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Cyclodextrin / Danube common rudd (*Scardinius erythrophthalmus* L.) oil complexes – synthesis and characterization

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Abstract

Common rudd (*Scardinius erythrophthalmus* L.) is a fish species that are living in Europe, including the Danube river. It is a trend regarding the use of wild fish species for various food applications as well as related fields. Common rudd can be a valuable *omega-3* fatty acid source for such applications.

In this study, the separation and evaluation of the fatty acid profile of common rudd oil from Danube river had performed. Moreover, the protection/stabilization of *omega-3* containing common rudd oil by cyclodextrin complexation had also been realized. The gas chromatography-mass spectrometry analysis of the derivatized common rudd oil (to the corresponding methyl esters) reveals an important content of *omega-3* fatty acid glycerides (about 4.95 % of EPA – eicosapentaenoic acid and 2.5 % of DHA – docosahexaenoic acid, both as methyl esters). β-Cyclodextrin and 2-hydroxypropyl-β-cyclodextrin had used for molecular encapsulation of common rudd oil at 1:1 and 3:1 molar ratios by kneading method. Recovering yields of cyclodextrin/common rudd oil complexes were 65.4-82.3 %, higher for β-cyclodextrin cases. Thermogravimetry-differential thermogravimetry analysis of cyclodextrin/common rudd oil complexes reveals different behavior against the commercial β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin as well as between natural and semi-synthetically modified cyclodextrin/common rudd oil complexes. The main difference was observed on the mass loss corresponding to water release (or other solvents used for obtaining of complexes). This mass loss was higher for β-cyclodextrin/common rudd oil complexes (5.9 % and 7.7 % for the cyclodextrin/fish oil molar ratio of 1:1 and 3:1, respectively) in comparison with the 2-hydroxypropyl-β-cyclodextrin/common rudd oil complexes (2.7-2.9 % for both ratios). No significant behavior was observed for higher temperature intervals, revealing the thermal stability of both types of cyclodextrin/common rudd oil complexes.

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

1. Introduction

Common rudd (*Scardinius erythrophtalmus* L.) is a freshwater fish species from *Cyprinidae* family living in Europe. It can be found in groundwater, as well as in rivers from plain and hill regions. They are shoaling and schooling fishes [1,2]. Generally, common rudd species are living two-three years and have 20-30 cm length, but 17 years old of common rudd can be found [1,3-5]. Common rudd is

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classified as “Least concern fish species” (LC) and have less studied for the possibility to use the valuable components, especially *omega-3*-containing fish oil (The IUCN Red List of Threatened Species).

Polyunsaturated fatty acids and their glycerides in fish oils are the most important for the human health. Among these, *omega-3* fatty acids such as (all-*Z*)-5,8,11,14,17-eicosapentaenoic (EPA) and (all-*Z*)-docosa-4,7,10,13,16,19-hexaenoic acids (DHA) are the most important for the human health [6,7]. EPA have a positive impact for the neuronal diseases, while DHA influences the vasodilator mechanism and reduces the blood pressure, being important for the ameliorating the cardio-vascular diseases. Moreover, they cannot be biosynthesized by humans and must be taken from the diet. On the other hand, polyunsaturated fatty acids and their glycerides are easily oxidized and degraded during the fish oil separation, storage and processing for obtaining *omega-3* supplemented food products [8]. They are radically split under oxidative conditions and form free radicals or degraded compounds such as peroxi, oxi and *C*-terminal radicals, as well as hydroperoxides, aldehydes and oxo-acids. These degrading compounds are very harmful for humans and compounds such as aldehydes are “off-flavor” compounds.

There are only few studies regarding the fatty acid profile of common rudd oils. Some of them are related to the lipid profile of oils separated from the muscle, after various feeding conditions [9]. Important DHA concentrations of 6.4-10.1 % were observed for fishes fed with formulated commercial dry diet, while EPA was less concentrated (2.9-4.1 %). Wild common rudd species living in lakes of Turkey revealed higher contents of *omega-3* fatty acids, as derivatized methyl esters quantified by gas chromatography-mass spectrometry (GC-MS) [10]. DHA had a relative concentration of 17.9 %, while EPA had only 0.34 %. However, the *omega-3/omega-6* ratio for the common rudd oils were higher than 2. There are some studies on the bioaccumulation of heavy metals in the common rudd species from the Danube basin [3,5,11], but no lipid profile were determined for such species living in this region.

Disadvantages of polyunsaturated fatty acid glycerides from fish oils can be reduced by micro- and nanoencapsulation [12-15]. Among nanoencapsulation matrices, cyclodextrins have many advantages due to both geometrical and hydrophobic compatibility with the fish oil glycerides. Cyclodextrins are cyclic oligosaccharides formed by six to eight glucopyranose units for the natural α-, β- and γ-cyclodextrin [16-18]. Hydroxyl groups are oriented to the exterior of the structure having truncated cone architecture. The inner cavity is hydrophobic and can encapsulates hydrophobic molecules such as fatty acid moieties from the fish oils [19-23]. The solid host-guest complex can have better water solubility and stability against degradation due to the protection to the access of oxygen. On the other hand, such complexes have controlled release properties and can be useful for *omega-3* enhanced food products, because natural cyclodextrins are GRAS approved (Generally recognized as safe, GRAS, of the American Food and Drug Administration, FDA).

In this study, the nanoencapsulation of common rudd oil from Danube river by natural and semi-synthetically modified cyclodextrins have been investigated for the first time.

2. Methods

2.1. Fish samples

Common rudd samples were manually fished out from the Danube river (Iron Gates II – Călăraşi sector, Mehedinţi county, Romania) during September 2015, according to the European Directives related to the protection of animals at the time of killing (Council Regulation N° 1099/2009 of 24 September 2009), the protection of animals used for scientific purposes (Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010), the 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 (Regulation N° 853/2004 and N° 854/2004 of the European Parliament and of the Council of 29 April 2004), and the rules on organic production and labelling of organic products with regard to organic production, labelling and control (Commission Regulation N° 889/2008 of 5 September 2008). The main characteristics of common rudd samples were: mean length 29 cm and the mean weight/unit 294 g. All important parts of the fish were separated and refrigerated for one day until the isolation of the fish oil.

2.2. Separation of the fish oil

Common rudd oil was separated from the muscle part using the heating-pressing method [20,22]. Finely grinded muscle samples of 825 g were mixed with distilled water at a mass ratio of 1:2, boiled for 90 minutes in a pressured aluminum vessel (Tefal Classic 6L, Rumilly, Haute-Savoie, France), filtered at normal pressure and the residue was pressed in a steel-made pressing equipment (Naumann NM-120, SC SFR Home Equipment SRL, Bucharest, Romania). The raw fish oil was separated by centrifugation for 15 minutes at 3200 rpm and 20 °C (Heraeus AG, Hanau, Germany). The clear common rudd oil was dried over anhydrous sodium sulphate (p.a., Merck & Co., Inc., Kenilworth, NJ, USA) and stored at 4 °C until derivatization or cyclodextrin complexation. Common rudd oil was obtained as duplicate samples.

2.3. Derivatization of fish oil

Common rudd oil was derivatized to the corresponding fatty acid methyl esters (FAMEs) using methanol-boron trifluoride method. Approximately 175 mg of common rudd oil was transesterified to FAMEs in the presence of 5 mL $MeOH{\cdot}BF_3$ (20 % BF_3, Merck & Co., Inc., Kenilworth, NJ, USA) for 30 minutes in a 100 mL flask equipped with a reflux condenser. Then, 10 mL hexane (GC-grade, Sigma-Aldrich, St. Louis, MO, USA) was added and the refluxing was continued for another 30 minutes. The hexane solution of FAMEs was separated in the neck of the flask by adding sufficient NaCl saturated solution. The organic layer was separated and dried over anhydrous sodium sulphate.

2.4. Gas chromatography-mass spectrometry (GC-MS) analysis

Derivatized common rudd oil was analyzed by GC-MS in order to identify and quantify the corresponding FAMEs from the fish oil. A GC Hewlett Packard 6890 Series gas chromatograph coupled with a Hewlett Packard 5973 Mass Selective Detector (Agilent Technologies, Santa Clara, California, USA) have been used, with the following conditions: Zebron 5-MS column (30 m × 0.25 mm × 0.25 μm), temperature program of 50 °C (1 min), 50 - 300 °C (6 °C/min), 300 °C (5 min), with a solvent delay of 7 min, injection volume of 2 μL, scanning range of 50-500 amu, helium flow of 1 mL/min, ionization energy of 70 eV and source temperature of 150 °C.

The acquisition and handling of the GC-MS data have been performed using the Enhanced MSD ChemStation ver. D.02.00.275/2005 package (Agilent Technologies, Santa Clara, California, USA), while the structural identification of FAMEs was performed by two methods (using the retention indices for the standard FAMEs – FAME37, Sigma-Aldrich, St. Louis, MO, USA – determined with the help of C_8-C_{20} alkane standard mixture – Sigma-Aldrich, St. Louis, MO, USA (1), and 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)).

2.5. Obtaining of cyclodextrin / common rudd oil complexes

Cyclodextrin / common rudd oil complexes were obtained by kneading method [20,22]. The cyclodextrin/fish oil molar ratios were 1:1 and 3:1 (the mean molar mass of the common rudd oil was determined from the fatty acid profile as 900.4 Da). The kneading time was 30 minutes at ~50 °C, in the presence of ethanol:water mixture at a ratio of 2:1 (*v/v*). The cyclodextrin:solvent ratio was 0.2-0.4 (*m/v*). Two types of cyclodextrins had used: natural β-cyclodextrin (hydrate, >98 %, CycloLab, Budapest, Hungary) and a semi-synthetically modified structure, 2-hydroxypropyl-β-cyclodextrin (hydrate, D.S. ~4.5, >98 %, CycloLab, Budapest, Hungary). Complexes were then dried at room temperature, grinded and stored at 4 °C until further analyses. β-Cyclodextrin/common rudd oil complexes at 1:1 ratio were obtained as duplicates.

2.6. Thermogravimetry - differential thermogravimetry (TG-DTG) analysis

Water or moisture content of cyclodextrin/common rudd oil complexes, as well as the degradation of the host-guest supramolecular system compounds were evaluated by TG-DTG. A Netzsch TG 209F1 Libra equipment (Netzsch Group, Selb, Germany) with a program temperature of 30-400 °C and a heating rate of 10 °C/min had used. All determinations were performed under nitrogen, with a purge flow of 20 mL/min and a protective flow of 40 mL/min. The acquisition and handling of the TG-DTG data were performed using Proteus® Software for Thermal Analysis ver. 6.1.0 (Netzsch Group, Selb, Germany).

2.7. Statistical analysis

Classical analysis of variance (ANOVA) had used for data handling from the GC and TG-DTG analyses (Pearson correlation coefficient, *r*, *F* test and standard deviations, *s*).

3. Results and discussion

3.1. Fatty acid profile of common rudd oil

Common rudd oil was obtained with a relatively low yield of ~1.1 %, but this is due to the low lipid content of muscle part. Other common rudd parts have higher lipid content, but this aspect was not discussed here. However, the goal of the study was to evaluate the lipid profile from the comestible part of this fish.

GC-MS analysis of the derivatized common rudd oil reveals many fatty acids (as methyl esters) and other compounds, mainly aldehydes (as dimethyl acetals), but also oxo-acids (derivatized to methyl esters and dimethyl acetals) or vicinal dihydroxylated acids (as esters). Unfortunately, low concentrations of non-derivatized free acids were also identified (appropriately counted) (Figure 1).

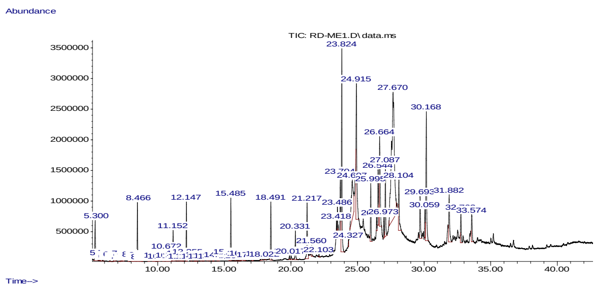

Figure 1. Gas chromatogram from the GC-MS analysis of the derivatized common rudd oil

Saturated fatty acids (SFAs) were the most concentrated in the common rudd oils, but *omega-3* fatty acids were also important (Table 1). SFAs were found in a relative concentration of 37.75 %, the most important being stearic, palmitic and myristic acids, with concentrations of 23.6, 12.6 and 1.6 %, respectively. All others FAs were mono- and polyunsaturated compounds (MUFAs and PUFAs), that were found in double relative concentrations for the second class (24.2 % for PUFAs and 12.9 % for MUFAs). By far, the most important FA from the MUFA class was oleic acid (relative concentration of 11 %, Table 1), while the PUFAs had a wide distribution in their concentrations. Thus, linoleic acid was quantified at 16.7 % in the common rudd oil (as methyl ester), while the valuable *omega-3* fatty acids, named (all-*Z*)-5,8,11,14,17-eicosapentaenoic (EPA) and (all-*Z*)-docosa-4,7,10,13,16,19-hexaenoic acids (DHA), were found in relative concentrations of 4.95 % and 2.5 %, respectively (Table 1).

It is well known that an *omega-3/omega-6* ratio higher than 0.2 is benefic for the human health, EPA and DHA from *omega-3* fatty acid class being very important for ameliorating cardio-vascular and neuronal diseases [8]. This ratio was more than double for the common rudd oil (0.44, Table 1). This value indicates that the common rudd oil from Danube river is a valuable *omega-3* source, even that the absolute fatty acid content (as glycerides) in the comestible part is relatively low.

Table 1. The relative concentrations of the main fatty acids (as methyl esters) identified and quantified by GC-MS from the common rudd oils (means of duplicate analysis, *RSD* <5 %)

Nº	MS Identification	*Retention Index*	*Area (%)*
1	Myristic acid, methyl ester	1725	1.60
2	Palmitoleic acid, methyl ester	1910	1.85
3	Palmitic acid, methyl ester	1933	12.55
4	Linoleic acid, methyl ester	2097	16.75
5	Oleic acid, methyl ester	2108	11.01
6	Stearic acid, methyl ester	2127	23.60
7	**EPA**, 5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	2256	4.95
8	**DHA**, 4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	2432	2.50
	Other compounds		*25.19*
	*Sum(SFA)**		37.75
	*Sum(MUFA)**		12.86
	*Sum(PUFA)**		24.20
	*Sum(omega-9)**		11.01
	*Sum(omega-6)**		16.75
	*Sum(omega-3)**		7.45
	*omega-3/omega-6**		0.44

* *SFA* – saturated fatty acid, *MUFA* – monounsaturated fatty acid, *PUFA* – polyunsaturated fatty acid, *omega-3/6/9* – fatty acids from *omega-3/6/9* classes, *omega-3/omega-6* – the ratio of the relative concentrations of *omega-3* and *omega-6* fatty acids (as methyl esters)

3.2. Cyclodextrin / common rudd oil complexes

Cyclodextrin / common rudd oil complexes were obtained by kneading method that has the advantage related to the minimum loss of components during the complexation process. On the other hand, the molecular encapsulation process is less appropriate in comparison with co-crystallization from solution. However, in this case of cyclodextrin / fish oil complexation is impossible to solubilize both components in the same solvent mixture and more hydrophobic solvents can be encapsulated instead the fish oil glycerides by competition. Thus, the recovering yields of 77.6 (±4.2) % were obtained for the β-cyclodextrin / common rudd oil complexes at 1:1 molar ratio. Higher yields were obtained for the corresponding complexes obtained at 3:1 molar ratio, probably due to a higher content of hydration water in the complexes containing much more cyclodextrin (see below for the water evaluation of complexes). A recovering yield of only 65.4 % was obtained for 2-hydroxypropyl-β-cyclodextrin / common rudd oil complex at 1:1 molar ratio.

Thermogravimetry is a widely used technique to evaluate the thermal behavior of cyclodextrin complexes during heating. Both cyclodextrin complex and starting cyclodextrin are hydrated and the mass loss up to ~140 °C can indicate the water or moisture content of such supramolecular systems. On the other hand, the mass loss after this temperature can indicate the dissociation of some encapsulated volatile compounds up to ~270 °C (and possibly strongly-retained water or solvent molecules). Generally, after this temperature, the degradation of cyclodextrin molecule appears.

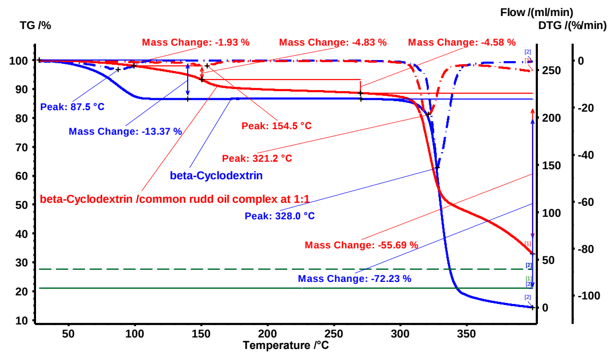

Figure 2. Superimposed thermograms from the TG-DTG analysis of β-cyclodextrin / common rudd oil complex obtained at a molar ratio of 1:1 and commercial β-cyclodextrin (only duplicates "*a*" were presented)

A significant mass loss up to ~270 °C for both considered temperature intervals (<140 °C and 140-270 °C) for β-cyclodextrin / common rudd oil complexes obtained at 1:1 molar ratio have been observed (Figure 2). Thus, the mass loss for the first interval was 5.94 (±1.17) % and slightly lower for the second interval, 3.72 (±1.22) %. On the other hand, these TG parameters clearly differ for the complex obtained at 3:1 molar ratio. It was observed a high water/solvent loss up to 140 °C of 7.7 % and only 0.4 % for the second interval. These values for the first interval are considerably lower than in the case of commercial β-cyclodextrin, having a mass loss of 13.3 (±0.1) % for the first interval and only 0.05 (±0.0) % for the second one. Similar differences also appear between DTG values of β-cyclodextrin and its complexes with common rudd oil.

The most important one is the considerably increases of the DTG peak corresponding to moisture release from 85.7 (±2.5) °C for β-cyclodextrin to 151.9 (±3.7) °C and 118.2 °C for β-cyclodextrin / common rudd oil complexes obtained at 1:1 and 3:1 molar ratio, respectively (Figures 2 and 3).

This thermal behavior of β-cyclodextrin / common rudd oil complexes revealed that the encapsulation of glycerides from fish oil occurs because of the considerable reduction of the content of hydration water molecules. Moreover, the remaining water molecules are more strongly-retained in the complex than in the commercial β-cyclodextrin.

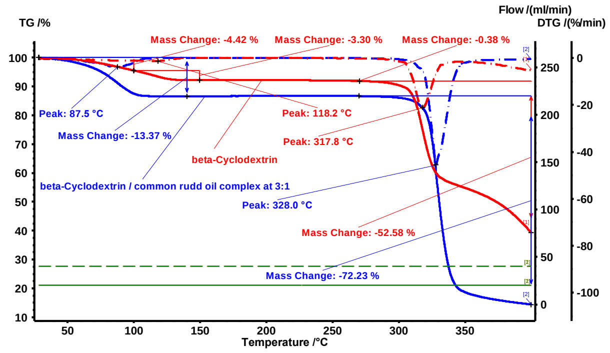

Figure 3. Superimposed thermograms from the TG-DTG analysis of β-cyclodextrin / common rudd oil complex obtained at a molar ratio of 3:1 and commercial β-cyclodextrin (duplicate "*a*")

The TG-DTG parameters have different values for 2-hydroxypropyl-β-cyclodextrin / common rudd oil complexes and the starting 2-hydroxypropyl-β-cyclodextrin. However, the mass loss corresponding to water release is significantly lower than in the case of β-cyclodextrin. These are only 2.94 % for 2-hydroxypropyl-β-cyclodextrin / common rudd oil complex and 4.4 % for the commercial 2-hydroxypropyl-β-cyclodextrin. The complex has a mass loss of 1.1 % for the second interval, more important than for the starting 2-hydroxypropyl-β-cyclodextrin. The DTG parameters clearly differ for the complex and hydrated 2-hydroxypropyl-β-cyclodextrin, such as for β-cyclodextrin.

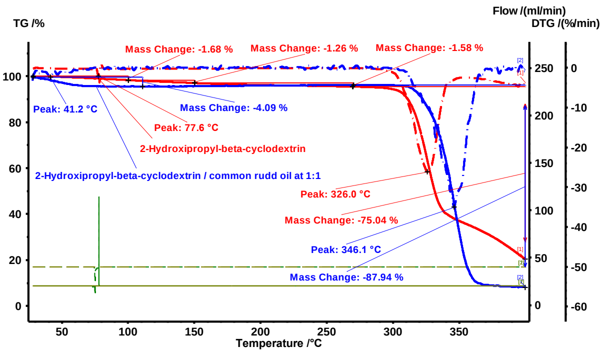

Figure 4. Superimposed thermograms from the TG-DTG analysis of 2-hydroxypropyl-β-cyclodextrin / common rudd oil complex obtained at a molar ratio of 1:1 and commercial 2-hydroxypropyl-β-cyclodextrin

The DTG temperature peak corresponding to moisture release (water and/or solvent molecules) appears at very low values for 2-hydroxypropyl-β-cyclodextrin than for the corresponding common rudd oil complex (34.3 °C and 70.6 °C, respectively, Figure 4). It seems that more surface water molecules exist in the starting 2-hydroxypropyl-β-cyclodextrin than in the corresponding complex.

There are no important differences between the decomposition of commercial cyclodextrins and their common rudd oil complexes. However, these differences are smaller for β-cyclodextrin in comparison with the case of 2-hydroxypropyl-β-cyclodextrin (328.4 °C and 317.8-319.1 °C for β-cyclodextrin and its complexes, 338.8 °C and 316.4 °C for 2-hydroxypropyl-β-cyclodextrin and its complexes) (Figures 2-4).

4. Conclusion

This is the first study on the cyclodextrin nanoencapsulation of Danube common rudd (*Scardinius erythrophthalmus* L.) oil. Significant EPA and DHA contents in the common rudd oil have been determined. Moreover, the *omega-3/omega-6* ratio is more than two times higher in comparison with the lowest accepted limit for a beneficial effect on human health. Consequently, this fish oil can be used on the ameliorating of some cardio-vascular or neuronal diseases.

The valuable *omega-3* fatty acid constituents of common rudd oil have disadvantages related to very low oxidative and thermal stability, as well as the difficulty to use this hydrophobic liquid system. Consequently, nanoencapsulation of common rudd oil constituents by natural β-cyclodextrin and its semi-synthetically modified 2-hydroxypropyl-β-cyclodextrin (both approved for food and/or pharmaceutical applications) was successful. The molecular encapsulation of the Danube fish oil constituents, including *omega-3*-based triglycerides, have been demonstrated by thermogravimetry-differential thermogravimetry. As a consequence, this new Danube common rudd oil-based nanomaterial can be proposed as valuable source for *omega-3* enhanced food supplements or fortified food products.

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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