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Synthesis and thermal analysis of β-cyclodextrin / Danube common carp (*Cyprinus carpio* L.) oil complexes

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Abstract

In this study the synthesis of new β-cyclodextrin / Danube common carp (*Cyprinus carpio* L.) oil supramolecular system have been performed. Kneading method at a molar ratio for β-cyclodextrin:fish oil mixture of 1:1 have been applied. The Danube common carp oil had a higher relative concentration of monounsaturated fatty acids of 41.2-42.3 %, especially oleic and palmitoleic acids, as was revealed by gas chromatography-mass spectrometry (GC-MS) analysis for the transesterified carp oil to the corresponding methyl esters. The main *omega-3* fatty acids were eicosapentaenoic acid (EPA) of 2.4 % and docosahexaenoic acid (DHA) of 0.6 %. However, the *omega-3/omega-6* ratio was higher than the lowest limit of 0.2, which support the quality of a fish oil for the beneficial effect for the human health.

β-Cyclodextrin / Danube common carp oil complexes were obtained by kneading method with an average of recovering yield of 56.4 (±8.9) %. The thermogravimetry-differential thermogravimetry (TG-DTG) analysis reveals the formation of the cyclodextrin-glyceride fatty acids inclusion compounds by reducing the water content of the complex, as well as the significant modification of the water bonding into the supramolecular structure. Thus, the water (or moisture) content of complexes, as TG mass loss, was 4.0 (± 0.17) % up to ~140 °C, but a mass loss of 4.7 (± 0.3) % for the temperature interval of 140-270 °C was also observed for complexes. The mass loss for β-cyclodextrin was significantly higher for the first interval (~13.3 %) and almost no mass loss was observed for the second interval. Moreover, the DTG peak corresponding to water release from the complex is significantly higher in comparison with the β-cyclodextrin (149.1 (±6.5) °C and 85.7 (±2.5) °C, respectively). These findings support the formation of the β-cyclodextrin / Danube common carp oil inclusion complexes and the possible use of these *omega-3*-based complexes for food applications (e.g. food supplements and fortified food products).

Keywords: common carp, *Cyprinus carpio* L., Danube fish oil, cyclodextrin complexes, molecular encapsulation, nanoencapsulation, nanoparticles, gas chromatography-mass spectrometry, GC-MS, thermogravimetry-differential thermogravimetry, TG-DTG

1. Introduction

Common carp is one of the most consumed fish in Europe. It originates from Middle Asia, but it spread in almost all freshwaters from Europe and Asia. It has a mean length of 40-80 cm, but some specimens can reach 14 kg.

Common carp is a schooling fish and is harmful for other fish species [1,2]. However, it was classified as vulnerable (VU) fish species by "The IUCN Red List of Threatened Species".

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There are differences between the lipid profile of the common carp from aquaculture and wild species. This is especially due to the differences on the diet. Monounsaturated fatty acids (MUFAs, as glycerides) were the most concentrated in the common carp from aquaculture, having values of 42-61.8% [3-5]. On the other hand, saturated (SFAs) and polyunsaturated fatty acids (PUFAs) were identified at 24-25.4% and 13.7-30.9%, respectively. Among these, *omega-3* fatty acids such as EPA ((all-*Z*)-5,8,11,14,17-eicosapentaenoic acid) and DHA ((all-*Z*)-docosa-4,7,10,13,16,19-hexaenoic acid), were the most concentrated in the lipid part separated from aquaculture carp muscle, having concentration of 1.8-5.2% and 4.1-8.9 %, according to Ljubojević and co-workers [6]. Other carp species (e.g. *Labeo rohita*, *Cirrhinus mrigala* and *Catla catla*) have been studied for their PUFAs composition, revealing higher contents of EPA and especially DHA [7]. There are some unusual fatty acid derivatives that were identified in common carp oil. It was the case of furan-containing fatty acids, which can be derived from some degradation reactions during the separation, processing or storing processes [8].

Heavy metal accumulation into fish species, particularly in oily parts, represents a disadvantage. For example, As, Cd, Hg, Pb, Cu and Zn have been identified in various common carp species living in a metallurgic area of Bulgaria or Bosnia and Herzegovina [9-11]. Moreover, mercury-based organic compounds have also been identified in some common carp species. It was the case of methyl-mercury, identified by Houserová and collaborators at low concentrations up to 53 μg/kg [12]. Among these toxic compounds, other harmful organic molecules can be identified in fish species, including common carp, such as halogenated pesticides or by-products from this industry or polyaromatic hydrocarbons [13-14].

The presence of high concentrations of PUFAs in the fish oils, particularly *omega-3* FAs at *omega-3/omega-6* ratio higher than 0.2 [15], makes these fish oils valuable for the human health. EPA and DHA are especially responsible for the beneficial effect on neuronal and cardio-vascular diseases, respectively [16-17]. Unfortunately, PUFAs are easily oxidized during the separation, purification, processing or application processes. Consequently, they must be protected against the access of oxygen/air by various methods such as micro- and nanoencapsulation [18-20].

Cyclodextrins (CDs) are suitable materials for molecular encapsulation of fish oil glycerides, protecting them against environmental degradative factors [21-24]. Moreover, CD/fish oil inclusion complexes are solid powders, with enhanced water solubility and higher stability, due to the specific structural architecture of CDs. These characteristics are due to the fact that CDs have hydrophobic inner cavities, which can well interact with the hydrophobic moieties such as fatty acids. On the other hand, the hydroxyl groups of those six, seven or eight glucose unit (which form α-, β- and γ-CD structure by α-(1-4)-linkages) provide water solubility for both natural CDs and their complexes [25-28].

In the present study, the nanoencapsulation of the main *omega-3* based components of the edible part of the Danube common carp (*Cyprinus carpio* L.) oil into the natural β-cyclodextrin (β-CD) for protection and controlled release of the bioactive compounds as new materials designated for food supplements and fortified food products have been performed for the first time.

2. Materials and Methods

2.1. Sampling and separation of Danube common carp oil

Common carp (*Cyprinus carpio* L.) samples were fished out from the Danube river in the spring of the year 2015. The mean length of the carp samples was 50 cm and the mean weight was 3728 g. The fresh muscle samples of the Danube common carp of 1484 g were mixed with 2968 mL of distilled water and heated for 1 ½ hours at ~110 °C and 1.5-1.6 atm in a pressure vessel (Tefal Classic 6L, Rumilly, Haute-Savoie, France). The mixture was cooled, filtered at normal pressure and the residue was pressed using a home steel-made equipment (Naumann NM-120, SC SFR Home Equipment SRL, Bucharest, Romania). The raw carp oil was centrifuged for fifteen minutes at 3200 rpm and 20 °C using a Heraeus AG centrifuge (Hanau, Germany). The clear Danube common carp oil was dried over anhydrous sodium sulphate (*p.a.*, Merck & Co., Inc., Kenilworth, NJ, USA) and stored in the refrigerator until further uses. Duplicate samples of Danube common carp oil have been obtained and analyzed.

The following EU directives and regulations were considered for catching, killing, and manipulating of fish samples: Council Regulation (EC) Nº

1099/2009 of 24 September 2009 on "The protection of animals at the time of killing", Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on "The protection of animals used for scientific purposes", Regulation (EC) Nº 853/2004 and Nº 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down "Specific hygiene rules for on the hygiene of foodstuffs, and for the organization of official controls on products of animal origin intended for human consumption", and Commission Regulation (EC) Nº 889/2008 of 5 September 2008 laying down detailed rules for the "Implementation of Council Regulation (EC) Nº 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control".

2.2. Derivatization and gas chromatography-mass spectrometry (GC-MS) analysis of Danube common carp oil

The fatty acid profile of the Danube fish oil have been determined by gas chromatography-mass spectrometry (GC-MS) analysis. Consequently, the common carp oil was derivatized to the more volatile fatty acid methyl esters (FAMEs) by transesterification of the corresponding natural glycerides using the borontrifluoride-methanol method (20 % BF_3, Merck & Co., Inc., Kenilworth, NJ, USA). Approximately 0.18 g of common carp oil was refluxed for ½ hours with 5 mL of BF_3:methanol solution in a 100 mL one-neck flask equipped with reflux condenser. The process was continued for another ½ hours after adding 10 mL of hexane (GC-grade, Sigma-Aldrich, St. Louis, MO, USA). The organic layer was separated on the top of the flask by adding saturated sodium chloride solution. The hexane solution of FAMEs was dried over anhydrous sodium sulphate, separated by decantation and analyzed by GC-MS.

FAMEs and other possible degradation compounds containing carboxyl and/or carbonyl groups (as methyl ester and/or dimethylacetal compounds after derivatization) were identified and quantified using a SCION 436-GC Bruker (Bruker Co., Billerica, MA, USA) system under the following conditions: BR-5ms GC column (30 m × 0.25 mm × 0.25 μm, Bruker Co., Billerica, MA, USA), temperature program of 50 °C for 1 min, 50-300 °C with a heating rate of 6 °C/min, and 300 °C for 5 min, solvent delay of 5 min, split ratio of 1:20, sample volume of 1 μL, scanning range of 50-500 amu, helium flow of 1 mL/min, temperature transfer of 250 °C, source temperature of 160 °C, and MS ionization energy of 70 eV. GC-MS data were handled using the MS Workstation 8 for SCIONTM (Bruker Co., Billerica, MA, USA). Two methods have been used for identification of FAMEs and other degradation compounds in the derivatized Danube common carp oil: the retention index (*RI*) of the singular compounds, obtained by means of GC-MS data for C_8-C_{20} linear alkane standard mixture (Sigma-Aldrich, St. Louis, MO, USA) and standard FAMEs for the main essential fatty acids (FAME37, Sigma-Aldrich, St. Louis, MO, USA), as well as by comparing the experimental MS spectra with those from the NIST/EPA/NIH Mass Spectral Library 2.0 (2011), using the NIST MS Search 2.0 package (NIST, Gaithersburg, MD, USA).

2.3. Obtaining of β-cyclodextrin / Danube common carp oil complexes

β-CD / Danube common carp oil complexes were obtained by kneading method. Approximately one mmole of carp oil (mean molar weight of 875.03 g/mol, according to FAMEs profile) was mixed for ½ hours with one mmole of hydrated β-CD (having a molar weight of 1135 g/mol as anhydrous β-CD or 1313 g/mol for commercial hydrated β-CD, >98%, CycloLab, Budapest, Hungary), in the presence of 2 mL of ethanol (>96% (*v/v*), Chimopar, Bucharest, Romania) and 4 mL distilled water in a preheated mortar (~50 °C). The β-CD / common carp oil complexes were dried at room temperature, finely grounded and stored in the refrigerator until further analyses.

2.4. Thermogravimetry - differential thermogravimetry (TG-DTG) analysis of β-cyclodextrin / Danube common carp oil complexes

The water or moisture content (including solvent or other volatiles) of β-CD and its Danube common carp oil complexes have been evaluated by TG-DTG analysis. A Netzsch TG 209F1 Libra (Netzsch Group, Selb, Germany) equipment have been used. TG-DTG were performed under the following conditions: temperature program of 30-400 °C, heating rate of 10 °C/min, dynamic flow of 20 mL/min, protective flow of 40 mL/min, and nitrogen atmosphere. Acquisition and handling of the TG-DTG data were performed using the Proteus® Software for Thermal Analysis ver. 6.1.0 package (Netzsch Group, Selb, Germany).

2.5. Statistical analysis

Danube common carp oil samples were obtained and analyzed by GC-MS in duplicate, while β-CD / Danube common carp oil complexes were prepared and analyzed in triplicate. Consequently, the classical analysis of variance (ANOVA) have been applied for these data (generally, results were presented as mean ± standard deviation, SD). Statistical evaluation of these data were performed using Microsoft Excel® 2013 program from the Microsoft Office Professional Plus 2013 package (Microsoft Corporation).

3. Results and discussion

3.1. Fatty acid profile of Danube common carp oil from muscle

Danube common carp oil was separated from the edible parts of the fish samples, after manual separation of the fresh muscle and heating-pressing for obtaining the raw carp oil. The purification of the raw oil was performed by centrifugation and drying over anhydrous sodium sulphate. The purified clear carp oil was obtained with a mean yield of 0.76 %.

Generally, fish oils consist of triglycerides of the essential fatty acids and they are difficult to identify and quantify because of the complexity of such structures. Consequently, these glycerides were derivatized to the corresponding methyl esters in order to enhance their volatility. The GC-MS analysis allows to evaluate the fatty acid profile of common carp oil. More than sixty compounds have been separated by GC for both derivatized Danube common carp oil samples (Figures 1 and 2).

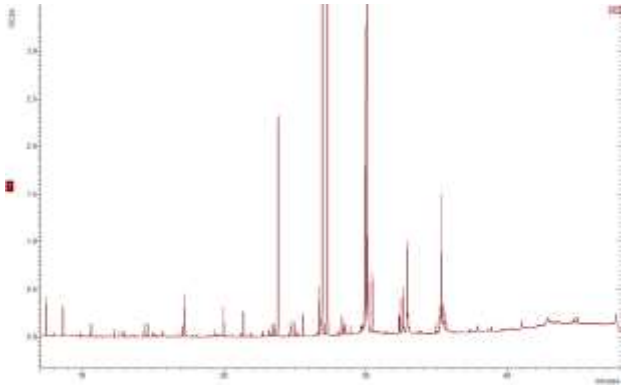

Figure 1. Gas chromatogram from the GC-MS analysis of the Danube common carp oil (duplicate "*a*")

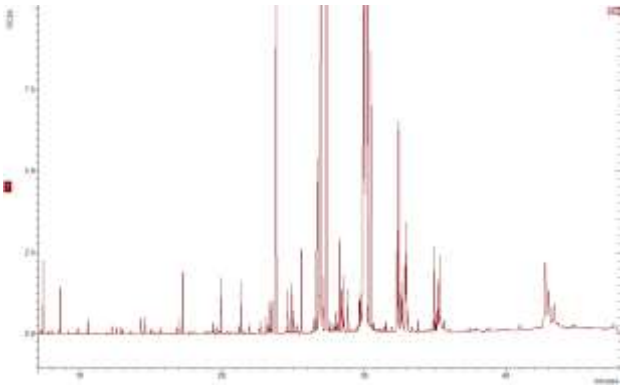

Figure 2. Gas chromatogram from the GC-MS analysis of the Danube common carp oil (duplicate "*b*")

The most concentrated fatty acids (as methyl esters) in Danube common carp oil were MUFAs, having a mean relative concentrations of 41.76 (± 0.76) % (Table 1). The main MUFA in common carp oil was oleic acid (as methyl ester), with a concentration of 19.20 (± 0.62) % (Figure 3). Other MUFAs such as palmitoleic and the *trans* isomer of oleic acid, i.e. elaidic acid, have been identified at significant concentrations of 15.39 (± 0.39) % and 2.98 (± 1.00) %, respectively (Table 1). However, other MUFAs were identified at lower concentrations (e.g., myristoleic acid methyl ester, 0.30 (± 0.02) %, Table 1).

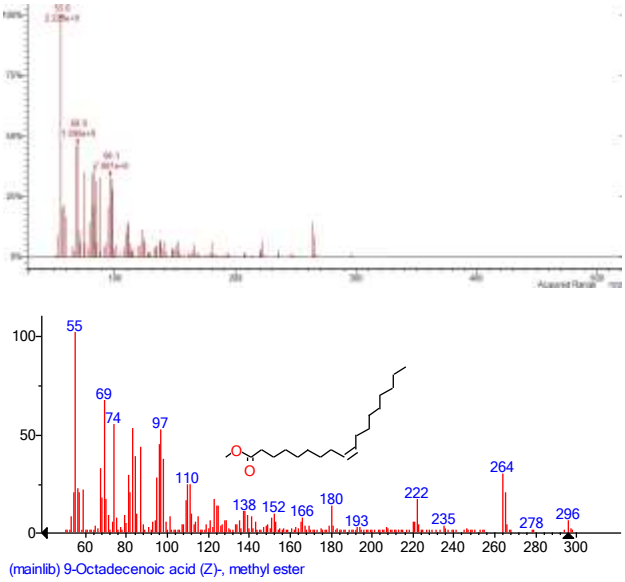

Figure 3. Experimental (top) and from the NIST database (bottom) MS spectra for oleic acid methyl ester (MUFA class), separated by GC-MS from the derivatized Danube common carp oil

The SFA class was represented by palmitic acid, as methyl ester. The overall relative concentrations of SFAs were in a narrow range of 26.6-27.6%, the main compound being identified at 17.53 (± 0.94) % (palmitic acid methyl ester, Table 1), close to the corresponding MUFA, palmitoleic acid. Other SFAs, such as myristic, stearic, pentadecanoic, lauric, arachidic, and heptadecanoic acids (as methyl esters), with the following relative concentrations, respectively: 5.1, 2.53, 0.65, 0.60, 0.38, and 0.31 % have been identified (Table 1).

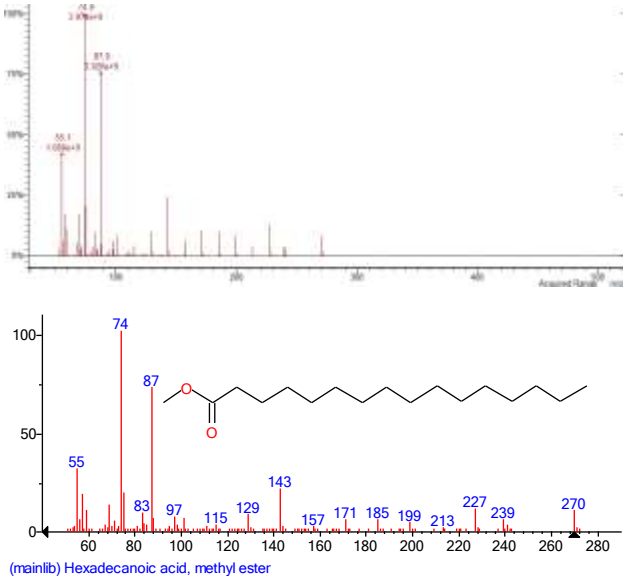

Figure 4. Experimental (top) and from the NIST database (bottom) MS spectra for palmitic acid methyl ester (SFA class), separated by GC-MS from the derivatized Danube common carp oil

The most valuable essential fatty acid-based components in fish oils are PUFAs, especially *omega-3* fatty acid glycerides. PUFAs were identified in Danube common carp oil at relative concentrations of 17.71 (± 4.16) %, *omega-3* fatty acids being the most concentrated. Among these, the PUFAs responsible for ameliorating neuronal and cardio-vascular diseases, i.e. EPA and DHA, were the most concentrated (2.38 (± 0.20) % and 0.60 (± 0.36) %, respectively; Figures 5 and 6). The sum of *omega-3* fatty acid concentrations (as methyl esters) in Danube common carp oil was 4.43 (± 0.59) %, other compounds being stearidonic and clupanodonic acids (0.41 and 0.30 %, Table 1). On the other hand, important *omega-6* PUFAs have been identified in common carp oil. It was the case of linoleic, linolenic, and arachidonic acids, with relative concentrations of 10.94 (± 2.44) %, 0.29 (± 0.08) %, and 0.77 (± 0.42) %, respectively (Table 1).

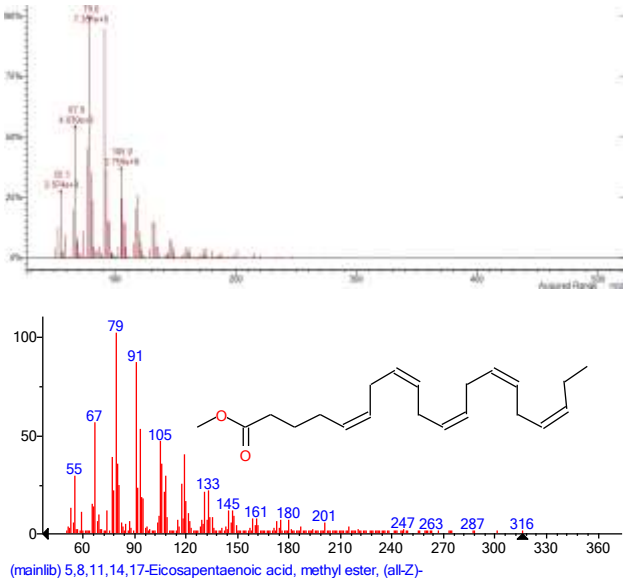

Figure 5. Experimental (top) and from the NIST database (bottom) MS spectra for (all-*Z*)-5,8,11,14,17-eicosapentaenoic acid methyl ester, EPA (PUFA and *omega-3* classes), separated by GC-MS from the derivatized Danube common carp oil

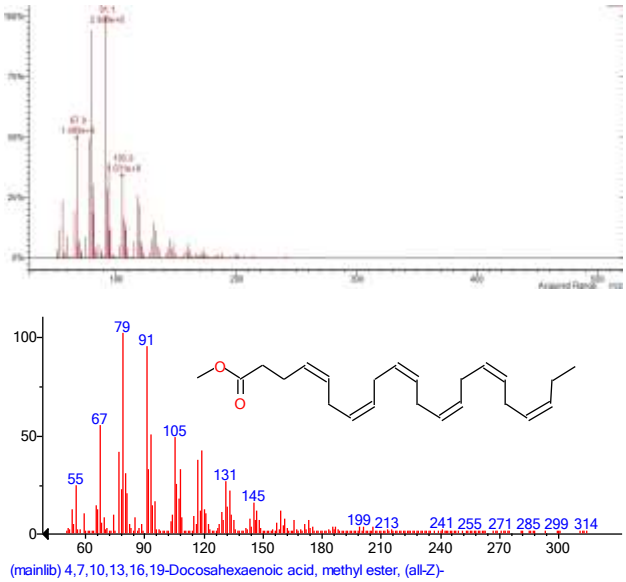

Figure 6. Experimental (top) and from the NIST database (bottom) MS spectra for (all-*Z*)-4,7,10,13,16,19-docosahexaenoic acid methyl ester, DHA (PUFA and *omega-3* classes), separated by GC-MS from the derivatized Danube common carp oil

It was established that an *omega-3/omega-6* ratio for the corresponding relative concentrations determined by GC-MS over than 0.2 is benefic for the human health. In the case of Danube common carp oil, this *omega-3/omega-6* ratio was almost two times higher (0.39 (± 0.15) %, Table 1), revealing the importance of the common carp oil, separated from the edible part of the fish species from the Romanian Danube river.

Table 1. Gas chromatography-mass spectrometry results for the derivatized Danube common carp oil (duplicate analysis)

Nº	**MS Identification**	***Retention Index* (*RI*)**	***Area* ± *SD* (%)**
1	Hexanal, dimethyl acetal	977	0.68 ± 0.07
2	Malondialdehyde, tetramethyl acetal	1026	0.70 ± 0.38
3	Heptane, 1,1-dimethoxy-	1075	0.10 ± 0.06
4	Propane, 1,1-dimethoxy-2-methyl-	1106	0.23 ± 0.12
5	Octanal, dimethyl acetal	1174	0.12 ± 0.06
6	Nonanal, dimethyl acetal	1274	0.23 ± 0.12
7	Octanoic acid, 6,6-dimethoxy-, methyl ester	1492	0.13 ± 0.05
8	Lauric acid, methyl ester	1522	0.60 ± 0.16
9	Undecane, 1,1-dimethoxy-	1593	0.53 ± 0.09
10	Tetradecenoic acid, (Z)-11-, methyl ester	1700	0.31 ± 0.01
11	Myristoleic acid, methyl ester	1709	0.30 ± 0.02
12	Myristic acid, methyl ester	1725	5.07 ± 0.39
13	Lauraldehyde, dimethyl acetal	1794	0.39 ± 0.06
14	Pentadecanoic acid, methyl ester	1823	0.65 ± 0.14
15	Palmitoleic acid, methyl ester	1910	15.39 ± 0.39
16	Palmitic acid, methyl ester	1933	17.53 ± 0.94
17	6-Hexadecenoic acid, 7-methyl, methyl ester, (*Z*)-	1977	0.10 ± 0.03
18	Hexadecanoic acid, 15-methyl-, methyl ester	1987	0.70 ± 0.29
19	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	2000	0.38 ± 0.20
20	Stearaldehyde, dimethyl acetal	2005	0.22 ± 0.10
21	Margaric acid, methyl ester	2024	0.31 ± 0.13
22	Linolenic acid, methyl ester	2073	0.29 ± 0.08
23	Stearidonic acid, methyl ester	2079	0.41 ± 0.11
24	Linoleic acid, methyl ester	2097	10.94 ± 2.44
25	Oleic acid, methyl ester	2108	19.20 ± 0.62
26	Elaidic acid, methyl ester	2111	2.98 ± 1.00
27	Stearic acid, methyl ester	2127	2.53 ± 0.92
28	Arachidonic acid, methyl ester	2250	0.77 ± 0.42
29	**EPA**, 5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-*Z*)-	2256	2.38 ± 0.20
30	13-Eicosenoic acid, methyl ester	2299	2.03 ± 0.72
31	Arachidic acid, methyl ester	2318	0.38 ± 0.48
32	**DHA**, 4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-*Z*)-	2432	0.60 ± 0.36
33	DPA, 7,10,13,16,19-Docosapentaenoic acid, methyl ester (clupanodonic acid)	2446	0.30 ± 0.17
	Other compounds		*12.52*
	Sum (SFA)*		27.11 ± 0.71
	Sum (MUFA)*		41.76 ± 0.76
	Sum (PUFA)*		17.71 ± 4.16
	Sum (omega-9)*		19.74 ± 0.47
	Sum (omega-6)*		12.06 ± 3.03
	Sum (omega-3)*		4.43 ± 0.59
	*omega-3/omega-6**		0.39 ± 0.15

* *SFA* – saturated fatty acid, *MUFA* – monounsaturated fatty acid, *PUFA* – polyunsaturated fatty acid, *omega-3/6/9* – *omega-3/6/9* fatty acid, methyl esters, *omega-3/omega-6* – the ratio of the *omega-3* and *omega-6* fatty acid methyl esters concentrations

3.2. β-Cyclodextrin / Danube common carp oil complexes

Complexes of β-CD / Danube common carp oil have been obtained by kneading method due to the difficulty to solubilize both β-CD and fish oil in hydrophilic solvents that is need for other complexation methods, such as co-precipitation or co-crystallization.

On the other hand, kneading method allows recovering a high proportion of complex, even the inclusion process is not the most appropriate. β-CD / Danube common carp oil complexes were obtained at 1:1 molar ratio in triplicate. The recovering yield of complexes (as percent ratio between the recovered complex and the sum of host-guest components, i.e. commercial hydrated β-CD and Danube common carp oil) was 56.4 (± 8.9) %.

The success of molecular encapsulation of fatty acid glycerides from common carp oil into the cavity of β-CD can be partially evaluated by thermal methods. Thermogravimetry – differential thermogravimetry (TG-DTG) allows evaluating the water / solvent (or other volatile compounds, including degradation derivatives) release from both β-CD / common carp oil complexes and commercial hydrated β-CD, as well as the thermal stability. Consequently, three TG-DTG ranges can be observed: (1) the range up to 140 °C is especially related to the dissociation of water molecules (both "surface" water at lower temperatures and "strongly-retained" water at higher temperatures) or solvent molecules, such as ethanol used in the complexation process; (2) the range of 140-270 °C is related to the dissociation of "strongly-retained" water and solvent molecules, as well as guest molecules (or degradation compounds), which can only be observed in the case of volatile bioactive compounds (is not the case of fish oil components, which especially contains non-volatile triglycerides); (3) the range over 270 °C reveals the degradation of both β-CD and common carp oil components (the thermal stability of these compounds).

There are significant differences between the TG-DTG curves for β-CD and β-CD / Danube common carp oil complexes. Thus, the TG mass loss for the first interval was only 4.00 (± 0.17) % for complexes in comparison with the value for commercial β-CD (13.31 (± 0.09) %, Table 2 and Figures 7-9).

Table 2. The thermogravimetry-differential thermogravimetry results for commercial β-cyclodextrin and β-cyclodextrin / Danube common carp oil complexes (as multiplicate analyses)

Code*	$ML_{<140°C}$ (%)	$ML_{140-270°C}$ (%)	$ML_{>270°C}$ (%)	$t_{1(DTG)}$ (°C)	$t_{2(DTG)}$ (°C)
β-CD(a)	13.24	0.05	74.77	83.9	328.9
β-CD(b)	13.37	0.05	72.23	87.5	328.0
β-CD	**13.31 ± 0.09**	**0.05 ± 0.00**	**73.50 ± 1.80**	**85.7 ± 2.6**	**328.5 ± 0.6**
β-CD / CRP (a)	4.15	4.39	59.52	147.7	316.9
β-CD / CRP (b)	4.03	4.99	56.72	143.4	315.5
β-CD / CRP (c)	3.81	4.72	57.19	156.1	316.8
β-CD / CRP	**4.00 ± 0.17**	**4.70 ± 0.30**	**57.81 ± 1.50**	**149.1 ± 6.6**	**316.4 ± 0.8**

* *β-CD* – β-cyclodextrin, *β-CD / CRP* – β-cyclodextrin / Danube common carp oil complex, $ML_{<140°C}$ – TG mass loss up to 140 °C, $ML_{140-270°C}$ – TG mass loss on the range of 140-270 °C, $ML_{>270°C}$ – TG mass loss over 270 °C, $t_{1(DTG)}$ – DTG temperature peak corresponding to the first temperature interval (related to water and solvent release), $t_{2(DTG)}$ – DTG temperature peak corresponding to the third temperature interval (related to β-cyclodextrin and guest compounds decomposition)

Moreover, the mass loss in the second interval is more significant for β-CD / common carp oil complexes than for commercial β-CD. This mass loss had values of 4.70 (± 0.30) % for complexes, while for β-CD this value was not significant (0.05%). This difference is due to the modification / redistribution of water molecules inside the β-CD / common carp oil complex, in comparison with those from the β-CD. The water or moisture content of complexes is considerable reduced, demostrating the inclusion process between triglycerides and β-CD. Moreover, the binding of water molecules in complexes in comparison with β-CD hydrate clearly differs. The water molecules are more "strongly-bonded" in β-CD / common carp oil complexes than in β-CD, which is revealed by the DTG peak temperature for the first interval (149.1 (± 6.6) °C and 85.7 (± 2.6) °C, respectively; Table 2 and Figures 7-9).

The mass loss over 270 °C also differ for both β-CD / Danube common carp oil complexes and β-CD. This value significantly decreases from 73.5 % for the starting β-CD to 57.81 (± 1.50) % for complexes. Similar behavior was observed for the corresponding DTG peak temperature (328.5 °C for β-CD and 316.4 °C for the complexes, Table 1 and Figures 7-9). Consequently, the solid residue, evaluated as the remaining percent over 270 °C, was only 13.15 (± 1.70) % for β-CD and 33.49 (± 1.35) % for the corresponding carp oil complexes.

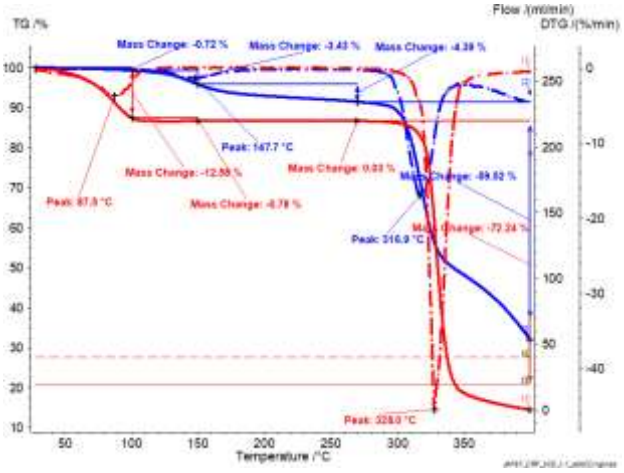

Figure 7. Thermogravimetry-differential thermogravimetry analysis of β-cyclodextrin / Danube common carp oil complex (triplicate "*a*") and β-cyclodextrin

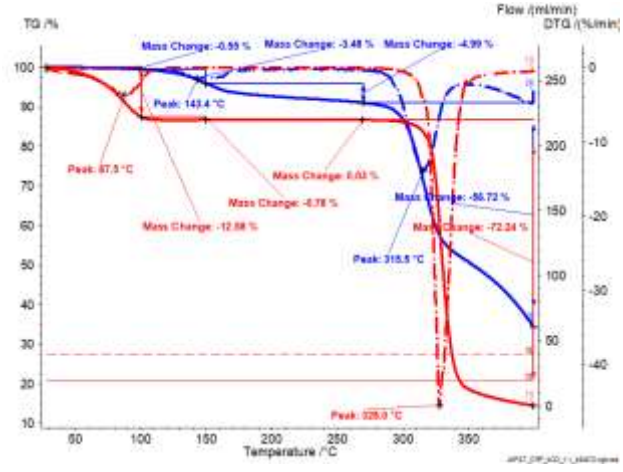

Figure 8. Thermogravimetry-differential thermogravimetry analysis of β-cyclodextrin / Danube common carp oil complex (triplicate "*b*") and β-cyclodextrin

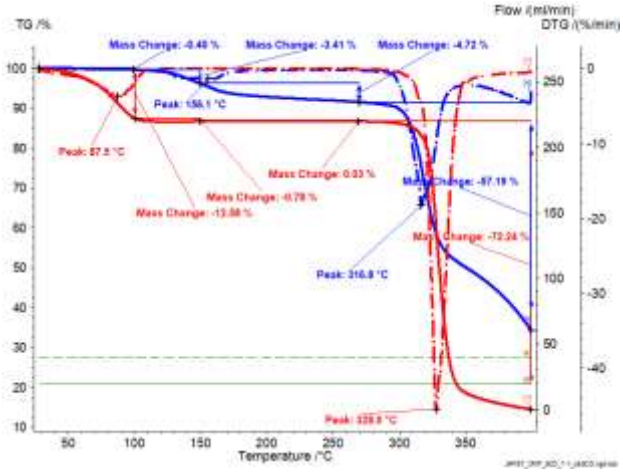

Figure 9. Thermogravimetry-differential thermogravimetry analysis of β-cyclodextrin / Danube common carp oil complex (triplicate "*c*") and β-cyclodextrin

4. Conclusion

β-Cyclodextrin / Danube common carp oil complexes at a molar ratio of 1:1 have been obtained by kneading method. The starting Danube carp oil revealed important content of PUFAs of 17.7%, the most important being *omega-3* fatty acid derivatives, especially EPA and DHA, with relative concentrations of approximately 3% for both compounds implied in ameliorating cardio-vascular and neuronal diseases. Moreover, the *omega-3/omega-6* ratio was two times higher in comparison with the standard limit for the beneficial effect to human health.

The formation of the host-guest molecular inclusion complexes between β-cyclodextrin and PUFA triglycerides (the most important derivatives in Danube common carp oil) have been evaluated by thermogravimetry-differential thermogravimetry, revealing the replacing of water molecules inside the β-cyclodextrin cavity by the hydrophobic Danube common carp oil-containing glycerides. Both water content and bonding of water molecules into the β-cyclodextrin and its Danube common carp oil complexes have been differentiated by this thermal technique. The corresponding values varies from 13.3% and 85.7 °C for β-cyclodextrin to 4.0 % and 149.1 °C for the corresponding Danube common carp oil complexes, respectively.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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References

1. *** *The Danube River Basin*, International Commission for the Protection of the Danube River, Vienna, 2009
2. Belitz, H.-D.; Grosch, W.; Scheberle, P., *Food chemistry*, Springer-Verlag, Berlin-Heidelberg, 2009, pp. 617-639

3. Aggelousis, G.; Lazos, E.S., Fatty acid composition of the lipids from eight freshwater fish species from Greece, *Journal of Food Composition and Analysis* **1991**, *4*, 68-76
4. Ljubojević, D.; Radosavljević, V.; Puvača, N.; Baloš, M.Z.; Đorpević, V.; Jovanović, R.; Ćirković, M., Interactive effects of dietary protein level and oil source on proximate composition and fatty acid composition in common carp (*Cyprinus carpio* L.), *Journal of Food Composition and Analysis*, **2015**, *37*, 44-50, doi: 10.1016/j.jfca.2014.09.005
5. Trbović, D.; Marković, Z.; Milojković-Opsenica, D.; Petronijević, R.; Spirić, D.; Djinović-Stojanović, J.; Spirić, A., Influence of diet on proximate composition and fatty acid profile in common carp (*Cyprinus carpio*), *Journal of Food Composition and Analysis* **2013**, *31*, 75-81, doi: 10.1016/j.jfca.2013.04.002
6. Ljubojević, D.; Trbović, D.; Lujić, J.; Bjelić-Cabrilo, O.; Kostić, D.; Novakov, N.; Ćirković, M., Fatty acid composition of fishes from inland waters, *Bulgarian Journal of Agricultural Science* **2013**, *19* (Suppl. 1), 62-71
7. Memon, N.N.; Talpur, F.N.; Bhanger, M.I.; Balouch, A., Changes in fatty acid composition in muscle of three farmed carp fish species (*Labeo rohita*, *Cirrhinus mrigala*, *Catla catla*) raised under the same conditions, *Food Chemistry* **2011**, *126*, 405-410, doi: 10.1016/j.foodchem.2010.10.107
8. Chvalová D.; Špicka J., Identification of furan fatty acids in the lipids of common carp (C*yprinus carpio* L.), *Food Chemistry* **2016**, *200*, 183-188, doi: 10.1016/j.foodchem.2016.01.029
9. Yancheva, V.; Stoyanova, S.; Velcheva, I.; Petrova, S.; Georgieva, E., Metal bioaccumulation in common carp and rudd from the Topolnitsa reservoir, *Bulgaria. Archives of Industrial Hygiene and Toxicology* **2014**, *65*, 57-66, doi: 10.2478/10004-1254-65-2014-2451
10. Yancheva, V.S.; Stoyanova, S.G.; Velcheva, I.G.; Georgieva, E.S., Histological response of fish gills to metal pollution: common carp, *Cyprinus carpio* L., and common rudd, *Scardinius erythrophthalmus* L., from Topolnitsa reservoir, *Bulgaria. Acta Zoologica Bulgarica* **2016**, *68*(1), 103-109
11. Djedjibegovic, J.; Larssen, T.; Skrbo, A.; Marjanovic, A.; Sober, M., Contents of cadmium, copper, mercury and lead in fish from the Neretva river (Bosnia and Herzegovina) determined by inductively coupled plasma mass spectrometry (ICP-MS), *Food Chemistry* **2012**, *131*, 469-476, doi: 10.1016/j.foodchem.2011.09.009
12. Houserová, P.; Kubán, V.; Kráčmar, S.; Sitko, J., Total mercury and mercury species in birds and fish in an aquatic ecosystem in the Czech Republic, *Environmental Pollution* **2007**, *145*, 185-194, doi: 10.1016/j.envpol.2006.03.027
13. Davodi, M.; Esmaili-Sari, A.; Bahramifarr, N., Concentration of polychlorinated biphenyls and organochlorine pesticides in some edible fish species from the Shadegan Marshes (Iran), *Ecotoxicology and Environmental Safety* **2011**, *74*, 294-300, doi: 10.1016/j.ecoenv.2010.07.045
14. Jürgens, M.D.; Johnson, A.C.; Jones, K.C.; Hughes, D.; Lawlor, A.J., The presence of EU priority substances mercury, hexachlorobenzene, hexachlorobutadiene and PBDEs in wild fish from four English rivers, *Science of the Total Environment* **2013**, *461-462*, 441-452, doi: 10.1016/j.scitotenv.2013.05.007
15. Rubio-Rodríguez, N.; Beltrán, S.; Jaime, I.; de Diego, S.M.; Sanz, M.T.; Carballido, J.R., Production of omega-3 polyunsaturated fatty acid concentrates: A review, *Innovative Food Science and Emerging Technologies* **2010**, *11*, 1-12, doi: 10.1016/j.ifset.2009.10.006
16. Domingo, J.L., Omega-3 fatty acids and the benefits of fish consumption: Is all that glitters gold? *Environment International* **2007**, *33*, 993-998, doi: 10.1016/j.envint.2007.05.001
17. He, K., Fish, long-chain omega-3 polyunsaturated fatty acids and prevention of cardiovascular disease – Eat fish or take fish oil supplement? *Progress in Cardiovascular Diseases* **2009**, *52*(2), 95-114
18. Chatterjee, S.; Judeh, Z.M.A., Encapsulation of fish oil with *N*-stearoyl-*O*-butylglyceryl chitosan using membrane and ultrasonic emulsification processes, *Carbohydrate Polymers* **2015**, *123*, 432-442, doi: 10.1016/j.carbpol.2015.01.072
19. Morales-Medina, R.; Tamm, F.; Guadix, A.M.; Guadix, E.M.; Drusch, S., Functional and antioxidant properties of hydrolysates of sardine (*S. pilchardus*) and horse mackerel (*T. mediterraneus*) for the microencapsulation of fish oil by spray-drying, *Food Chemistry* **2016**, *194*, 1208-1216, doi: 10.1016/j.foodchem.2015.08.122
20. Tirgar, M.; Jinap, S.; Zaidul, I.S.M.; Mirhosseini, H., Suitable coating material for microencapsulation of spray-dried fish oil, *Journal of Food Science and Technology* **2015**, *52*(7), 4441-4449, doi: 10.1007/s13197-014-1515-3
21. Hădărugă, D. I.; Birău (Mitroi), C.L.B.; Gruia, A.T.; Păunescu, V.; Bandur, G.N.; Hădărugă, N.G., Moisture evaluation of β-cyclodextrin/fish oils complexes by thermal analyses: A data review on common barbel (*Barbus barbus* L.), Pontic shad (*Alosa immaculata* Bennett), European wels catfish (*Silurus glanis* L.), and common bleak (*Alburnus alburnus* L.) living in Danube river, *Food Chemistry* **2017**, *236*, 49-58, doi: 10.1016/j.foodchem.2017.03.093

22. Lee, C.-M.; Kim, M.-H.; Na, H.-S.; Kim, J.; Lee, K.-Y., The effect of caseinate on inclusion complexes of γ-cyclodextrin for oxidative stabilization of fish oil, *Biotechnology and Bioprocess Engineering* **2013**, *18*, 507-513, doi: 10.1007/s12257-012-0752-4
23. Ünlüsayin, M.; Hădărugă, N.G.; Rusu, G.; Gruia, A.T.; Păunescu, V.; Hădărugă, D.I., Nano-encapsulation competitiveness of omega-3 fatty acids and correlations of thermal analysis and Karl Fischer water titration for European anchovy (*Engraulis encrasicolus* L.) oil/β-cyclodextrin complexes, *LWT - Food Science and Technology* **2016**, *68*, 135-144, doi: 10.1016/j.lwt.2015.12.017
24. Hădărugă, N. G.; Hădărugă, D. I.; Păunescu, V.; Tatu, C.; Ordodi, V. L.; Bandur, G.; Lupea, A. X., Thermal stability of the linoleic acid/α- and β-cyclodextrin complexes. *Food Chemistry* **2006**, *99*, 500-508, doi: 10.1016/j.foodchem.2005.08.012
25. Duchêne, D.; Bochot, A., Thirty years with cyclodextrins, *International Journal of Pharmaceutics* **2016**, *514*, 58-72, doi: 10.1016/j.ijpharm.2016.07.030
26. Kurkov, S.V.; Loftsson, T., Cyclodextrins, *International Journal of Pharmaceutics* **2013**, *453*, 167-180, doi: 10.1016/j.ijpharm.2012.06.055
27. Astray, G.; Gonzalez-Barreiro, C.; Mejuto, J. C.; Rial-Otero, R.; Simal-Gandara, J., A review on the use of cyclodextrins in foods. *Food Hydrocolloids* **2009,** *23*, 1631-1640, doi: 10.1016/j.foodhyd.2009.01.001
28. Del Valle, E. M. M., Cyclodextrins and their uses: a review. *Process Biochemistry* **2004,** *39*, 1033-1046, doi: 10.1016/S0032-9592(03)00258-9