (EC) No. 1881/2006 the maximum level set for Hg is 0.50mg/kg w.w., which is less than our values.

The level of Ni concentrations in all of the collected samples was between 0.023 mg.kg^{-1} and 0.067 mg.kg^{-1}, which is less than the value allowed by the joint FAO/WHO/Expert Committee on Food Additives (ECFA) (JECFA, 1993), EC (2001), Bulgarian Food Codex (Anonymous, 2004) and Stoyanova et al. (2016). In comparison with other studies, Ni levels were lower than those reported in fish from the Tajan River, Iran (Eslami et al., 2011), and from Sakarya, Turkey (Küpeli et al., 2014).

The range of Pb levels among the collected samples was 0.094-0.169 mg.kg^{-1}, which is less than the limit allowed by the joint FAO/WHO/ECFA (JECFA, 1993). The Pb concentration in the study of samples from Mandra Lake varies between 0.15 mg.kg^{-1}w.w. for freshwater beam up to 0.27 mg.kg^{-1} w.w. for carassius (Peycheva et al., 2017). In a study performed in Věstonice reservoir in Czech Republic the highest lead concentrations were found in tissues of asp (0.12 mg.kg^{-1} w.w.) and carp (0.09 mg.kg^{-1} w.w.) while the lowest lead concentrations were found in pikeperch tissues (0.01 mg/kg w.w.) and in pike gonads (0.03 mg.kg^{-1} w.w.) (Kenšová et al., 2010). The mean lead concentration measured in rainbow trout in Western Anatolia, Turkey is 0.08 mg.kg^{-1} w.w., which is less than the values found in this study (Yabanl et al., 2014). The EC (2006) set a maximum level for Pb in muscle meat of fish as 0.30 mg/kg w.w. or the values are below that level.

Health risk assessment

Fish is one of the most important food sources and thus, intake of trace elements from capture fish is of great concern for human health (Krishna et al., 2014). To evaluate the health risk to people in Bulgaria, the THQ of heavy metal was calculated based on the concentrations of metal uptake in fish muscle and daily consumption of fish. The estimated hazard index (HI), target hazard quotient (THQ) and target risk (TR) of the observed heavy metals through consumption of fish separately for males and females was given in Table 6.

In this study, THQs values for As, Cd, Hg, Ni and Pb by consumption of common carp and bighead carp from Plovdiv region were determined well below 1 no matter if we consider males or females. Therefore, it was concluded that *Cyprinus carpio* and *Aristichthys nobilis* samples pose no health risk for an average consumer. Otherwise a potential health risk should not be free for regular or excessive consumers, even if the food is considered safe for an average consumer. Olmedo et al. (2013) found out that the seafood products from the central market of Granada are safe for an average consumer but underlined that a potential risk should be considered for regular or excessive consumers.

Storelli (2008) reported HI of albacore (*T. thynnus*) from the Adriatic Sea as 2.08, and concluded a potential human health risk due to the consumption of albacore. Still, in our study, HI value was between 0.150 and 0.443 showing that consumers would not chance significant health risks from the intake of heavy metals through consumption of *Cyprinus carp*io and *Aristichthys nobilis*. It is noticeable from these results that the HI values are higher for the females rather than males. Mol et al. (2017) found that HI in *Thunnus thynnus* from the northern Levantine Sea was only 0.03 and concluded it is no potential health risk for Turkish adults.

The calculated average value of carcinogenic target risk (TR) of common and bighead crap was performed for Pb, As, and Ni, since only those elements from the analyzed ones show carcinogenicity (Table 6). The values calculated in this study are compared to those in literature (Bhupander et al., 2011; Strivastava et al., 2014; Peycheva et al., 2016) and the guidelines values (10^{-6}-10^{-4}), indicating that the analyzed fish species from the Plovdiv region are safe for human consumption.

Conclusion

Two carp species – common carp and bighead carp, farmed in the Plovdiv region, were studied in order to assess the quality of fish species in details and to evaluate their safety based on the content of five toxic elements. The studied carp species contained appreciable amounts of proteins and low lipid content. In both species, the main lipid class was NL, the lowest – PL. The observed differences in FA profile of each lipid fraction showed that bighead carp contained more than 33% PUFAs of total FAs, whereas common carp showed significantly lower PUFAs, especially in NL fractions. It was found

Table 6. Risk values (THQ, HI and TR) of each metal contaminant in muscle of two freshwater carp species

	Target Hazard Quotient (THQ)					Hazard	Target Risk (TR)		
	As	Cd	Hg	Ni	Pb	Index (Hi)	As	Ni	Pb
Females									
Common carp	0.013	0.002	0.162	0.0002	0.005	0.182	7.15×10^{-6}	8.10×10^{-6}	2.15×10^{-5}
Bighead carp	0.210	0.008	0.216	0.0007	0.009	0.443	1.12×10^{-4}	7.44×10^{-5}	4.03×10^{-5}
Males									
Common carp	0.011	0.002	0.133	0.0002	0.004	0.150	5.73×10^{-6}	6.49×10^{-6}	1.21×10^{-8}
Bighead carp	0.173	0.006	0.177	0.0006	0.007	0.364	8.94×10^{-5}	1.01×10^{-4}	2.27×10^{-8}

that the examined fish were good sources of EPA+DHA n-3 PUFAs bound to polar lipids (0.235-0.377 g.100^{-1}g ww) which increased their lipid quality. High beta-carotene and vitamin D_3 amounts and low cholesterol content found in this research showed the great potential of these carp species to supply our daily nutrient requirements when included in a proper healthy diet. Moreover, the concentrations of the five toxic elements (As, Pb, Ni, Cd and Hg) did not exceed the maximum limits, according to world food standards for human health safety. The presented data were used for calculation of several indices to assess the quality of carp species tissue. Nutrition quality indices AI and TI were below 1.0, while h/H was up to 3.98, which confirmed the good cardio protective (antiatherogenic, antithrombotic and hypocholesterolaemic) properties of carp lipids. The values for THQs and HI were below 1.0, and TR were below 10^{-4} showing that adverse health effects might not occur when considering different carp consumption patterns, which pose no potential health risk to consumers. In conclusion, we can summarize that the high nutritional quality, high levels of bioactive compound with antioxidant properties, low values of SFA, cholesterol and toxic elements of both common carp and bighead carp make them advisable for well-balanced and healthy diet and suitable for pharmaceutical utilization. Further investigations are needed in order to evaluate the changes due to annual and reproductive cycle. In addition, it is noteworthy to focus on the functional properties of the carp species tissue.

References

Abbott SK, Else PL, Atkins TA and Hulbert AJ, 2012**.** Fatty acid composition of membrane bilayers: importance of diet polyunsaturated fat balance. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1818, 1309-1317.

Andrej J, Stranai I, Kacaniova M, Massanyi P and Valent M, 2006. Toxic/hazardous substances and environmental engineering. Journal of Environmental Science and Health, Part A, 41**,** 1071.

AOAC, 1991. Moisture in meat and meat products. Air Drying Method, Official methods of analysis: Association of Official Analytical Chemists, 16th Edition, Washington D.C., USA.

Berger A, 2014. Polar lipids-phospholipids and glycolipids - an enhanced omega-3 structure. Almega PL™ Qualitas Health, 1-11.

Bernhoft RA, 2012. Mercury toxicity and treatment: a review of the literature. International Journal of Environmental Research Public Health, 1-10.

Bhupander K and Mukherjee DP, 2011. Assessment of human health risk for arsenic, copper, nickel, mercury and zinc in fish collected from tropical wetlands in India. Advances in Life Science and Technology, 2, 13-24.

Bligh E and Dyer WJ, 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physics, 37, 913-917.

Bogdanovic T, Ujevic I, Sedak M, Listes E, Simat V, Petricevic S and Poljak V, 2014. As, Cd, Hg and Pb in four edible shellfish species from breeding and harvesting areas along the eastern Adriatic Coast. Croatian Food Chemistry, 146, 197-203.

Böhm M, Schultz S, Koussoroplis AM and Kainz MJ, 2014. Tissue-specific fatty acids response to different diets in common carp (*Cyprinus carpio* L.). PloS one, 9, 947-59.

Bulgarian State Standard (BSS) 9374:1982. Meat and meat quality. Determination of crude protein in meat. 2-nd ed. Bulgarian Institute of Standardization, Sofia, Bulgaria (Bg).

BSS EN ISO 12966-2:2017. Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters. Part 2: Preparation of methyl esters of fatty acids, p. 37 (Bg).

BSS EN ISO 12966-4:2015**.** Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters. Part 4: Determination by capillary gas chromatography, p. 30 (Bg).

Buchtova H, Svobodova Z, Kocour M and Velíšek J, 2008**.** Chemical composition of edible parts of three-year-old experimental scaly crossbreds of common carp (*Cyprinus carpio*, L., 1758). Acta alimentaria, 37, 311-322.

Carta G, Murru E, Banni S and Manca C, 2017. Palmitic acid: Physiological role, metabolism and nutritional implications. Frontiers in physiology, 8, 902.

Chapman PM, Allen HE, Godteredsen KZ and Graggen MN, 1996. Evolution of bioaccumulation factors in regulating metals. Environment Sciences Technology, 30, 448.

Ćirković M, Ljubojević D, Đorđević V, Novakov N and Petronijević R, 2012. Chemical composition of body including fatty acids of four cyprinids fish species cultured at the same conditions. Archiva Zootechnica, 15, 37-50

Commission Regulation (EC) No. 116/2010 of 9 February 2010 amending Regulation (EC) No. 1924/2006 of the European Parliament and of the Council with regard to the list of nutrition claims.

Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (displayed 10 February 2014)].

Connor SL, Gustafson JR, Artaud-Wild SM, Favell DP and Classick-Kohn CJ, 1986. The cholesterol/saturated fat index an indication of the hypercholesterolemic and atherogenic potential of food. Lancet, 327, 1229-1232

Dadar M, Adel M, Ferrante M, Nasrollahzadeh Saravi H, Copat C and Oliveri Conti G, 2016. Potential risk assessment of trace metals accumulation in food, water and edible tissue of rainbow trout (*Oncorhynchus mykiss*) farmed in Haraz River, northern Iran. Toxin Review, 35, 141-146.

de Oliveira Sales R and Maia EL, 2012. Chemical composition and lipids classes of the freshwater fish tilapia do Nilo, *Oreochomis niloticus*. Revista Brasileira de Higiene e Sanidade Animal, 6, 17-30.

Dey S, Misra K and Homechoudhuri S, 2015. Analysis of major lipid classes and their fatty acid composition of an Indian minor carp *Puntius sophore* in order to evaluate its nutritional aspects. International Journal of Advances Research in Biological Sciences, 2, 100-119.

Dobreva DA, Merdzhanova A, Makedonski L and Stancheva M, 2014. Seasonal changes in fatty acid composition and fat soluble vitamins content of grass carp and common carp. Agricultural Science and Technology, 6, 271-277.

Dobreva DA, Panayotova V, Stancheva R and Stancheva M, 2017. Simultaneous HPLC determination of fat soluble vitamins, carotenoids and cholesterol in seaweed and muscle tissue. Bulgarian Chemical Communications, 49, G, 112-117.

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2012. Scientific opinion on the tolerable upper intake level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). EFSA Journal, 10, 2815-2863.

Eslami S, Hajizadeh Moghaddam A, Jafari N, Nabavi SF, Nabavi SM and Ebrahimzadeh MA, 2011. Trace element level in different tissues of Rutilus frisii kutum collected from Tajan River. Iran. Biology Trace Elements Research, 143, 965-973.

European Commission (EC), 2001. Council Directive 91/493/EEC of 22 July 1991 laying down the health conditions for the production and the placing on the market of fishery products. O.L. L268, 0015-0034 (24/09/1991).
FAO, 2000. Compilation of legal limits for hazardous substances in fish and fishery products. FAO Fishery Circular No. 464, Rome, pp. 5-10.
FAO, 2010. Nutrition elements of fish http://www.fao.org/fishery/topic/12319/en on 26/8/2010
FAO/INFOODS Guidelines for converting units, denominators and expressions, 2012. Version 1.0., FAO, Rome.
FDA, 2001. Fish and fisheries products hazards and controls guidance, 3rd ed., Center for Food Safety and Applied Nutrition, US Food and Drug Administration, USA.
Fidan AF, Cigerci H, Konuk M, Kucukkurt I, Aslan R and Dundar Y, 2008. Determination of some heavy metal levels and oxidative status in *Carassius carassius* L., 1758 from Eber Lake. Environmental Monitoring and Assessment, 147, 35-42.
Godavarthy P and Sunila Kumari Y, 2014. Glycolipids as potential energy molecules during starvation in climbing perch, *Anabas testudineus* (Bloch). International Journal of Zoology, 2,1-5.
Guler GO, Kiztanir B, Aktumsek A, Citil OB and Ozparlak H, 2008. Determination of the seasonal changes on total fatty acid composition and ω3/ω6 ratios of carp (*Cyprinus carpio* L.) muscle lipids in Beysehir Lake (Turkey). Food Chemistry, 108, 689-694.
Heshmati A, Karami-Momtaz J , Nili-Ahmadabadi A and Ghadimi S, 2017. Dietary exposure to toxic and essential trace elements by consumption of wild and farmed carp (*Cyprinus carpio*) and Caspian kutum (*Rutilus frisii kutum*) in Iran. Chemosphere, 173, 207-215.
HMSO, 2001. Nutritional aspects of cardiovascular disease: Report on health and social subjects. Department of Health, London, UK.
Hong H, Fan H, Wang H, Lu H, Luo Y and Shen H, 2015. Seasonal variations of fatty acid profile in different tissues of farmed bighead carp (*Aristichthys nobilis*). Journal of Food Science and Technology, 52, 903-911.
Hosseini, SM, Karaminasab M, Batebi-Navaei M, Aflaki F, Monsefrad F, Regenstein JM and Vajdi R, 2015. Assessment of the essential elements and heavy metals content of the muscle of Kutum (*Rutilus frisii kutum*) from the south Caspian Sea and potential risk assessment. Iranian Journal Fishery Sciences 14, 660-671.
Jacobs S, Sioen I, De Henauw S, Tous N, Maulvault AL, Fait G, Pons FC and Verbeke W, 2014. Risk–benefit perception and consumption of seafood in European consumers. Archives of Public Health, 72, 8.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1993. Evaluation of certain food additives and contaminants: 41st Report of the Joint FAO/WHO Expert Committee on Food Additives. WHO, Geneva. Technical Reports Series No. 837.
Jovíc M, Stankovíc A, Slavkovíc-Beskoski L, Tomíc I, Degetto S and Stankovic S, 2011**.** Mussels as a bio-indicator of the environmental quality of the coastal water of the Boka Kotorska Bay (Montenegro). Journal of Serbian Chemical Societe, 76, 933-946
Kenšová R, Čelechovská O, Doubravová J and Svobodová Z, 2010. Concentrations of metals in tissues of fish from the Věstonice reservoir. Acta Veterinaria Brno, 79, 335-345.
Krishna PV, Jyothirmayi V and Madhusudhana Rao, 2014. Human health risk assessment of heavy metal accumulation through fish consumption, from Machilipatnam Coast, Andhra Pradesh, India. International Research Journal of Public and Environmental Health, 1, 121-125.
Küpeli T, Altunda H and Imamoglu M, 2014. Assessment of trace element levels in muscle tissues of fish species collected from a river, stream, lake, and sea in Sakarya. The Scientific World Journal, 496107-496107.
Ljubojevic D, Trbovic D, Lujic J, Bjelic-Cabrilo O, Kostic D, Novakov N and Cirkovic M, 2013. Fatty acid composition of fishes from inland waters. Bulgarian Journal of Agriculture Sciences, 19, 62-71.
MNSPA (Multiannual National Strategic Plan for Aquaculture in Bulgaria), 2014-2020. Available on:
https://ec.europa.eu/fisheries/cfp/aquaculture/multiannual-national-plans_en (Bg).
Moallem S, Karimi G, Khayyat MH, Bozorgi M, Nili-Ahmadabadi A and Nazari F, 2010. Exposure assessment for mercury from consumption of marine fish in Iran. Toxicological & Environmental Chemistry, 92, 1213-1218.
Mol S, Karakulak FS and Ulusoy S, 2018. Potential health risks due to heavy metal uptake via consumption of *Thunnus thynnus* from the northern Levantine Sea. Toxin Reviews, 37, 56-61.
Mráz J and Pickova J, 2009. Differences between lipid content and composition of different parts of fillets from crossbred farmed carp (*Cyprinus carpio*). Fish physiology and biochemistry, 35, 615.
Mráz J, Máchová J, Kozák P and Pickova J, 2012. Lipid content and composition in common carp–optimization of n-3 fatty acids in different pond production systems. Journal of Applied Ichthyology, 28, 238-244**.**
Muñoz-Olivas R and Cámara C, 2001. Speciation related to human health. Trace element speciation for environment, food and health, 331-353.
Olmedo P, Hernandez AF and Pla A, 2013. Determination of essential elements (Cu, Mn, Se and Zn) in fish and shellfish samples. Risk and nutritional assessment and mercuryselenium balance. Food Chemistry Toxicology, 62, 299–307.
Özyurt G, Polat A, Loker, GB, 2009. Vitamin and mineral content of pike perch (*Sander lucioperca*), common carp (*Cyprinus carpio*), and European catfish (*Silurus glanis*). Turkish Journal of Veterinary and Animal Sciences, 33, 351-356.
Petursdottir H, Gislason A, Falk-Petersen S, 2008. Lipid classes and fatty acid compositions of muscle, liver and skull oil in deep-sea redfish Sebastes mentella over the Reykjanes Ridge. Journal of Fish Biology, 73, 2485-2496.
Peycheva K, Makedonski L and Stancheva M, 2017**.** Human exposure to some toxic and essential elements through freshwater fish consumption in Bulgaria. Bulgarian Chemical Communications, 49, G, 37-42.
Peycheva K, Panayotova V and Stancheva M, 2016. Assessment of human health risk for copper, arsenic, zinc, nickel, and mercury in marine fish species collected from Bulgarian black sea coast. International Journal of Fisheries and Aquatic Studies, 4, 41-46.
Prabhakara Rao, Balaswamy PG, Narsing Rao K, Jyothirmayi and GT, 2013. Lipid classes, fatty acid and phospholipid composition of roe lipids from *Catla catla* and *Circinus mrigala*. International Food Research Journal, 20, 275-279
Regulation No. 1 /22.01.2018 for the physiological norms of nutrition of the population. Ministry of Health, Bulgaria (Bg).
Regulation No. 31 /29.07.2004 on maximum levels of pollutants in food staffs. Ministry of Health, Bulgaria State Gazette No 88/ 08.10.2004 (Bg).
Santos-Silva J, Bessa RJB and Santos-Silva F, 2002. Effect of genotype, feeding system and slaughter weight on the quality of light

lambs. II. Fatty acid composition of meat. Livestock Production Science, 77, 187-194

Scherr C, Gagliardi ACM, Miname MH and Santos RD, 2015. Fatty acid and cholesterol concentrations in usually consumed fish in Brazil. Arquivos brasileiros de cardiologia, 104, 152-158.

Simopoulos A, 2013. Dietary omega-3 fatty acid deficiency and high fructose intake in the development of metabolic syndrome, brain metabolic abnormalities, and non-alcoholic fatty liver disease. Nutrients, 5, 2901-2923.

Srivastava SC, Verma P, Verma AK and Singh AK, 2014. Assessment for possible metal contamination and human health risk of *Pangasianodon hypophthalmus* (Sauvage, 1878) farming, India. International Journal of Fisheries and Aquatic Studies, 1, 176-181.

Stanek M, Borejszo Z, Dąbrowski J and Janicki B, 2011. Fat and cholesterol content and fatty acid profiles in edible tissues of spiny-cheek crayfish, *Orconectes limosus* (Raf.) from Lake Gopło (Poland). Archives of Polish Fisheries, 19, 241-248.

Steffens W and Wirth M, 2005. Freshwater fish - an important source of n-3 polyunsaturated fatty acids: a review. Archives of Polish Fisheries, 13, 5-12.

Steffens W, 2006. Freshwater fish-wholesome foodstuffs. Bulgarian Journal of Agricultural Science, 12, 320.

Stoyanova S, Sirakov I, Velichkova K and Staykov Y. 2016. Heavy metal content in the meat of common carp (*Cyprinus carpio* L.) and rainbow trout (*Oncorhynchus mykiss* W.), cultivated under different technologies. Agricultural Sciences and Technology, 8, 90-93.

Subotić S, Spasić S, Višnjić-Jeftić Ž, Hegediš A, Krpo-Ćetković J, Mićković B, Skorić S and Lenhardt M, 2013. Heavy metal and trace element bioaccumulation in target tissues of four edible fish species from the Danube River (Serbia). Ecotoxicology and environmental safety, 98,196-202.

Swapna HC, Rai AK, Bhaskar N and Sachindra NM, 2010. Lipid classes and fatty acid profile of selected Indian fresh water fishes. Journal of food science and technology, 47, 394-400.

Sweet LI and Zelikoff JT, 2001. Toxicology and immunotoxicology of mercury: comparative review in fish and humans. Journal Toxicology Environmental Health B Critical Review, 4, 161-205.

Tocher DR, 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Review Fishery Sciences, 11, 107−184.

Ulbricht T and Southgate D, 1991. Coronary heart disease: seven dietary factors. Lancet, 338, 985-992.

USEPA (US Environmental Protection Agency), 1989. Guidance manual for assessing human health risks from chemically contaminated, fish and shellfish, Washington DC (EPA-503/8-89-002).

USEPA, 2000. Guidance for assessing chemical contaminant data for use in fish advisories. 1: Fish Sampling and Analysis, 3rd ed., Washington, DC (EPA-823-B-00-007).

USEPA, 2004. Integrated Risk Information System (IRIS) on lead and compounds (inorganic). National Center for Environmental Assessment Washington, DC: Office of Research and Development. CASRN 7439-92- 1. Last revised 2004.

Virbickas T and Sakalauskienė G, 2006. Heavy metals in fish muscle in the rivers of Lithuania. Acta Zoologica Lituanica, 16, 271-278.

WHO (World Health Organization), 2000. Health criteria other supporting information. In: Guidelines for Drinking Water Quality, 2nd ed., Geneva, 2, pp. 31-388.

Yabanlı M, Yozukmaz A, Alparslan Y and Acar Ü, 2014. Evaluation of heavy metals and selenium contents in the muscle tissues of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) in Western Anatolia. Journal of Food, Agriculture & Environment, 12, 165-168.

Yancheva V, Stoyanova S, Velcheva I, Petrova S and Georgieva E, 2014. Metal bioaccumulation in common carp and rudd from the Topolnitsa reservoir, Bulgaria. Arhivies of Industrial Hygiene and Toxicology, 65, 57-66.

Yuangsoi B, Jintasataporn O, Tabthipwon P and Kamel C, 2010. Utilization of carotenoids in fancy carp (*Cyprinus carpio*): astaxanthin, lutein and carotene. World Applied Sciences Journal, 11, 590-598.

Zhang Y, Zhang B, LiL, Wang Z, Xie S, Shen Y and Li J, 2012. Radiation induced reduction: an effective and clean route to synthesize functionalized graphene. Journal of Materials Chemistry, 22, 7775-7781.

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Todorov N and Mitev J, 1995. Effect of level of feeding during dry period, and body condition score on reproductive performance in dairy cows,IXth International Conference on Production Diseases in Farm Animals, September 11–14, Berlin, Germany.

Thesis:

Hristova D, 2013. Investigation on genetic diversity in local sheep breeds using DNA markers. Thesis for PhD, Trakia University, Stara Zagora, Bulgaria, (Bg).

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