

QUALITY EVALUATION OF DIETARY LIPID OF CHANNEL CATFISH (*ICTALURUS PUNCTATUS*) FROM BULGARIA

A. MERDZHANOVA, D. DOBREVA and M. STANCHEVA
Medical University, Department of Chemistry, BG – 9002 Varna, Bulgaria

Abstract

MERDZHANOVA, A., D. DOBREVA and M. STANCHEVA, 2015. Quality evaluation of dietary lipid of channel catfish (*Ictalurus punctatus*) from Bulgaria. *Bulg. J. Agric. Sci.*, Supplement 1, 21: 202–207

Fish lipids are important components of diet due to their significance as energy, essential fatty acids and fat soluble vitamins sources. No data is available on fatty acid (FA) composition and fat soluble vitamins content of freshwater channel catfish from Bulgarian fish market. The objectives of the present work were to investigate the total lipid content, FA profile, lipid quality indices (atherogenic, thrombogenic), fat soluble vitamins (A, E and D₃) as well as relative daily intake of vitamins of Channel catfish (*Ictalurus punctatus*). The potential nutritional and medicinal value of FA composition and vitamins content to consumers were evaluated. The FA composition was analyzed by GC–MS. Fat soluble vitamins were analyzed simultaneously using RP–HPLC. The FA distribution of catfish is: SFA>MUFA>PUFA. The n3/n6 and PUFA/SFA ratios were greater than the recommended by FAO/WHO. A portion of 100 g contained 0.245 g of EPA+DHA n-3 PUFA. Catfish tissue presented significant amounts of vitamin E ($1374.5 \pm 158.1 \mu\text{g} \cdot 100 \text{ g}^{-1} \text{ ww}$), followed by vitamin A ($36.2 \pm 0.7 \mu\text{g} \cdot 100 \text{ g}^{-1} \text{ ww}$) and D₃ ($17.7 \pm 0.7 \mu\text{g} \cdot 100 \text{ g}^{-1} \text{ ww}$). This species is excellent source of fat soluble vitamins, especially for vitamin D₃ – one survey provides more than 300% of the RDI established in Bulgaria. This study provides specific nutritional information with respect to the consumption of channel catfish for nutrient balance as foodstuff. Since fish tissue is a valuable source of essential nutrients, a detailed analysis for evaluation the nutrient composition and content on fish lipids is needed.

Key words: channel catfish, fatty acids, lipid quality indices, vitamin E, vitamin A, vitamin D₃

Abbreviations: FA – fatty acid; GC–MS – gas chromatography with mass detection; RP–HPLC–reverse phase – high pressure liquid chromatography; SFA – Saturated fatty acids; MUFA – monounsaturated fatty acid; PUFA – polyunsaturated fatty acid; n-3 PUFA – omega-3 polyunsaturated fatty acid; n-6 PUFA – omega-6 polyunsaturated fatty acid; EPA – eicosapentaenoic fatty acid, n-3 PUFA; DHA – docosahexaenoic fatty acid, n-3 PUFA; RDI – recommended daily intake

Introduction

Fishes are regarded as important natural food sources of various beneficial components such as omega-3 (n-3) FA and fat soluble vitamins, which are necessary for a healthy diet. The nutritional benefits of fish consumption are mainly attributed to the effects of n-3 PUFAs (Kris-Etherton et al., 2003). Numerous studies have explored and supported the antiatherogenic, antithrombotic, and antiarrhythmic effects

of these PUFAs (Lee et al., 2006). PUFA can affect platelet function by interacting with membrane proteins, but their effect depends on the FA chain length and the degree of saturation. Individual saturated fatty acids (SFA) such as lauric (C12:0), myristic (C14:0) and palmitic (C16:0) increase LDL cholesterol and platelet aggregation (Kris-Etherton et al., 2003; Lee et al., 2006). Several studies have shown that freshwater fishes have a high capacity for transformation of C18 essential fatty acids (EFA) as 18:3 n-3 (alpha-linoleic

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

acid n-3, ALA) and 18:2 n-6 (linolenic acid n-6, LA) into 20:5 n-3 (eicosapentaenoic acid n-3, EPA), 22:6 n-3 (docosahexaenoic acid n-3, DHA) and 20:4 n-6 (arachidonic acid n-6, AA) and thus they could be a good source of these PU-FAs to a consumer (Tocher, 2003; Steffens, 2006).

Fat soluble vitamins control a variety of biologically important processes in the human body. All-trans retinol participates in photoreception, reproduction, bone growth, regulates gene expression etc. Cholecalciferol promotes and enhances the absorption of calcium and phosphorus. Alpha-tocopherol is an important antioxidant – protects membrane structures, essential fatty acids and vitamins A from oxidation (Ribarova, 2007; Anderson, 2008).

Channel catfish is benthopelagic freshwater species. They feed primarily on small fish, crustaceans (e.g. crayfish), clams and snails; also feed on aquatic insects and small mammals (Fishbase). The main warm water fish species, which have been an object of breeding in Bulgaria, are the cyprinid fish species, which are the predominant fish in the world aquaculture with 54% of total production (FAO, 2011). According to Hadjinikolova et al. (2010) recently, have been increased the production capacity of channel catfish (*Ictalurus punctatus*) in Bulgaria with a maximum production capacity of 171.2 t in 2005 and 236 t in 2007. The chemical composition of channel catfish edible tissue is little known despite the increasing importance of this species in some Central and Eastern European countries. Although the importance of this species, the data concerning fat soluble vitamins, FA composition and lipid quality indices on channel catfish in the Bulgarian scientific literature is lacking. Due to these facts, the aim of the present work was to determine FA composition, lipid quality indices, and fat soluble vitamins content of commercially important channel catfish (*Ictalurus punctatus*).

Materials and Methods

Fish species

Samples of channel catfish were obtained from Varna local fish market (autumn, 2012). All fishes were immediately frozen at -20°C and stored in a fridge at the same conditions. Six specimens were used for lipid, FA and fat soluble vitamin analysis. The biometric characteristics of fishes were determined and noted.

Standards and reagents

Fatty Acid Methyl Esters (FAME) mix standard (SUPELCO 37 FAME Mix), nonadecanoic acid and methyl ester nonadecanoic acid standards were purchased from Sigma-Aldrich™. All-trans-retinol was purchased from Fluka, cholecalciferol, alpha-tocopherol, and other HPLC-grade re-

agents – from Sigma-Aldrich™. All used chemicals were of analytical, HPLC and GC grade (Sharlau, Spain).

Lipid extraction and fatty acid analysis

Portions of freshly prepared homogenate (5.000 ± 0.001 g) were extracted in triplicate with chloroform: methanol (1:2 v/v) according to Bligh and Dyer (1959). Total lipid content of edible tissue was determined ($n = 6$) and the results are presented as g per 100 g wet weight ($\text{g} \cdot 100 \text{ g}^{-1} \text{ ww}$). The chloroform fraction was methylated by base-catalyzed transmethylation using 2M KOH in methanol and n-hexane (EN ISO 5509: 2000). The hexane layer was separated and analyzed by GC-MS. Gas chromatography was performed by a FOCUS Gas Chromatograph equipped with Polaris Q MS detector (Thermo Scientific, USA). The capillary column used was a TR-5 MS, 30 m length, film thickness 0.25 μm , 0.25 mm i.d. The optimum temperature gradient was 40°C to 280°C ($5^{\circ}\text{C}/\text{min}$). Helium was used as a carrier gas at a flow rate 1 ml/min. Three parallel analyses were made from each methylated sample. Peaks were identified according to: Retention Time (RT) based on available FAME mix standard (SUPELCO 37 FAME Mix C4 – C24) and mass spectra (ratio m/z) – compared to internal Data Base (Thermo Sciences Mass Library, USA). The quantisation was done by the method of external calibration. The results were expressed as the percentage of each FA with respect to the total fatty acids, and as $\text{g} \cdot 100^{-1} \text{ ww}$ (EN ISO 5508:2000).

Lipid quality indices (LQI)

Nutrition qualities are estimated by several indices and ratios of fatty acid composition: the indices of atherogenicity (IA), thrombogenicity (IT), cholesterolemic index (h/H); n-6/n-3 and PUFA/SFA ratios, according to Simopolous (2013).

Ulbricht and Southgate (1991) suggest two indices – IA and IT which might better describe the atherogenic and thrombogenic potential of different unsaturated FA. IA indicates the relationship between the sum of the main saturates and that of the main unsaturated, the former being considered pro-atherogenic (favouring the adhesion of lipids to cells of the immunological and circulatory systems), and the latter anti-atherogenic (inhibiting the aggregation of plaque and diminishing the levels of esterified FA, cholesterol and phospholipids, thereby preventing the appearance of micro- and macro coronary diseases). IT shows the tendency to form clots in the blood vessels. This is defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic FA (MUFA, n-6 PUFA and n-3PUFA) Ulbricht and Southgate (1991). h/H presents the functional effects of different PUFAs of cholesterol metabolism (hypo- and hyper-cholesterolemic effect), and calculated according to Santos-Silva et al. (2002).

Fat soluble vitamin's analysis

Prior to analysis the fishes were defrosted; the head, tail, fins, and viscera of the fish were removed. The fishes were filleted and homogenized and used as raw material for the preparation of random fish tissue sample. The sample preparation was performed using the method of Dobрева et al. (2011).

HPLC system (Thermo Scientific Spectra SYSTEM) equipped with analytical column ODS2 Hypersil™ 250 x 4.6 mm, 5 μ was used. The mobile phase was composed of 97:3 = MeOH: H₂O with a flow rate 1.0 ml/min. The qualitative analysis was performed by comparing the retention times of standard solutions all-trans-retinol, cholecalciferol and alpha-tocopherol. Retinol and cholecalciferol were monitored by UV detection at λ_{\max} = 325 nm and λ_{\max} = 265 nm, respectively. Alpha-tocopherol was detected by fluorescence at λ_{ex} = 288 nm and λ_{em} = 332 nm. The quantisation was done by the method of external calibration.

Statistical analysis

All analytical determinations were performed in triplicate. The results were expressed as average and standard deviation (mean \pm SD), and the data were presented as μg or mg per 100 g wet weight ($\mu\text{g}\cdot 100\text{ g}^{-1}\text{ ww}$; $\text{mg}\cdot 100\text{ g}^{-1}\text{ ww}$). The obtained data was analyzed using Graph Pad Prism 5 software.

Results and Discussion

Total lipid content

The total lipid (TL) content of fish depend on a variety of factors such as water temperature, food type and availability, reproductive behavior and individual differences (Steffens, 2006). TL of analyzed channel catfish was $3.90 \pm 0.25\text{ g}\cdot 100\text{ g}^{-1}\text{ wet weight}$. The higher TL content ($5.4\text{ g}\cdot 100\text{ g}^{-1}\text{ ww}$) was observed for channel catfish in earlier investigation (Robinson et al., 2001; Li et al., 2009). Nettleton and Elmhurst (2001) reported lower TL content in wild channel catfish ($2.90\text{ g}\cdot 100\text{ g}^{-1}\text{ ww}$) and, three time higher values ($11.3\text{ g}\cdot 100\text{ g}^{-1}\text{ ww}$) for cultured catfish.

Fatty acid composition

It is known that FA composition differs among herbivorous, carnivorous and omnivorous fish species. It was found that fish lipids vary greatly in the percentage of saturated FA (SFA) and unsaturated FA and usually they account as 20–40% SFA and 60–80% unsaturated FA (Henderson and Tocher, 1987). In this work the FA distribution in analyzed catfish is SFA>MUFA>PUFA. This FA pattern was significantly different compared to our earlier study of Danube catfish composi-

tion (MUFA>SFA>PUFA) (Stancheva et al., 2014) and similar to results for US cultivated catfish (Sathivel et al., 2002).

The most dominant SFA were C14:0, C16:0 and C18:0, and they presented the following distribution: C16:0>C14:0>C18:0. This result is in agreement with data presented by Zhang et al. (2014) for Yangtze catfish and Robinson et al. (2001) and Li et al. (2009) for Mississippi channel catfish. The main observed differences in literature are in specified amounts of these acids. Table 1 shows the total saturated, mono- and polyunsaturated FAs in channel catfish.

Table 1

Fatty acids profile (% of total FA) of channel catfish (mean \pm SD)

Fatty acids	mean \pm SD		mean \pm SD
Saturated FA		C 18:1 n9 c	18.63 \pm 1.05
C 12:0	0.15 \pm 0.01	C 20:1	0.74 \pm 0.13
C 13:0	1.26 \pm 0.05	C 22:1 n9	0.32 \pm 0.01
C 14:0	2.30 \pm 0.10	C 24:1	0.17 \pm 0.01
C 16:0	24.53 \pm 1.15	Σ MUFA	35.39
C 17:0	0.46 \pm 0.02	Polyunsaturated FA	
C 18:0	4.29 \pm 0.11	C 18:3 n6	0.62 \pm 0.02
C 20:0	0.82 \pm 0.02	C 18:2 n6 t	0.06 \pm 0.01
C 21:0	0.14 \pm 0.01	C 18:2 n6 c	13.50 \pm 1.00
C 22:0	0.66 \pm 0.02	C 18:3 n3	1.55 \pm 0.16
C 23:0	0.19 \pm 0.01	C 20:5 n3	2.68 \pm 0.24
C 24:0	1.36 \pm 0.14	C 20:4 n6	1.24 \pm 0.08
Σ SFA	36.16	C 20:3 n3	0.84 \pm 0.03
Monounsaturated FA		C 20:3 n6	0.95 \pm 0.05
C 14:1	0.88 \pm 0.02	C 20:2	2.54 \pm 0.14
C 16:1	14.39 \pm 0.35	C 22:6 n3	3.85 \pm 0.23
C 17:1	0.20 \pm 0.01	C 22:2	0.62 \pm 0.02
C 18:1 n9 t	0.03 \pm 0.01	Σ PUFA	28.45

However MUFA level in the present study was lower than those reported by previous study (Robinson et al., 2001; Nettleton and Elmhurst, 2001; Sathivel et al., 2002; Li et al., 2009). Oleic acid (C18:1n-9) was the most abundant MUFA, which accounted 52.8% of total MUFAs. The second FA was palmitoleic acid (C16:1n-7). Freshwater fish are able to biosynthesize C18:1n-9, but it also has an exogenous origin and its content reflects the fish tissue FA composition (Mraz, 2011). High levels of C18:1n-9 and C16:1n-7 has been reported as a characteristic property of freshwater fish tissues (Steffens, 2006). The other individual MUFAs were characterized by extremely low level (below 1%).

In analyzed species lowest level was observed for PUFA groups. Linolenic acid (C18:2n-6, LA) was most abundant PUFA (50.47% of total PUFAs). Moreover, LA is a normal constituent of the freshwater cultivated fish and therefore it

contains higher LA levels compared to wild carnivores fish (Mraz, 2011). The presented results are in agreement with data presented by Nettleton and Elmhurst (2001) for cultivated catfish and significantly higher compared to Yangtze River catfish (Zhang et al., 2014). The second most abundant PUFA is DHA (13.5% of total PUFA). In this study EPA level was 9.42% of total PUFA, whereas Li et al. (2009) did not detect this acid in Mississippi cultivated channel catfish. Observed PUFA distribution of channel catfish is: LA>DHA>EPA. EPA and DHA, found only in fish and seafood, possess extremely beneficial properties for the prevention of human coronary artery disease. In this work, the channel catfish was a good source of EPA and DHA. The percentages of EPA + DHA were 6.53% (23% of total PUFA). The amount of EPA + DHA in analyzed catfish was found to be lower compared to the results of Zhang et al. (2014), but higher than those reported by Robinson et al. (2001) for channel catfish. Discrepancy between the presented results and those reported by Stancheva et al. (2014) was established. The authors reported significantly higher EPA and DHA levels for wild Danube catfish. One possible reason for the least favorable PUFA composition of the analyzed farmed channel catfish could be related to the type and quantity of the available food. Furthermore some authors suggest that temperature influences significantly FA composition. Higher temperature leads to decreased proportion of unsaturated FA (Tocher, 2003; Mraz, 2011).

Despite the differences in the FA profile it can be concluded that the analyzed fish species are good sources of essential n-3 and n-6 PUFA. n-3 and n-6 PUFA levels, n-6/n-3 and PUFA/SFA ratios, IA, IT, h/H indices in studied fish are presented in Table 2. The analyzed channel catfish are characterized by lower levels of n-3 PUFAs (37.33 % of total PUFA) compared to n-6 PUFA levels (57.54% of total PUFA).

Table 2

FA groups (% of total FA), FA ratios, lipid quality indices, EPA and DHA n-3 PUFA content (g.100 g⁻¹ ww) (means ± SD)

Fatty acids	mean ± SD
n-3	10.62 ± 0.68
n-6	16.37 ± 0.80
n-6/n-3	1.54
PUFA/SFA	0.79
n-3	0.371 ± 0.02
EPA	0.095 ± 0.005
DHA	0.135 ± 0.015
EPA+DHA	0.245 ± 0.015
IA	0.55 ± 0.01
IT	0.53 ± 0.01
h/H	1.57 ± 0.03

The ratio of n-6/n-3 PUFAs of total lipids of freshwater fishes ranges mostly between 0.5 and 3.8 (Tocher, 2003). Also, the n-6/n-3 ratio has been suggested to be a useful indicator for comparing relative nutritional values of fish. Simopolous (2013) suggested that a decrease in the human dietary n-6/n-3 PUFA ratio is essential to help prevent coronary heart disease by reducing the plasma lipids and to reduce the risk of cancer. In the present study this ratio was 1.54, due to higher n-6 PUFA values, and was found similar to earlier results presented in literature for catfish species (Robinson et al., 2001; Zhang et al., 2014) and significantly lower compared to Nettleton and Elmhurst (2001) study. Several studies have found inverse correlation between the PUFA/SFA ratios and cardiovascular diseases and suggested that the replacement of SFA with PUFA in the human diet will decrease similar health problems (Simopolous, 2013). This ratio is another key indicator for evaluation of fish nutrition quality. Department of Health (1994) and Wood et al. (2008) recommend values of PUFA/SFA ratio greater than 0.40. In this study the PUFA/SFA ratio was found higher than cut-off value in analyzed species (Table 2). Zhang et al. (2014) found similar results for wild Yangtze catfish (China), whereas Nettleton and Elmhurst (2001) reported significantly higher value for Mississippi river channel catfish (4.2). On the contrary Li et al. (2009) presented significantly lower PUFA/SFA ratio (0.27) and discussed disadvantages of US channel catfish edible tissue. The nutritional value of analyzed species is also assessed by lipid quality indices, which depend on the relative proportions of some individual saturated and unsaturated fatty acids. The current lipid quality indices presented values lower than those found in lamb, beef and rabbit meats (1.0–2.0). Higher values of IA and IT (>1.0) are detrimental to human health, whereas higher h/H levels (>1.0 ± 0.2) are recommended (Wood et al., 2008). The presented IA and IT values (Table 2) are beneficial for human nutrition. Zhang et al. (2014) reported similar IA (0.58) and IT (0.51) rates compared to our results and highlighted that Yangtze catfish species have potential of high-quality fish such as rainbow trout and salmon. To our knowledge, no data is available in literature for lipid quality indices for channel catfish from the inland waters in Bulgaria. Moreover, the European Food Safety Authority (EFSA, 2012) recommended daily intake of 0.250 to 0.500 g EPA and DHA based on cardiovascular risk considerations for European adults. The percentage values of these FA were recalculated to g.100 g⁻¹ of filets according to FAO/INFOODS Guidelines (2012). A 100 g of edible portion of fillets of analyzed samples contained 0.245 g of EPA+DHA n-3 PUFA. In order to meet the above norm 200 g portion of channel catfish provides 0.5 g EPA and DHA n-3 PUFA. Zhang et al. (2014) and Li et al. (2009) presented significant lower EPA+DHA content (0.068 g.100 g⁻¹ ww) for Yangtze catfish species and for Mississippi channel catfish (0.080 g.100 g⁻¹ ww) compared to our results.

Table 3

Vitamin contents $\mu\text{g}\cdot 100\text{ g}^{-1}\text{ ww}$, (mean \pm SD) and percentage contribution of fat soluble vitamins from analyzed fish

	All-trans-retinol	Cholecalciferol	α -tocopherol
Analyte/ $100\text{ g}^{-1}\text{ ww}$	36.2 ± 0.7	17.7 ± 0.7	1374.5 ± 158.1
% RDI	4.8%	354%	9.2%
ADI* for Bulgaria	$750\ \mu\text{g}^{**}$	$5\ \mu\text{g}$	15 mg

*Average daily intake; ** Average value of the recommended daily intake for adults (male and female)

Vitamins content

The information on the fat soluble vitamin's content in channel catfish in the scientific literature is limited. This ascertainment is confirmed by the study of Casallas et al. (2012) for nutrition quality of nine different species of catfish.

In presented investigation significant differences in retinol, cholecalciferol and alpha-tocopherol contents were established. The quantities of fat soluble vitamins provided by 100 g raw fish tissue and calculated as percentages of the relative daily intake (RDI) are presented in Table 3.

Vitamin A content in channel catfish fish edible tissue is higher than those presented by Stancheva et al. (2014) for Danube River European catfish, whereas data presented in Self Nutrition Data Facts ($15.0\ \mu\text{g}\cdot 100\text{ g}^{-1}\text{ ww}$) is two times lower from ours. In the case of vitamin D_3 , studied samples contained higher amounts compared to those reported by same Database ($12.5\ \mu\text{g}\cdot 100\text{ g}^{-1}\text{ ww}$) and for Danube River's catfish ($3.1\ \mu\text{g}\cdot 100\text{ g}^{-1}\text{ ww}$).

Retinol and α -tocopherol contents in European catfish raw tissue were presented by Özyurt et al. (2009). Their research showed significant differences compared to our results. The cited contents were $6.30\ \mu\text{g}\cdot 100\text{ g}^{-1}\text{ ww}$ for retinol and $800.0\ \mu\text{g}\cdot 100\text{ g}^{-1}\text{ ww}$ for α -tocopherol, respectively. According to Danish Food Composition Databank (2009) information for raw edible catfish tissue, the amounts of retinol, cholecalciferol, and α -tocopherol are 18.0, 1.3, and $2400.0\ \mu\text{g}\cdot 100\text{ g}^{-1}\text{ ww}$, respectively. Retinol and cholecalciferol contents are considerable lower, especially those for cholecalciferol, while tocopherol amount is two times higher than the contents obtained in this study. The observed discrepancies may be caused by the season or other environments factors which affect fish vitamin metabolism.

There are dietary standards in Bulgaria for relative daily intake (RDI) of fat soluble vitamins, which are in accordance with the European Union standards with the exception of those for vitamin D_3 ($5\ \mu\text{g}$ for adults in Bulgaria against $10\ \mu\text{g}$ per day in the European Union) (Ordinance No 23, 2005; DRI, 2011).

Fat soluble vitamins provided by 100 g raw fish tissue as a percentage of the average daily allowance were compared with the RDI values for retinol, cholecalciferol and α -tocopherol amounts. According to the Bulgarian dietary standards for aver-

age daily intake of fat soluble vitamins, analyzed fish show low percentage for the daily recommended intake of retinol (4.8% RDI, Table 3). One portion (100 g) provides two times more α -tocopherol (9.2% RDI) compared to vitamin A. In contrast, cholecalciferol content in studied fish surpasses the recommended daily needs by 354% of RDI (Table 3).

Conclusion

This is the first study of the FA composition, fat soluble vitamins contents, FA ratios, lipid quality indices and EPA and DHA n-3 PUFA contents of the traditionally consumed in Bulgaria channel catfish. Its main objective is to assess the nutrition quality of fish edible tissue in terms of fatty acid composition and fat-soluble vitamin contents.

Observed FA distribution of channel catfish edible tissue is: SFA>MUFA>PUFA. Catfish species showed higher n-6 PUFA levels (16.37%) than n-3 PUFA (10.62%). PUFA/SFA (0.79) and n-6/n-3 (1.54) ratios were within the recommended levels. The values of IA, IT indices were below and h/H was higher than 1.00, which determined the good anti-thrombogenic, anti-atherogenic and hypo-cholesterolemic properties of fish lipids. 200 g portion of the analyzed species provides the recommended 0.500 g EPA + DHA n-3 PUFA.

The fat soluble vitamins showed significant differences. This freshwater fish could be assigned as valuable sources of vitamin D_3 – up to $17.79\ \mu\text{g}\cdot 100\text{ g}^{-1}\text{ ww}$ and 100 g fillets per serving provide almost three times of the established RDI in Bulgaria.

From human health aspect, the analyzed channel catfish appear to be quite nutritious in terms of the corresponding biologically active components.

References

- Anderson, J. and L. Young, 2008. Fat-Soluble Vitamins. Food and Nutrition series, Colorado State University. 9.315.
- BDS EN ISO 5508: 2000: Animal and vegetable fats and oils – preparation of methyl esters of fatty acids.
- BDS EN ISO 5509: 2000: Gas chromatographic Analysis of methyl esters of fatty acids.

- Bligh, E. and W. J. Dyer**, 1959. A rapid method of total lipid extraction and purification. *Canadian J. Biochemistry and Physiology*, **37**: 913–917.
- Crus Casallas, N. E., P. E. Crus Casallas and H. S. Mahecha**, 2012. Characterization of the Nutrition Quality of the meat of some Species of Catfish: A Review. *Rev. Fac. Nal. Agr. Medellin*, **65** (2): 6799–6709.
- Danish Food Composition Databank**, Department of Nutrition, National Food Institute 2007, http://www.foodcomp.dk/v7/fcdb_grpsearchres.asp?MainGrp=08.
- Department of Health**, 1994: Nutritional aspects of cardiovascular disease: Health and social subjects. HMSO, London.
- Dobрева, D. A., B. Galunska and M. Stancheva**, 2011. Liquid chromatography method for the simultaneous quantification of fat soluble vitamins in fish tissue. *Scripta Sci. Medica*, Varna Medical University, **43**: 276–279.
- DRI**, Dietary Reference Intakes for Calcium Vitamin D. Available online: http://books.nap.edu/openbook.php?record_id=13050.
- 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** (7): 2815. Available online: www.efsa.europa.eu.
- FAO**, Fisheries and Aquaculture Department, 2011. Fisheries and Aquaculture topics. Composition of fish, Available online: www.fao.org/fishery/topic/12318/en.
- FAO/INFOODS**, 2012. Guidelines for Converting Units, Denominators and Expressions, version 1.0 Prepared by U. R. Charrondierre, D. Rittenshober, V. Nowak, R. Wijesinha-Bettoni, B. Stadlmayr, D. Haytowitz, D. Persijn.
- Hadjinikolova, L., T. Hubenova and A. Zaikov**, 2010. Status and development tendencies of freshwater aquaculture production in Bulgaria. *Bulg. J. Agri. Sci.*, **16** (3): 398–405.
- Henderson, R. J. and D. R. Tocher**, 1987. The lipid composition and biochemistry of freshwater fish. *Progress Lipid Research*, **20**: 281–346. http://www.fishbase.us/country/CountrySpeciesSummary.php?id=290&c_code=100 <http://nutritiondata.self.com/facts/finfish-and-shellfish-products/4036/2>
- Kris-Etherton, P., W. Harris and L. Appel**, 2003. Fish Consumption, Fish Oil, Omega-3 Fatty Acids, and Cardiovascular Disease. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **23**: 20–30.
- Lee, K. W., A. D. Blann, Y. Gregory and H. Lip**, 2006. Effects of omega-3 polyunsaturated fatty acids on plasma indices of thrombogenesis and inflammation in patient's post-myocardial infarction. *Thrombosis Research*, **118**: 305–312.
- Li, M. H., E. H. Robinson, C. S. Tucker, B. B. Maning and L. Khoo**, 2009. Effect of dried algae *Schizochytrium sp.*, a rich source of docosahexaenoic acid, on growth, fatty acid composition and sensory quality of channel catfish (*Ictalurus punctatus*). *Aquaculture*, **292** (3–4): 232–236.
- Mraz, J.**, 2011. Lipid Quality of Common Carp (*Cyprinus carpio*) in Pond Culture. [Licentiate Thesis.] Swedish University of Agricultural Sciences, Uppsala: 52.
- Nettleton, J. A. and I. L. Elmhurst**, 2001. Fatty Acids in Cultivated and Wild Fish. Microbehavior and Macroresults: Proceedings of the Tenth Biennial Conference of the International Institute of Fisheries Economics and Trade, July 10–14, 2000, Corvallis, Oregon, USA. Compiled by Richard S. Johnston and Ann L. Shriver. International Institute of Fisheries Economics and Trade (IIFET), Corvallis, available on: <http://hdl.handle.net/1957/35415>.
- Ordinance No 23 / 19.07.2005** on the Physiological Feeding of Population. Available online: http://bg.wikipedia.org/wiki/Физиологични_норми_за_хранене#cite_note-echo-9.
- Ostermeyer, U. and T. Schmidt**, 2005. Vitamin D and provitamin D in fish. *European Food Research and Technology*, **222**: 403–413.
- Ozyurt G, A. Polat and G. B. Loker**, 2009. Vitamin and mineral content of pike perch (*Sander lucioperca*), common carp (*Cyprinus carpio*), and European catfish (*Silurus glanis*). *Turkish J. Vet. and Animal Sci.*, **33**: 351–356.
- Robinson E., M. Li and D. Oberle**, 2001. Nutrient characteristics of pond-raised channel catfish. Mississippi Agricultural and Forestry Experiment Station, *Research Report*, **22** (16): 1–5, Available online: <http://www.msucare.com/16609/1.2M>.
- Ribarova, F.**, 2007. Food and Vitamins. Sofia, Bulgaria.
- Santos-Silva, J. S., R. J. B. Bessa and F. S. Santos**, 2002. Effect of genotype, feeding system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat. *Livestock Product Science*, **77**: 187–194.
- Sathivel, S., P. Witoon, C. Grimm, J. King and S. Lloyd**, 2002. Fatty acid composition of crude oil recovered from catfish viscera. *JA-OCS*, **79** (10): 989–992.
- 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.
- Stancheva, M., A. Merdzhanova, D. Dobрева and L. Makedonski**, 2014. Common Carp (*Cyprinus caprio*) and European Catfish (*Silurus glanis*) from the Danube River as Sources of Fat Soluble Vitamins and Fatty Acids. *Czech J. of Food Sci.*, **32** (1): 16–24.
- Steffens, W.**, 2006. Freshwater fish – wholesome foodstuffs. *Bulg. J. Agric. Sci.*, **12**: 320–328.
- Tocher, D.**, 2003. Metabolism and functions of lipid and fatty acid in teleost fish. *Reviews in Fisheries Sciences*, **11**: 107–184.
- Ulbricht, T. and D. Southgate**, 1991. Coronary heart disease: seven dietary factors. *Lancet*, **338**: 985–992.
- Wood, J. D., M. Enser, A. V. Fisher, G. R. Nute, P. R. Sheard, R. Richardson, S. I. Hughes and F. M. Whittington**, 2008. Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, **78**: 343–358.
- Zhang, Z., L. Liu, C. Xie, D. Li, J. Xu, M. Zhang and Min Zhang**, 2014. Lipid content, fatty acid profiles and nutrition quality of nine wild caught freshwater fish species of the Yangtze Basin, China. *Jour. of Food and Nutr. Res.*, **2** (7): 388–394.