Preprint Title	Discrimination of β-cyclodextrin/hazelnut (*Corylus avellana* L.) oil/ flavonoid glycoside and flavonolignan ternary complexes by Fourier-transform infrared spectroscopy coupled with principal component analysis
Authors	Nicoleta G. Hadaruga, Gabriela Popescu, Dina Gligor (Pane), Cristina L. Mitroi, Sorin M. Stanciu and Daniel I. Hadaruga
Publication Date	03 Jan. 2023
Article Type	Full Research Paper
Supporting Information File 1	Supporting Information File 1.pdf; 5.1 MB
ORCID® iDs	Daniel I. Hadaruga - https://orcid.org/0000-0002-6326-413X

Discrimination of β-cyclodextrin/hazelnut (*Corylus avellana* L.) oil/flavonoid glycoside and flavonolignan ternary complexes by Fourier-transform infrared spectroscopy coupled with principal component analysis

Nicoleta G. Hădărugă [1,2], Gabriela Popescu [3], Dina Gligor (Pane) [2], Cristina L. Mitroi [1], Sorin M. Stanciu [4], Daniel I. Hădărugă [2,5,*]

[1] Department of Food Science, University of Life Sciences "King Mihai I" from Timişoara, Calea Aradului 119, 300645 - Timişoara, Romania

[2] Doctoral School "Engineering of Vegetable and Animal Resources", University of Life Sciences "King Mihai I" from Timişoara, Calea Aradului 119, 300645 - Timişoara, Romania

[3] Department of Rural Management and Development, University of Life Sciences "King Mihai I" from Timişoara, Calea Aradului 119, 300645 - Timişoara, Romania

[4] Department of Economy and Company Financing, University of Life Sciences "King Mihai I" from Timişoara, Calea Aradului 119, 300645 - Timişoara, Romania

[5] Department of Applied Chemistry, Organic and Natural Compounds Engineering, Polytechnic University of Timişoara, Carol Telbisz 6, 300001-Timişoara, Romania

Corresponding author: Daniel Ioan Hădărugă – daniel.hadaruga@upt.ro

Abstract

This is the first study aiming the discrimination of β-cyclodextrin (β-CD)/hazelnut (*Corylus avellana* L.) oil/antioxidant ternary complexes through Fourier-transform infrared spectroscopy coupled with principal component analysis (FTIR-PCA). These innovative materials combine the characteristics of the three components in order to enhances the

properties of ternary complexes such as the onsite protection against oxidative degradation of hazelnut oil unsaturated fatty acid glycerides, increased apparent water solubility and bioaccessibility of the hazelnut oil components and antioxidants, or controlled release of bioactive compounds (fatty acid glycerides and antioxidant flavonoids, namely hesperidin, naringin, rutin and silymarin). The appropriate method for obtaining the ternary complexes was kneading at various molar ratios (1:1:1 and 3:1:1 for β-CD hydrate:hazelnut oil (average molar mass of 900 g/mol):flavonoid). Recovering yields of the ternary complexes were in the range of 51.5-85.3%, higher for 3:1:1 samples. Their thermal stability was evaluated by thermal analyses. Discrimination of the ternary complexes was easily performed through the FTIR-PCA coupled method, especially based on the stretching vibrations of CO groups in flavonoids and/or CO/CC groups in ternary complexes at 1014.6(±3.8) and 1023.2(±1.1) cm^{-1} along the second PCA component (PC_2), respectively. The wavenumbers are more appropriate for discrimination than the corresponding intensities of the specific FTIR bands. On the other hand, ternary complexes were clearly discriminated from the starting β-CD hydrate along the first component (PC_1) by all FTIR band intensities and along the PC_2 by the wavenumber of the asymmetric stretching vibrations of the CH groups at 2922.9(±0.4) cm^{-1} for ternary complexes and 2924.8(±1.4) cm^{-1} for β-CD hydrate. The first two PCA components explain 70.38% from the variance of the FTIR data (from a total number of 26 variables). Other valuable classifications were obtained for the antioxidant flavonoids, with a high similarity for hesperidin and naringin, according to FTIR-PCA, as well as for ternary complexes depending on molar ratios. The FTIR-PCA coupled technique is a fast, nondestructive and cheap method for evaluation of the quality and similarity/characteristics of these new types of cyclodextrin-based ternary complexes having enhanced properties and stability and that can have applications in food supplements or functional food products.

Keywords

Antioxidant; Cyclodextrin; Flavonoid; Hazelnut vegetable oil; Ternary supramolecular inclusion complex

Introduction

Cyclodextrins (CDs) are studied for more than one hundred years due to their unique properties related to the spatial macrocyclic structure that involves six to eight α-D-glucopyranose (Glc*p*) units for the natural α-, β- and γ-CD [1-3]. All hydroxyl groups are oriented to the exterior of the macrocycle, providing high water solubility. On the other hand, the tetrahydropyrane moieties of the Glc*p* units provide the hydrophobic property of the CD cavity [4]. As a consequence, CDs can molecular nanoencapsulate hydrophobic molecules or hydrophobic moieties of the bioactive compounds that are geometrically compatibles [5]. The supramolecular inclusion complexes can provide enhanced water solubility and bioavailability/bioaccessibility to the nanoencapsulated bioactive compounds, higher oxidative and thermal stability or photostability of the labile compounds and their controlled release [6, 7].

Vegetable oil and animal fat components that especially consist of fatty acid (FA) triglycerides are appropriate guest molecules for obtaining CD-based complexes. The hydrophobic long-chain thin moieties of the FA glycerides allow obtaining CD:FA glyceride complexes at various molar ratios [8, 9]. Among the enhancing of the apparent water solubility and bioaccessibility of the oil and fat components, the oxidative stability of the polyunsaturated FA glycerides or free FAs are significantly increased by CD nanoencapsulation. Thus, high thermal stability of the free linoleic acid encapsulated into α-CD by co-crystallization, even the degradation temperature was increased to 100 °C [10].

Omega-3 FA glycerides such as eicosapentaenoic and docosahexaenoic acid glycerides (EPA and DHA glycerides) from fish oils are less stable against oxidation. Their thermal and oxidative stabilities were significantly increased by CD nanoencapsulation such as for common barbel (*Barbus barbus* L.), Pontic shad (*Alosa immaculata* Bennett), European wels catfish (*Silurus glanis* L.), common bleak (*Alburnus alburnus* L.), common nase (*Chondrostoma nasus* L.), Atlantic salmon (*Salmo salar* L.), and European anchovy (*Engraulis encrasicolus* L.) oils [11-14]. Their stability and the level of degradation compounds were determined by thermal methods (thermogravimetry-differential thermogravimetry, TG-DTG, and differential scanning calorimetry, DSC) and gas chromatography-mass spectrometry (GC-MS), respectively. The addition of sodium caseinate during the CD complexation of fish oils can further increase the oxidation stability and retardation of odor [15]. Poultry lipids have high contents of mono- and polyunsaturated FA glycerides, especially oleic and linoleic acid glycerides. The chicken lipids stability was significantly increased by β-CD complexation, as was demonstrated by both thermal (TG-DTG and DSC) and chromatographic (GC-MS for the degradation compounds, i.e. aldehydes, formylated carboxylic acids, or dicarboxylic acids) methods [16]. Also, vegetable oils containing unsaturated FA moieties were stabilized by CD complexation. Common bean (*Phaseolus vulgaris* L.) oil has 55.7-58.8% polyunsaturated FA relative content (as methyl esters), with an important fraction of omega-3 α-linolenic acid (ALA) of 14.1-18.9%. It was stabilized by β-CD complexation, with an increased content of the omega-3 FAs into the nanoencapsulated oil of >14% [17]. Essential oil components are also compatible guests for CD nanoencapsulation, even as unique compounds or essential oil mixtures (e.g., linalool, nerolidol, nootkatone, or sweet basil – *Ocimum basilicum* L., caraway – *Carum carvi* L., coriander – *Coriandrum sativum* L., fennel – *Foeniculum vulgare* Mill., dill – *Anethum graveolens* L., garlic – *Allium sativum* L., juniper – *Juniperus communis* L., clove – *Syzygium*

aromaticum (L.) Merr. & L.M., and perilla – *Perilla frutescens* (L.) Britton essential oils, respectively) [18-24].

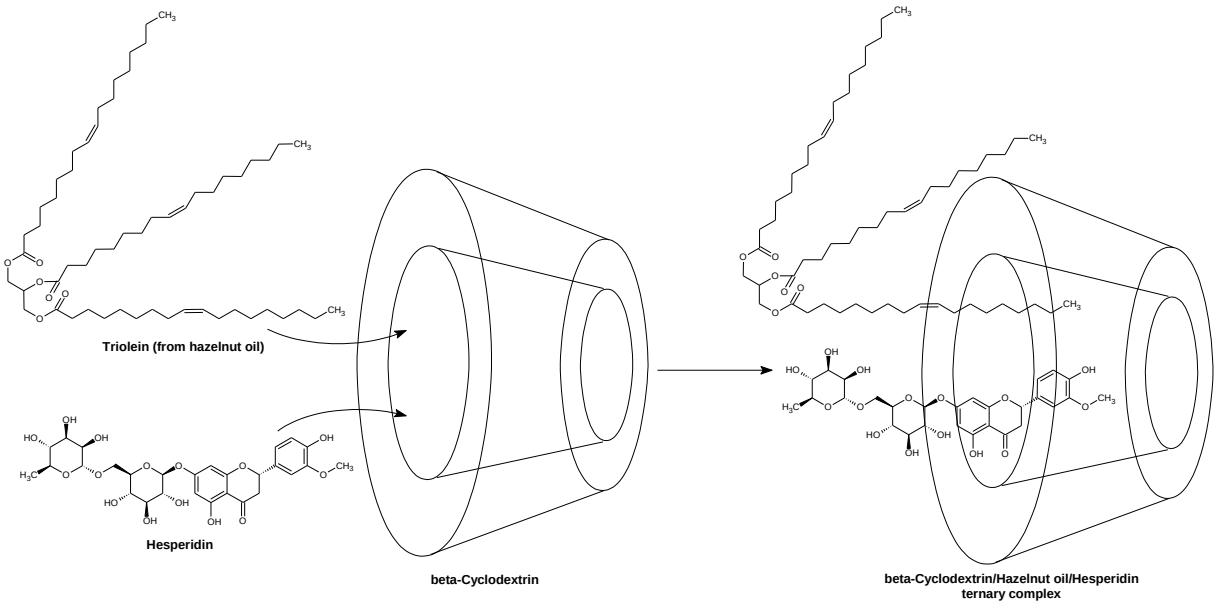
Among vegetable oils, hazelnut (*Corylus avellana* L.) oil is a valuable source of oleic acid bound in various triglyceride combinations. The highest content was observed for triolein, OOO (61-77.5% relative concentration), but OOL (glyceryl 1,2-dioleate 3-linoleate) and OOP (glyceryl 1,2-dioleate 3-palmitate) also had high relative contents of 10.5-22.8 and 6.4-11.0%, respectively [25]. The fatty acid profile of the hazelnut oil revealed a significantly high content of oleic acid (as methyl ester, determined by GC-MS) of 74.2-82.8%, among linoleic acid and even ALA (9.8-18.7 and ~0.1%, respectively) [26, 27]. The very high content of unsaturated fatty acid glycerides significantly decreases the hazelnut oil stability. Only one study was performed on the nanoencapsulation of hazelnut oil by γ-CD by co-precipitation method and the thermal decomposition of the complex was evaluated by TG [28].

A way of enhancing the oxidative stability of oils and fats is the addition of antioxidants. Among food grade antioxidants, natural polyphenols such as flavonoids and flavonoid-based extracts are widely used [29-35]. Generally, flavonoids have a high number of phenolic hydroxyl groups that provide the antioxidant activity. On the contrary, the presence of highly hydrophilic groups such as saccharide moieties for flavonoid glycosides reduces the level of hydrophobic interaction with the CD cavity. However, less hydrophilic moieties of flavonoid glycosides or flavonolignans can favorable interact with CDs (i.e., 4-hydroxyphenyl, 3,4-dihydroxyphenyl- and 3-methoxy-4-hydroxyphenyl moieties in the hesperidin, naringin, and rutin aglycones or silibinin). There are many studies revealing the interaction of flavonoids, flavonoid glycosides and flavonolignans by CDs, especially for obtaining binary complexes [36-43].

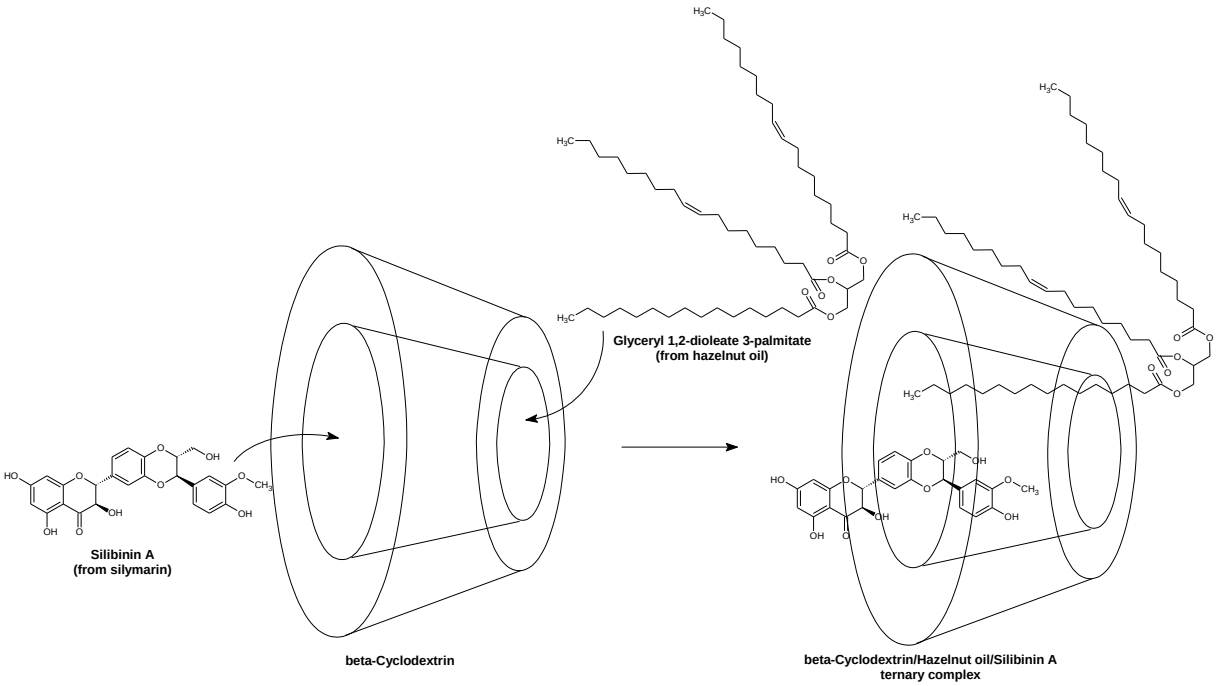
In a ternary complex (considering the vegetable oil as a single component), an antioxidant can protect onsite the labile FA glycerides by co-nanoencapsulation into CD cavity. However, it is very difficult to evaluate the way of interaction in such multicomponent system. There are some studies on the CD-based ternary complexes, but they don't deal neither with triglyceride based vegetable oils nor flavonoid glycosides/flavonolignans. Most of these studies are related to controlled release from the CD complexes of various drugs such as diosmin and polyethylene glycol, haloperidol and lactic acid, cyclosporine A and polyvinyl alcohol, ketoprofen and phospholipids, dihydroartemisinin and lecithin, cefixime and L-arginine, flurbiprofen and naproxen/ketoprofen/ethenzamide [44-53].

Fourier-transform infrared spectroscopy (FTIR) is a very fast, nondestructive and cheap method for evaluation of such ternary complexes. The coupling of FTIR or other spectroscopic or chromatographic techniques with a multivariate statistical analysis method (e.g., principal component analysis, PCA) allows evaluating the similarity/dissimilarity of the complexes, as well as the identification of the variables that have significance for these classifications. FTIR-PCA was successfully applied for discrimination of the raw and thermally processed chicken lipids stabilized by nanoencapsulation in β-CD or the raw and recrystallized β-CD from water and alcohol-water solutions [4, 16]. In other studies, PCA was coupled with GC-MS for classifying β-CD/*Ocimum basilicum* L. essential oil complexes or the raw and thermally processed Mangalitza (*Sus scrofa domesticus*) lipid fractions, as well as with spectrophotometry for discriminating of organic apples (*Malus domestica* Borkh.) on the basis of antioxidant properties and radical scavenging kinetics [21, 54, 55].

The goal of the study was the synthesis for the first time of β-CD/hazelnut (*Corylus avellana* L.) oil/flavonoid glycoside or flavonolignan ternary complexes (Figure 1) and discrimination of these complexes by FTIR-PCA. The new ternary complexes can provide the onsite protection against oxidative degradation of hazelnut oil components, in combination with the protection/stabilization through the CD nanoencapsulation. Moreover, the apparent water solubility, bioaccessibility, bioavailability and controlled release of guest bioactive compounds can also be enhanced by ternary complexation.



(a)



(b)

Figure 1: Schematic representation of the interactions between host (β-cyclodextrin) and guest molecules (flavonoid glycoside/flavonolignan and a fatty acid triglyceride from the hazelnut oil): (a) both triolein from hazelnut oil and hesperidin interact with β-cyclodextrin from the secondary face; (b) glyceryl 1,2-oleate 3-palmitate (from hazelnut oil) interact with the β-cyclodextrin from the primary face, while silibinin A (the main component from silymarin) interact with β-cyclodextrin from the secondary face

Results and Discussion

Synthesis and thermal analysis of ternary complexes

The complexity of the starting materials, especially the hazelnut oil, as well as the differences among their characteristics (hydrophobicity and water solubility) suggest the kneading method as the most appropriate one for obtaining β-CD/hazelnut (*Corylus avellana* L.) oil/flavonoid glycoside or flavonolignan ternary complexes. Kneading allows to recover much more CD complexes in comparison with co-crystallization method because of the lower solvent volumes used for preparation. On the other hand, similar methods to kneading such as

spray-drying do not provide an intimate contact for the three types of components for enough time to reach the association-dissociation equilibrium [1, 21, 56]. In this study, the recovery yields were in the range of 51.5-85.3%, significantly higher for 3:1:1 complexes. Equimolar X1H, X1N, X1R and X1S ternary complexes were obtained with the yields of 57.7(±8.8), 54.6(±1.9), 74.3(±1.8) and 64.7(2.6)%, respectively. For 3:1:1 ternary complexes (single samples) these yields were in the range of 74.5-85.3%. These differences can be explained by the level of hydration, as was determined by TG (see below). The mass loss for 1:1:1 complexes is at a half in comparison with the water content of β-CD, as was indicated by the manufacturer (6.4-7.4% for complexes and 14% for β-CD hydrate). On the other hand, the mass loss of the 3:1:1 complexes is much higher (e.g., 11.8% for X3N complex). As a consequence, the 1:1:1 complexes lose much hydration water than the 3:1:1 complexes, more probably due to a high level of complexation for the first cases. This aspect is confirmed by thermal analysis, especially by DSC.

Both TG-DTG and DSC thermal analyses provide information about the molecular inclusion of guest molecules into the β-CD cavity. Unfortunately, these methods cannot differentiate between the encapsulated components and entrapment efficiency. However, the study aimed to discriminate such ternary complexes only on the basis of FTIR (fast, cheap and nondestructive) and less to evaluate the competitiveness to molecular encapsulation of such multicomponent mixtures (highly hydrophobic FA triglycerides, mono- and diglycerides, free FAs, as well as more hydrophilic flavonoid glycoside, namely hesperidin, naringin and rutin, or flavonolignan – silibinins). According to TG-DTG and DSC analyses, ternary complexes are very stable, at least up to 200 °C and even more if DSC behavior is considered. TG and DTG plots are quite similar for ternary complexes at 1:1:1 molar ratio, in comparison with the β-CD hydrate up to ~200 °C. The only significant difference was observed for the mass loss

corresponding to the water/moisture release up to ~110 °C, with values of 6.37-7.38% and 9.45% for β-CD hydrate, respectively. Lower mass loss for β-CD hydrate in comparison with the water content provided by the manufacturer (maximum 14% by oven drying) can be due to the TG protocol, which assumes the pre-equilibration of the microbalance prior analysis. Consequently, the surface water can be partially loss prior the starting of the analysis. However, the difference of 2-3% for the ternary complexes at 1:1:1 molar ratios can be explained by partially replacing the water molecules during the molecular encapsulation of guest molecules (FA triglycerides and flavonoids). On the other hand, the mass loss for the 3:1:1 ternary complexes is similar to β-CD hydrate or even higher (see Supporting Information File 1, Figures S1-S4 and Tables S1 and S2). This means that an important fraction of β-CD is not involved in the formation of complexes and remain as β-CD hydrate. These observations are in agreement with other studies on the complexation of vegetable (common bean lipids) and fish (common barbel, Pontic shad, European wels catfish, common bleak) oils by CDs [11, 17]. Moreover, this TG behavior does not depend on the method of synthesis (kneading or co-crystallization) or the method of water determination (TG as mass loss or Karl Fischer water titration, KFT) [6, 57]. It was observed that the difference between the water content or TG mass loss up to ~110 °C is lower for CD/flavonoid binary complexes in comparison with the CD/fish (Atlantic salmon or European anchovy) oil binary complexes [12, 14, 37]. TG results are in agreement with the DSC data, where the endothermic calorimetric effect corresponding to water/moisture release is lower for the ternary complexes (378 J/g for X1N and 432 J/g for β-CD hydrate, Supporting Information File 1, Figure S5 and Table S3). There are two aspects that can be observed in DSC and not in TG-DTG analyses. First, the presence of two types of water molecules in the ternary complexes appears at two specific DSC peak temperatures of 44.5 °C for surface water and 82.0 °C for the strongly retained water molecules. If the surface water-related temperature is quite similar to the β-CD

hydrate, the strongly retained water have higher DSC peak temperature value for β-CD (94.7 °C). This observation sustain the partial replacing of strongly retained water molecules during the complexation process. The second observation on DSC results is related to the absence of the endothermal-exothermal calorimetric peak from 218.9 °C in the case of X1N ternary complex. It means that this complex obtained by kneading has amorphous structure, in comparison with the β-CD hydrate, which reveals a calorimetric peak in this region due to the transition of anhydrous β-CD (after water release) from the crystalline to amorphous state [6]. Finally, TG indicates a mass loss of 1.4-4.0% in the range of 110-275 °C for 1:1:1 ternary complexes and only 1.25% for 3:1:1 complexes, in comparison with almost no mass loss for β-CD hydrate (0.05%). The degradation of β-CD appears after 275 °C, with a maximum degradation rate at 299.4-326.0 °C by DTG (the highest for β-CD) and ~322 °C by DSC. The degradation of the encapsulated hazelnut oil components (especially triglycerides) appear at higher temperature of 394-407 °C (DTG and DSC).

Fourier transform infrared spectroscopy (FTIR) of ternary complexes

FTIR is a fast method that allows evaluating the presence of a compound in a complex through the specific bands. β-CD consists of seven α-D-glucopyranose units 1→4 linked into a macrocycle. As a consequence, the FTIR specific bands especially appear for OH, CC and CH/CH_2 bonds and groups. However, CD specific bands also appear for CH group in the CD ring and α-type glycosidic bonds. Thus, the broad FTIR band corresponding to the stretching vibration of the O-H bonds in β-CD and hydration water molecules appear at ~3301 cm^{-1}. A weak band for the asymmetric stretching vibrations of the C-H groups appear at 2924.8(±1.4) cm^{-1}, while the bending vibrations (in-plane, asymmetric and symmetric) of OH and CH groups appear as weak bands in the range of 1205-1643 cm^{-1}. The stretching vibrations of the C-O and C-C groups in glucoside moieties appear as medium-strong bands in the range of

998-1152 cm^{-1}. A specific band for CD appear at 939.2(±1.8) cm^{-1} and is assigned to the stretching vibrations of the C-H groups from the β-CD ring. Also, the band from 852.9(±0.8) cm^{-1} is assigned to the bending vibrations of the C-C-H groups related to the α-type glycosidic bonds in CDs. Other bands appear at wavenumbers lower than 800 cm^{-1} and were tentatively assigned to the banding vibrations of the CH and OCC groups (574-754 cm^{-1}), as well as to the stretching vibrations of the CC bonds at 526.3(±1.3) cm^{-1} [58, 59]. Relevant data on the FTIR analysis of β-CD is presented in Figures 2, 3 and Supporting Information File 1 (Figures S6-S11 and Table S4).

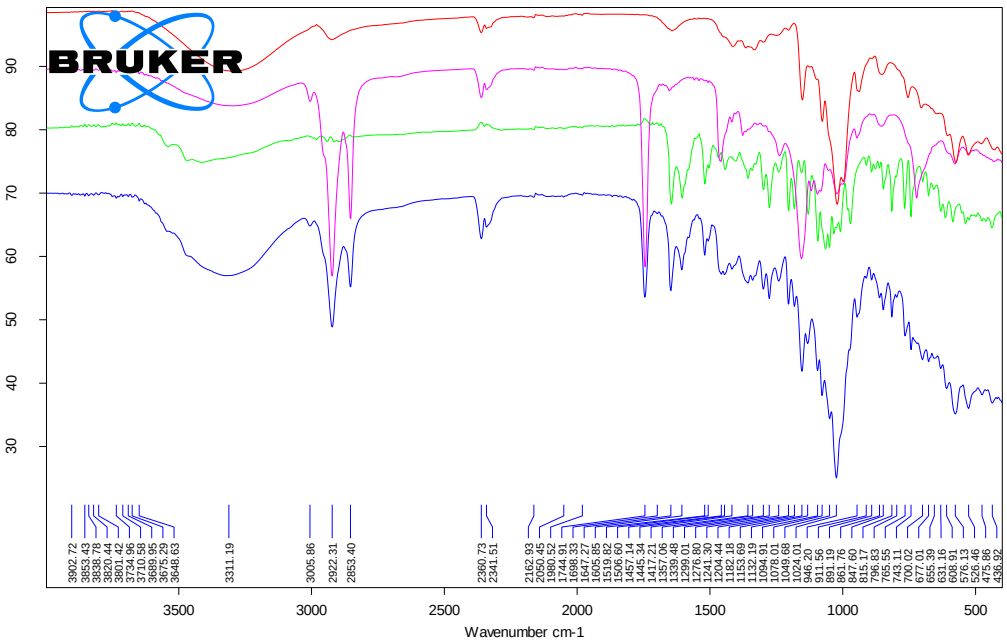


Figure 2: Superposition of the FTIR spectra for β-cyclodextrin/*Corylus avellana* oil/Hesperidin ternary complex at 1:1:1 molar ratio (blue), β-cyclodextrin hydrate (red), *C. avellana* oil (pink) and hesperidin (green)

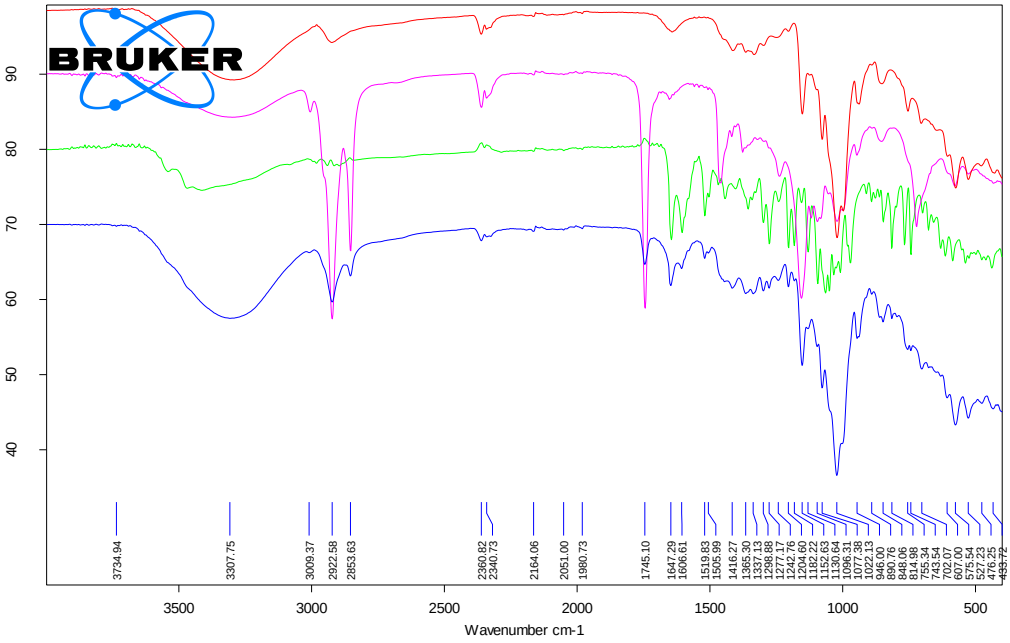


Figure 3: Superposition of the FTIR spectra for β-cyclodextrin/*Corylus avellana* oil/Hesperidin ternary complex at 3:1:1 molar ratio (blue), β-cyclodextrin hydrate (red), *C. avellana* oil (pink) and hesperidin (green)

Vegetable oils and animal fats especially contain FA triglycerides, but mono-, diglycerides and free FAs also exist. As a consequence, the broad band corresponding to the stretching vibrations of the O-H groups only appear from the free fatty acids and water. In the hazelnut samples, this band was observed at 3287.8(±10) cm^{-1}. Very useful in this study was the weak band at 3005(±0.2) cm^{-1}, which correspond to the symmetric stretching vibrations of the =CH groups from the mono- and polyunsaturated FA moieties (especially oleic acid, but also palmitoleic and linoleic acids). The asymmetric and symmetric stretching vibrations of the CH groups provide strong bands at 2952.5(±0.3), 2922.5(±0), and 2853.2(±0) cm^{-1} due to the high number of CH_2 and CH_3 groups in the triglyceride structures. Another important and characteristic FTIR band for glycerides is that corresponding to the stretching vibrations of the esteric C=O groups that appear very strong at 1744(±0) cm^{-1} for hazelnut oil. The stretching vibration of the *cis*RHC=CHR' group was observed at 1652.7(±0.3) cm^{-1}, but as a

281 weak band. Medium and strong bands are those related to the bending vibrations of the CH_2
282 and CH_3 groups at 1458.7(±0.2) cm^{-1}, bending vibrations of the CH_2 groups at 1236.8(±1.3)
283 and 1158.1(±2.3) cm^{-1}, the stretching vibrations of the C-O groups at 1027.9(±5.7) cm^{-1}, as
284 well as the out-of-plane bending vibrations in the C-H groups at 722(±0.1) cm^{-1}. Some
285 degradation/isomerization of oils can be observed at 956.7(±8.7) cm^{-1}, where the band
286 corresponding to the bending vibrations of the C=C groups in *trans*RHC=CHR' groups
287 appear (sometimes at slightly higher values). Details of the FTIR analysis of hazelnut oil
288 samples can be seen in Figures 2, 3 and Supporting Information File 1 (Figures S6-S11 and
289 Table S5) [60].
290
291 Hesperidin, naringin and rutin are flavonoid glycosides derived from the corresponding
292 flavanones (hesperetin and naringenin) and flavonol (quercetin), respectively. They also have
293 a disaccharide moiety connected to the aglycones through the etheric linkage with the
294 hydroxyl groups from the 7 and 3 positions (Figure 1a). On the other hand, silibinins (the
295 main components from silymarin) are flavanonol derivatives, having a coniferyl alcohol
296 moiety connected through the hydroxyl groups from the 3' and 4' positions of the aglycone
297 (Figure 1b). FTIR analysis of these flavonoids revealed stretching and bending vibrations
298 corresponding to OH bonds (phenolic or alcoholic, glycosidic and from the water molecules),
299 CH bonds (especially from CH_2 and CH_3 groups), bands corresponding to the aromatic CC
300 bonds and to the carbonyl C=O bond. The most relevant FTIR band for these compounds is
301 the asymmetric stretching vibrations of the C=O bonds, $\nu^{as}_{C=O}$, which appears around 1633-
302 1651 cm^{-1}. The highest value was observed for silymarin at 1634.1(±0.4) cm^{-1} and the lowest
303 one for rutin at 1651(±0.1) cm^{-1}. Herperidin and naringin have approximately the same values
304 for this band (~1645 cm^{-1}). The stretching vibrations of the O-H bonds in phenolics,
305 glycosidic, or water hydroxyls appear as a broad band in with maximum wavenumbers in the

range of 3263-3541 cm^{-1}. Asymmetric and symmetric stretching vibrations of the C-H bonds in CH_3 and CH_2 groups appear at 2931-2941 cm^{-1}, but FTIR bands also appear at 2982, 2907-2914 and 2876-2897 cm^{-1} in flavonoid glycosides. In these compounds the bending vibrations of the aromatic CC groups appear at 1583-1604 cm^{-1} and ~1518 cm^{-1}, some of these bands being superimposed by the stretching vibrations of the C-C group in the ring C of aglycones. The stretching of a C-C group also appear in silymarin/silibinins at 1509.9(±0.6), while this value is significantly lower for flavonoid glycosides (1502-1504 cm^{-1}). Other bending vibrations were observed for CH bonds in the range of 1393-1468 cm^{-1}, while the stretching vibrations for CC, CO bonds and the bending vibrations for HOC, OCH, HCC groups were superimposed in the range of 1011-1364 cm^{-1}. It must be highlighted the stretching vibration of the O-C groups in all flavonoids, which appear at 968-995 cm^{-1}. Finally, out-of-plane bending vibrations of CH groups and twisting bending vibrations of COH and HCCC groups appear in the range of 742-921 cm^{-1} [61-66]. All wavenumber values corresponding to specific FTIR bands as well as the superimposed FTIR spectra of flavonoids with the other components of the ternary complexes are presented in Figures 2, 3 and Supporting Information File 1 (Figures S6-S11 and Tables S6-S9).

Ternary complexes reveal the medium and strong FTIR bands of the above-mentioned host and guest components. However, FTIR bands that appear in specific regions where no other bands exist can also be relevant for the presence of the compound in the complex. It is the case of the weak band corresponding to the symmetric stretching vibrations of the =CH groups from unsaturated glycerides in the hazelnut oil, which appear at 3006.5(±1), 3006.4(±0.6), 3006.3(±1.1) and 3006.6(±1.6) cm^{-1} for the X1H, X1N, X1R and X1S ternary complexes at 1:1:1 molar ratios, respectively. These values are slightly higher by 1.1-3.1 cm^{-1} for all 3:1:1 ternary complexes (see Figures 2, 3 and Supporting Information File 1, Figures

S6-S11 and Tables S6-S9). The strong bands corresponding to the asymmetric and symmetric stretching vibrations of the C-H bonds in the aliphatic CH_3/CH_2 groups, as well as to the stretching vibrations of the esteric C=O groups in triglycerides from hazelnut oil are clearly visible in all ternary complexes at 2922-2924, 2853-2854 and 1744-1745 cm^{-1}, respectively. These values are very close to that corresponding to the starting hazelnut oil. Among other glyceride-related bands, those from 1453-1458 (bending vibrations of the CH_2 and CH_3 groups), 1236-1244 and 1152-1153 cm^{-1} (bending vibrations of the CH_2 groups) are also representative in the ternary complexes. They generally appear at lower values in the first case and significantly higher values in the last case in comparison with the raw hazelnut oil (see Supporting Information File 1, Figures S6-S11).

For flavonoids, the most relevant FTIR bands for the ternary complexes were those corresponding to the asymmetric stretching vibrations of the C=O groups in the range of 1637-1652 cm^{-1} for ternary complexes and the stretching vibrations of the C-C group in the ring C of flavonoid glycosides or the bending vibrations of the aromatic CC groups in the range of 1598-1608 cm^{-1}, but without specific variations in comparison with the starting compounds. The same remark can be made for the band from the range 1268-1299 cm^{-1}, which can be attributed to the in-plane bending vibrations of the CH and OCH groups, to the stretching vibrations of the C-C groups in flavonoid glycosides or to the stretching vibrations of the C-O groups in silymarin components (lower values). Another band that appear in all ternary complexes and was assigned to flavonoids is that from the 807-821 cm^{-1}, which corresponds to the out-of-plane bending vibrations of the C-H groups. They are significantly lower in rutin and rutin-related complexes.

β-CD is the host compounds of the above-mentioned biologically active compounds and its contents vary in 1:1:1 and 3:1:1 complexes. Among the wavenumbers corresponding to the characteristic bands from the FTIR of β-CD, their intensities are also relevant for discrimination of ternary complexes. However, many β-CD-related bands are weak or have at least medium intensities in the range of 1200-4000 cm^{-1}. The most relevant for ternary complexes were the medium-strong intensity bands at 1152-1154 cm^{-1} (stretching vibrations of the C-O-C groups in glucoside moieties), 1077-1080 cm^{-1} (stretching vibrations of the C-C groups), 1022-1026 cm^{-1} (stretching vibrations of the C-O groups), 944-947 cm^{-1} (stretching vibrations of the C-H groups from the β-CD ring), and other two medium intense bands at 574-576 and 522-529 cm^{-1}, which were tentatively assigned as bending vibrations of the O-C-C groups and stretching vibrations of the C-C groups, respectively (see Figures 2, 3 and Supporting Information File 1, Figures S6-S11 and Tables S4, S6-S9).

Discrimination of ternary complexes by Fourier transform infrared spectroscopy coupled with principal component analysis (FTIR-PCA) of ternary complexes

Taking into account the differences between the wavenumbers and intensities of specific stretching and bending vibrations of β-CD hydrate, raw hazelnut oil and flavonoids in the "pure" form and as ternary complexes, a multivariate statistical analysis technique was applied for discriminating these samples and identifying the important FTIR variables for such classifications. PCA is a widely used multivariate statistical analysis technique that can extract the valuable information from a large dataset. It is the case of FTIR data (both wavenumbers and intensities), where were assigned 20, 17, 34 and 33 FTIR bands for β-CD hydrate, hazelnut oil, flavonoids and ternary complexes, respectively (see Supporting Information File 1, Tables S4-S9). On the other hand, not all FTIR bands corresponding to the starting compounds can be seen and assigned in the ternary complexes. PCA works with a

complete variable matrix. As a consequence, only those FTIR bands that were identified in both the starting materials and ternary complexes were considered for PCA analysis (see Table 1 and Supporting Information File 1, Tables S10-S12). This matrix is translated and rotated in a way for obtaining the maximum variance of the data. The new axes are denominated Factors or Principal Components (PCs). The translation coordinates will provide the scores plots that reveal the similarities/dissimilarities between cases (samples), while the representation of the rotation coordinates of the axes (direction cosines) will give information about the influence of variables to the classification of cases. Only few PCs will extract the useful information from the dataset. As a consequence, the large number of variables will be reduced to only 2-4 PCs that will explain the variance of the data.

Discrimination of flavonoid glycosides and flavonolignans

Twenty two variables were considered for the discrimination of flavonoids (flavonoid glycosides – hesperidin, "H", naringin, "N", rutin, "R", and flavonolignans – silymarin, "S"), which corresponds to wavenumbers and intensities of the FTIR bands identified in all flavonoids (Supporting Information File 1, Table S10). The flavonoid samples were clearly grouped, as was observed in the PC_2 or PC_3 *vs.* PC_1 scores plot (Supporting Information File 1, Figures S12 and S13. Better results were obtained if only wavenumbers were used as PCA variables (Figure 4). All flavonoid glycosides are classified in the positive region of the PC_1, in comparison with flavonolignans (silymarin components). According to FTIR-PCA analysis, hesperidin, naringin and rutin are more similar and all of them are dissimilar to silymarin. This classification is especially due to the bands corresponding to stretching vibrations of the C=O groups and bending vibrations for the CH groups for the positive region of PC_1 and to stretching vibrations of the CO and CC bonds for the negative part (Table 1 and Supporting Information File 1, Figures 14-18 and Table S10). In this latter case, only the first

three PCs explain 97.41% of the variance of the FTIR data, with the biggest value for PC_1 (47.29%), as is observed for eigenvalues greater than 1 in Figure S19 (Supporting Information File 1).

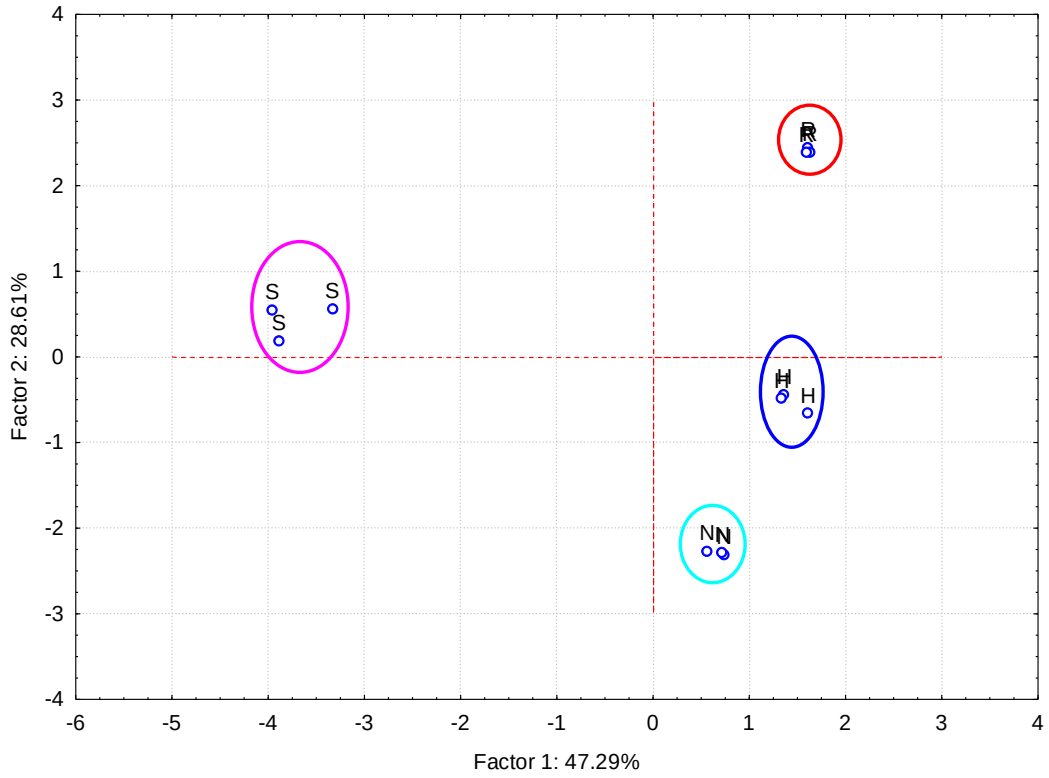


Figure 4: PC_2 *versus* PC_1 scores plot from the FTIR-PCA analysis of the flavonoid glycoside and flavonolignan antioxidants (codes: "H" – hesperidin, "N" – naringin, "R" – rutin and "S" – silymarin); only wavenumbers of the FTIR bands were used as input variables

Table 1: Factor coordinates (principal components, PCs) of the variables, based on correlations, from the FTIR-PCA analysis of the flavonoid glycoside and flavonolignan antioxidants; only wavenumbers ("v" – for stretching vibrations, "d" – for bending vibrations) of the FTIR bands were used as input variables

	PC_1	**PC_2**	**PC_3**
v(OH)	0.763	-0.616	-0.182
vas(CH)	-0.090	0.565	-0.780
vs(CH)	0.233	-0.781	-0.563
d(OH)/vas(C=O/C=C)	0.930	0.323	0.165

d(arC#C)	0.595	0.714	0.353
d1(CH2/3)	-0.350	0.026	-0.931
v1(CO)/d1(CO)	-0.416	0.797	-0.435
d1(CH)	0.986	-0.142	-0.061
v(CO)/v(CC)	0.937	0.128	-0.321
v(CO)/v(CC/CO)	-0.940	0.077	0.302
d4(CH)	-0.557	-0.739	0.049

Discrimination of ternary complexes and β-CD hydrate samples

In the same way, ternary complexes and native β-CD hydrate samples were classified according to specific FTIR wavenumbers and intensities of the bands identified in all samples. β-CD hydrate samples were classified in the top-right region of the PC_2 *vs.* PC_1 scores plot (codes "Y"), in comparison with the ternary complexes in the center-left and bottom of the plot. Moreover, such grouping can also be observed for some ternary complexes types (e.g., "X1H" in the left and "X3R" in the top-left of the plot) (Figure 5). Few FTIR variables are responsible for the discrimination of ternary complexes and β-CD samples, especially those related to band intensities corresponding to bending vibrations of CH_2 groups and stretching vibrations of various bonds including those from CCO, CCC, CO and COC systems (PCA results are not presented).

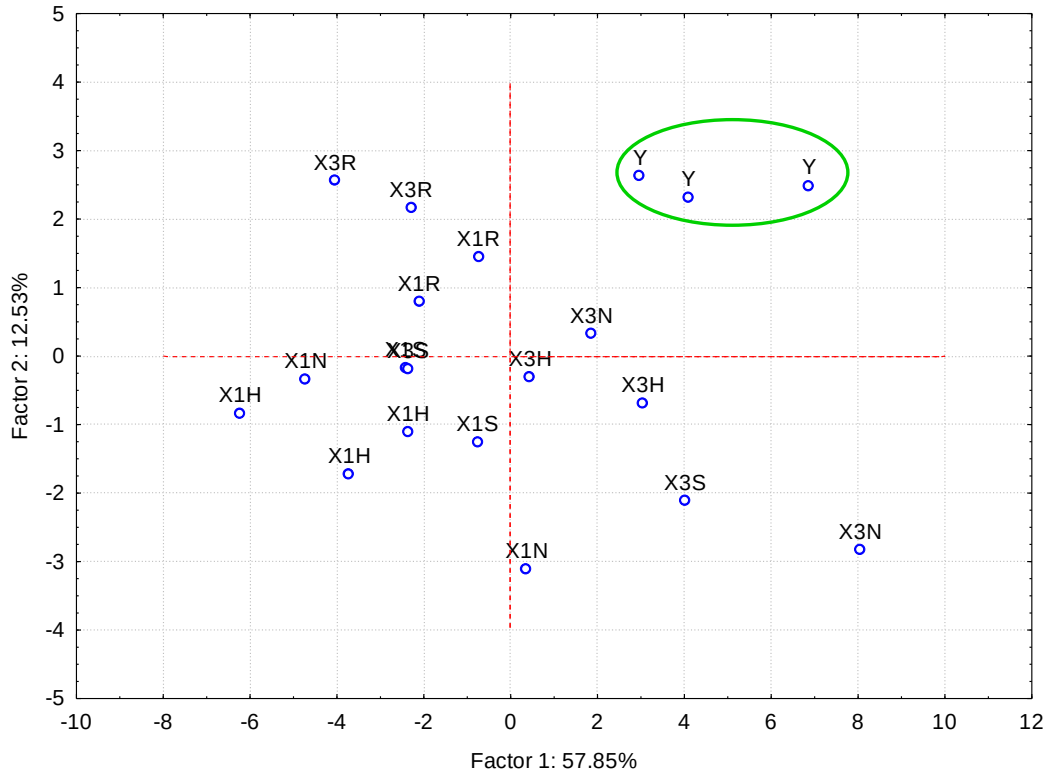


Figure 5: PC_2 *versus* PC_1 scores plot from the FTIR-PCA analysis of the β-CD/hazelnut oil/flavonoid ternary complexes (codes: "X1H/N/R/S" and "X3H/N/R/S" for the 1:1:1 and 3:1:1 ternary complexes with hesperidin/naringin/rutin/silymarin, respectively) and β-CD hydrate (code: "Y"); all wavenumber and intensity of the FTIR bands were used as input variables

Discrimination of ternary complexes and flavonoids

More interesting were the results obtained for the FTIR-PCA analysis of ternary complexes and flavonoids. A total of 18 FTIR variables (both wavenumbers and intensities, Supporting Information File 1, Tables 11 and 12) were identified in all ternary complexes and flavonoids. They were used as input variables for discrimination of complexes and guest compounds. Also, the wavenumbers and intensities sets were used separately for the discrimination. Flavonoids were clearly classified in the left side of the PC_2 *vs.* PC_1 scores plot (Figure 6). Wavenumber of the band corresponding to the stretching vibrations of the CO and CC bonds

445 for the positive side, as well as the intensity on the band corresponding to the asymmetric
446 stretching vibration of the CH bond for the negative side of the PC_1 were the most important
447 for this classification (see also Supporting Information File 1, Figure S20 for the PC_3 *vs.* PC_1
448 scores plot, Figures S21, S22 for the corresponding loadings plots and Table S11 for the
449 influence of variables to the classification). Better results were obtained if only wavenumbers
450 were used as input variables for the FTIR-PCA analysis of ternary complexes and the starting
451 flavonoids. All flavonoids were grouped in the right side of the PC_2 *vs.* PC_1 scores plot, with
452 higher similarity for hesperidin, naringin and rutin (Figure 7). On the other hand, all ternary
453 complexes were located in the left side of this plot, also sub-classified according to the
454 presence of a specific flavonoid. In a similar manner, ternary complexes based on silymarin
455 are dissimilar with the other complexes, which have a high level of similarity. These
456 observations are also sustained by the other scores plots, all with very good classifications of
457 the samples (Figures 8 and 9). Responsible for these classifications are the variables
458 corresponding to the FTIR band related to symmetric and asymmetric stretching vibrations of
459 the CH bonds (positive PC_1), stretching vibrations of the CC and CO bonds (negative PC_1),
460 stretching and bending of C=O and OH/CH, respectively (negative PC_2) (Figures 10 and 11,
461 Supporting Information File 1, Table S12). These valuable discrimination results used only
462 the first three PCs, which explain almost all the variance of the FTIR data, as was presented in
463 Figure 12 (85.69%).
464

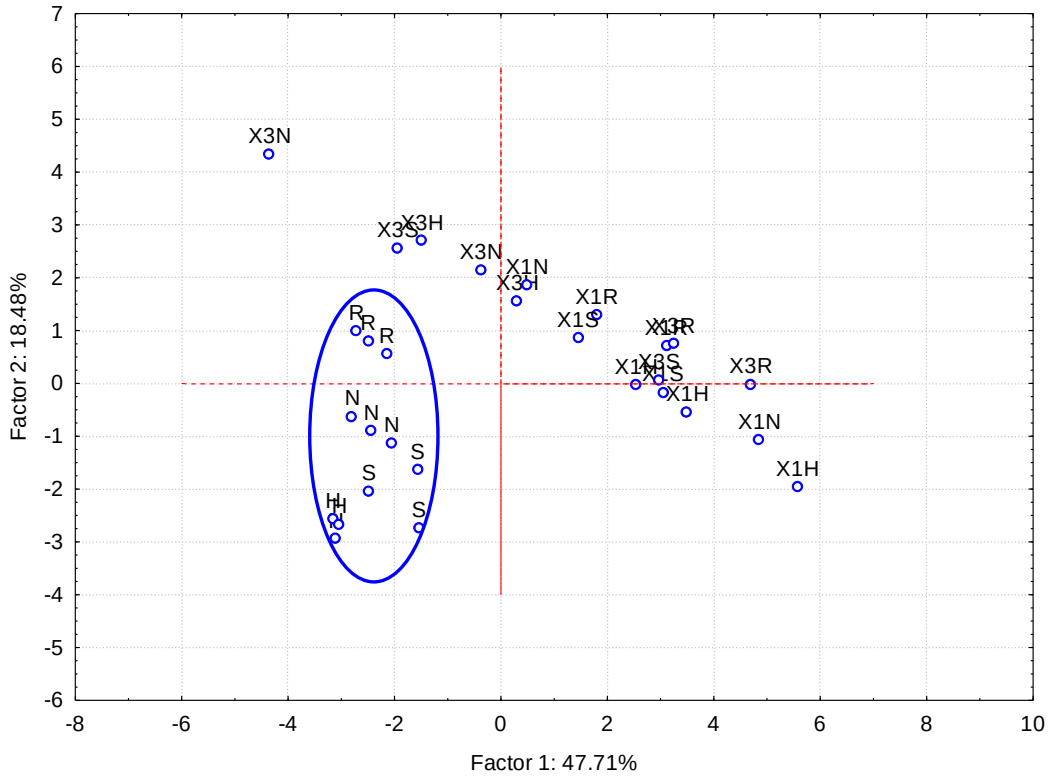


Figure 6: PC_2 *versus* PC_1 scores plot from the FTIR-PCA analysis of the β-CD/hazelnut oil/flavonoid ternary complexes (codes: "X1H/N/R/S" and "X3H/N/R/S" for the 1:1:1 and 3:1:1 ternary complexes with hesperidin/naringin/rutin/silymarin, respectively) and flavonoids (codes: "H" – hesperidin, "N" – naringin, "R" – rutin and "S" – silymarin); all wavenumber and intensity of the FTIR bands were used as input variables

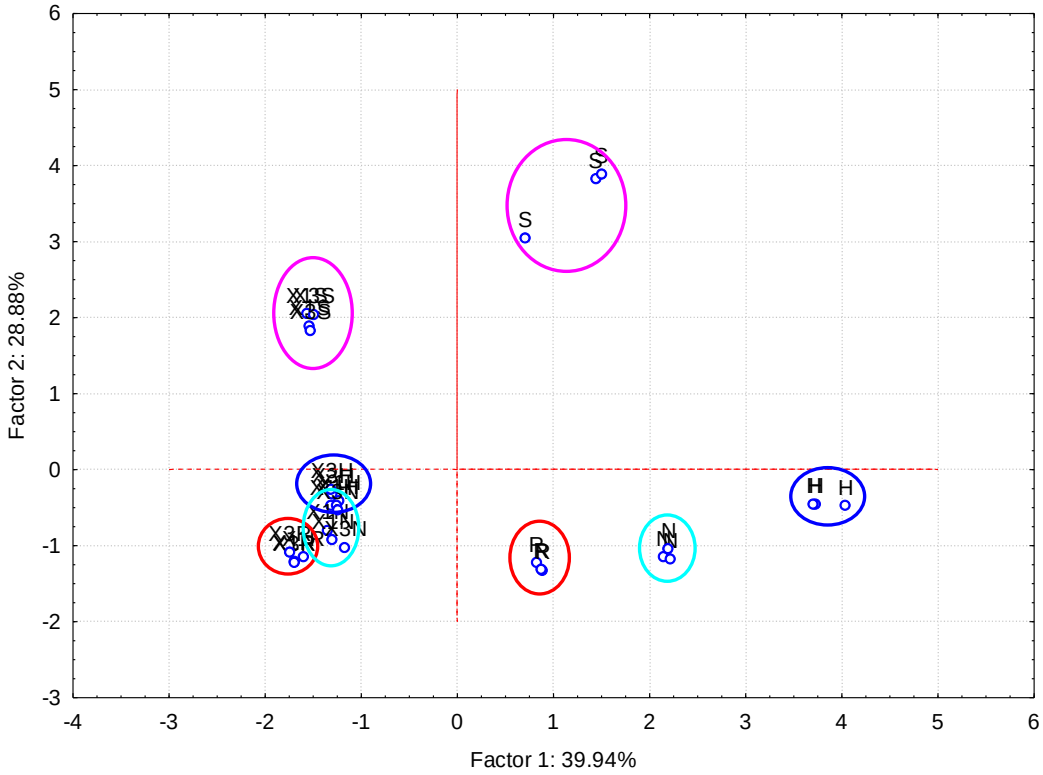


Figure 7: PC_2 *versus* PC_1 scores plot from the FTIR-PCA analysis of the β-CD/hazelnut oil/flavonoid ternary complexes (codes: "X1H/N/R/S" and "X3H/N/R/S" for the 1:1:1 and 3:1:1 ternary complexes with hesperidin/naringin/rutin/silymarin, respectively) and flavonoids (codes: "H" – hesperidin, "N" – naringin, "R" – rutin and "S" – silymarin); only wavenumbers of the FTIR bands were used as input variables

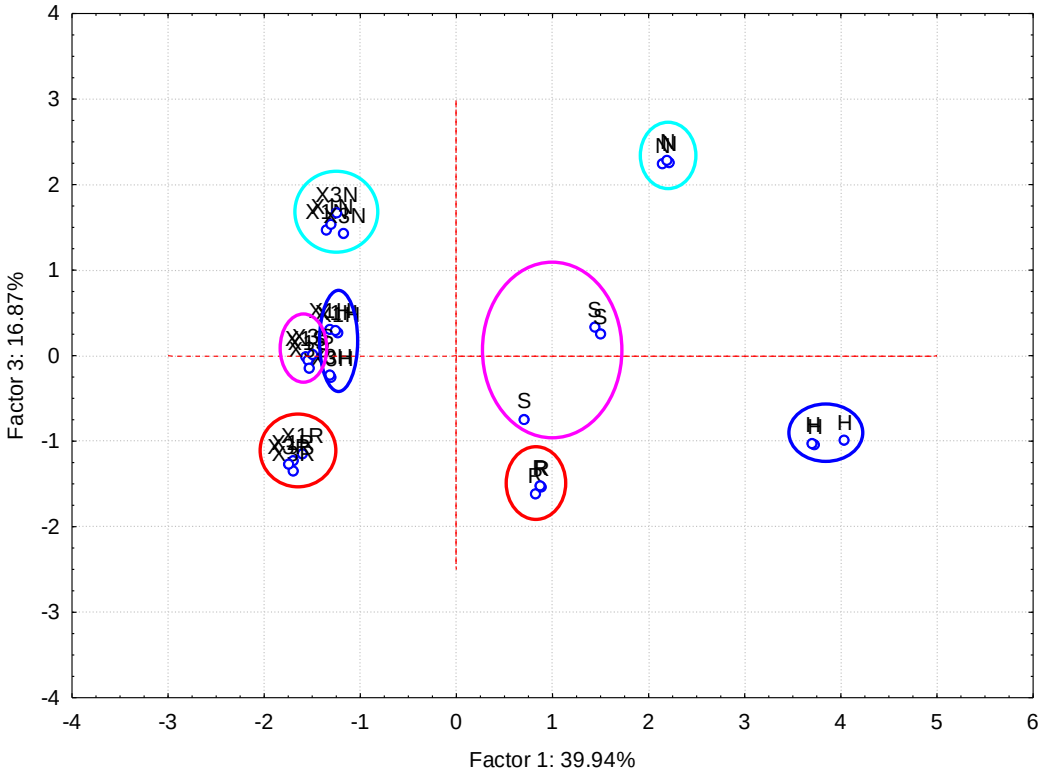


Figure 8: PC_3 *versus* PC_1 scores plot from the FTIR-PCA analysis of the β-CD/hazelnut oil/flavonoid ternary complexes (codes: "X1H/N/R/S" and "X3H/N/R/S" for the 1:1:1 and 3:1:1 ternary complexes with hesperidin/naringin/rutin/silymarin, respectively) and flavonoids (codes: "H" – hesperidin, "N" – naringin, "R" – rutin and "S" – silymarin); only wavenumbers of the FTIR bands were used as input variables

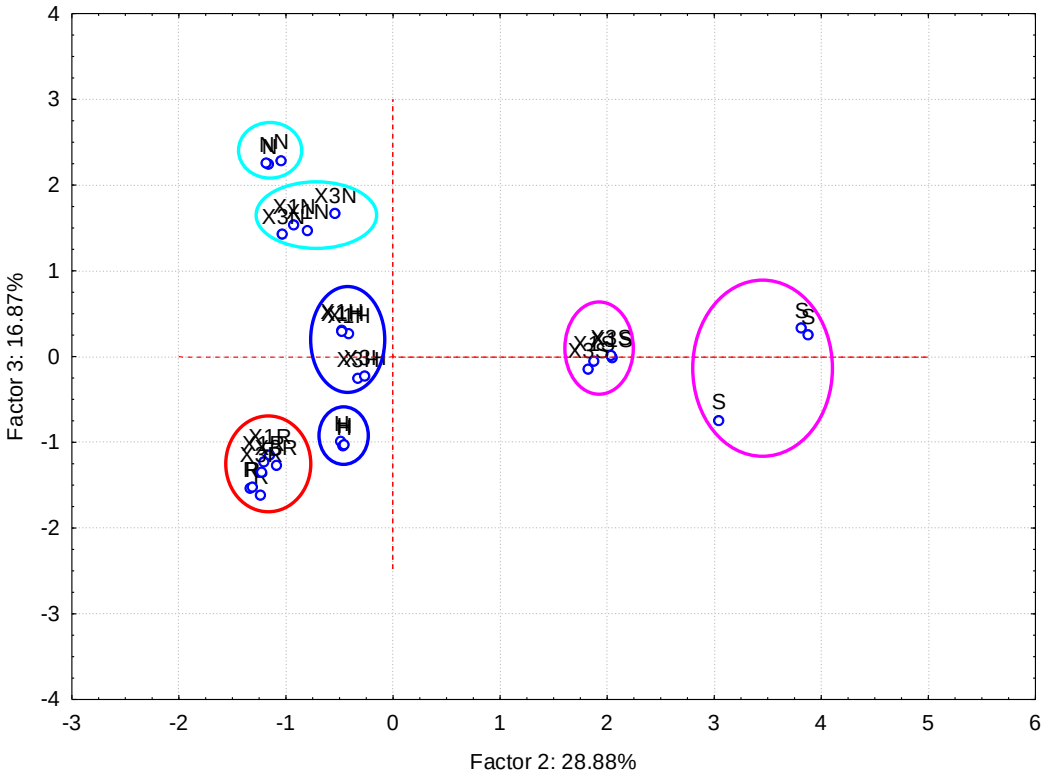
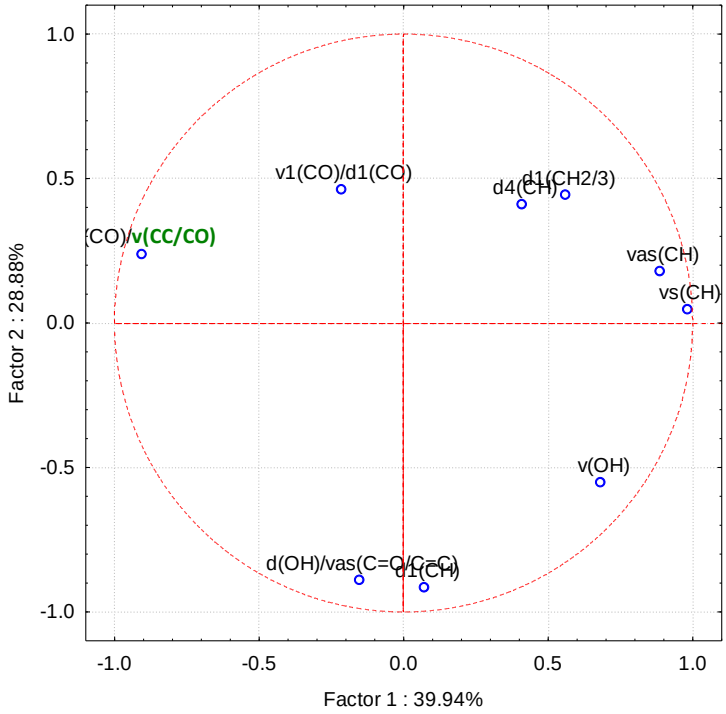


Figure 9: PC_3 *versus* PC_2 scores plot from the FTIR-PCA analysis of the β-CD/hazelnut oil/flavonoid ternary complexes (codes: "X1H/N/R/S" and "X3H/N/R/S" for the 1:1:1 and 3:1:1 ternary complexes with hesperidin/naringin/rutin/silymarin, respectively) and flavonoids (codes: "H" – hesperidin, "N" – naringin, "R" – rutin and "S" – silymarin); only wavenumbers of the FTIR bands were used as input variables



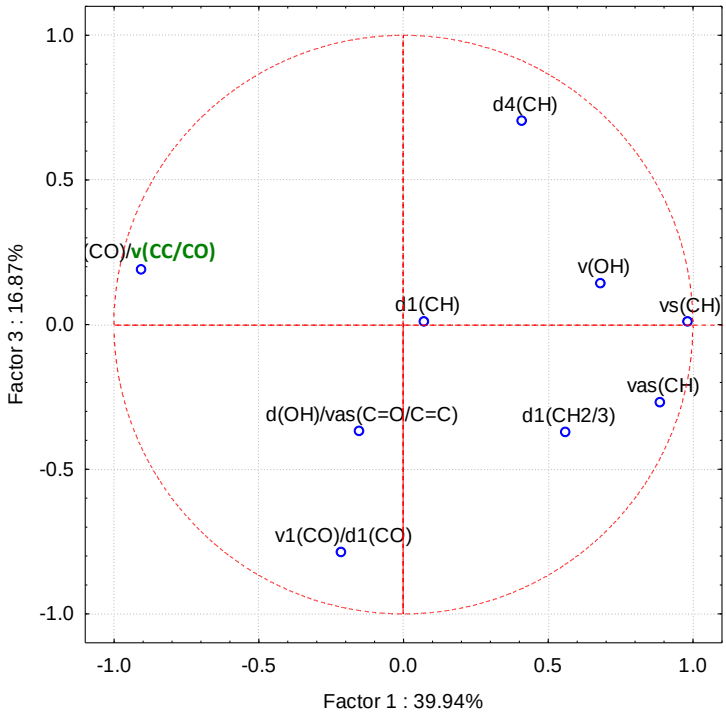
495

496 **Figure 10:** PC_2 *versus* PC_1 loadings plot from the FTIR-PCA analysis of the β-CD/hazelnut

497 oil/flavonoid ternary complexes and flavonoids; only wavenumbers of the FTIR bands were

498 used as input variables (see Table S13 for codes)

499



500

Figure 11: PC_3 *versus* PC_1 loadings plot from the FTIR-PCA analysis of the β-CD/hazelnut oil/flavonoid ternary complexes and flavonoids; only wavenumbers of the FTIR bands were used as input variables (see Table S13 for codes)

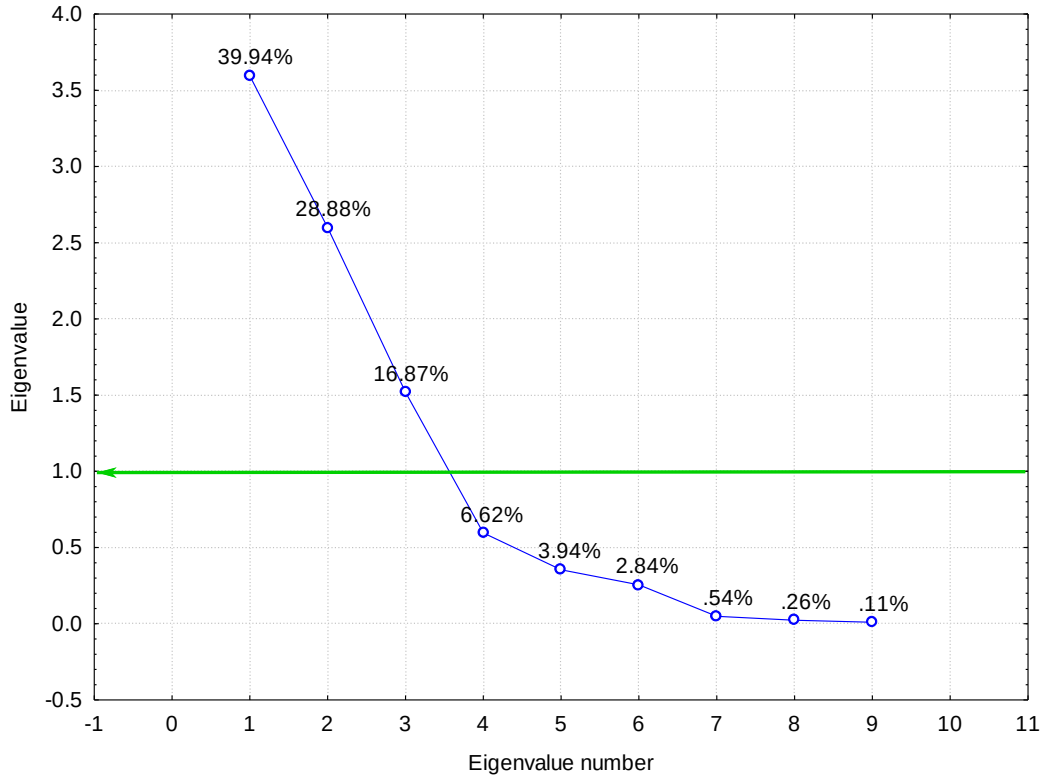


Figure 12: Eigenvalues of the correlation matrix from the FTIR-PCA analysis of the β-CD/hazelnut oil/flavonoid ternary complexes and flavonoids; only wavenumbers of the FTIR bands were used as input variables (see Table S13 for codes); the first three PCs can be retained, which explain 85.69% from the variance of the data

Conclusion

The β-CD/hazelnut oil/flavonoid ternary complexes are innovative materials synthesized for the first time, which combine the valuable properties of the specific components, host – β-CD and guest – antioxidant and essential FA glyceride compounds. β-CD encapsulation enhances the apparent water solubility of both hazelnut triglyceride components (e.g., triolein) and

flavonoid glycosides/flavonolignans. They both have significantly lower water solubility and thus low bioaccessibility and bioavailability, which are enhanced by β-CD co-encapsulation. On the other hand, the encapsulated flavonoid molecule can act as onsite antioxidant and protect the labile hazelnut oil components that contain unsaturated FA moieties. The thermal/oxidative stability of ternary complexes is similar to β-CD hydrate, as was evaluated by TG and DSC. Moreover, the formation of the molecular inclusion complexes is supported by thermal analysis (partial replacing of hydration water by biologically active molecules and disappearance of the DSC peak corresponding to crystalline-amorphous transition). In the present study, a synthesis method for these ternary complexes that is more appropriate from the applicative point of view had used. Also, a very fast, cheap and nondestructive technique, namely FTIR-PCA, was used for discrimination between ternary complexes (by the antioxidant used or by the molar ratio) and the starting components. β-CD/hazelnut oil/flavonoid ternary complexes at 3:1:1 had spectroscopic and thermal behavior more close to the native β-CD hydrate, in comparison with the 1:1:1 complexes. This observation indicate that not all FA moieties separately interact with the β-CD host molecules, as was the reason to use such non-equimolar ratios. This is due to the steric hindrance that exist in a theoretical 3:1 interaction for α-CD/triglyceride supramolecular system. On the other hand, ternary complexes and flavonoids were very well classified and discriminated by FTIR-PCA, especially through the type of antioxidant used. Finally, these new bioactive materials can be used in food supplements and functional foods, but further synthesis methods and analyses (slow co-crystallization, X-ray diffraction and nuclear magnetic resonance) are needed for the elucidation of the interactions in such complex supramolecular systems.

Experimental

Vegetable samples and chemicals

Hazelnut (*Corylus avellana* L.) oil was obtained from kernel of the fruit by Soxhlet extraction. Wild hazelnuts were collected from the Apuseni Mountains (Transylvania, Romania, 46°22'46" N and 23°16'47" E) in September-October 2018 and were kept at room temperature, in the dark and dry atmosphere for six months. Then, the kernels were manually separated, finely ground and subjected to Soxhlet extraction using a 250 mL equipment. One hundred of hazelnut kernels were extracted five times with 300 mL of petroleum ether anhydrous (ACS reagent, 40-60 °C boiling range, Sigma-Aldrich, St. Louis, MO, USA). The extract was distilled and evaporated to dryness until no petroleum ether remains. The oil separation yield was ~50%. The hazelnut oil was kept at -20 °C until further analyses and β-CD complexation.

β-CD hydrate, Kleptose®, was kindly donated by Roquette Frères S.A. (Lestrem, France) and has a purity of >98%, a water content of 14.0% and maximum 0.5% α-CD and γ-CD. Flavonoid glycosides and flavonolignans used in the complexation process were hesperidin (code "H", $C_{28}H_{34}O_{15}$, M = 610.56 g/mol, purity ≥80%, other flavonoid glycosides as impurities), naringin hydrate (code "N", $C_{27}H_{32}O_{14}\cdot 2H_2O$, M = 580.50 g/mol, purity ≥95%), rutin hydrate (code "R", $C_{27}H_{30}O_{16}\cdot xH_2O$, M = 610.52 g/mol, purity ≥94%) and silymarin (code "S", $C_{25}H_{22}O_{10}$, M = 482.44 g/mol, ~70% silibinin A, other flavonolignans as impurities) and were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA). Ethanol used for complex synthesis was of 96% concentration (v/v) and was purchased from ChimReactiv (Bucharest, Romania). The analysis of the fatty acid profile of the hazelnut oil required the derivatization (transesterification) of the FA glycerides to the corresponding fatty acid methyl esters (FAMEs) [11, 13]. The derivatization involved methanol-boron trifluoride (20% BF_3), hexane (GC grade) and anhydrous sodium sulfate. All were purchased from Merck & Co., Inc., Rahway, NJ, USA. Sodium chloride (reagent grade) used for the

separation of FAMEs was purchased from Reactivul (Bucharest, Romania). The identification of the FAME components of the hazelnut oil involves FAME37 standard mixture, as well as C_8-C_{20} linear alkane standard mixture for the determination of specific retention index (RI) of compounds (both purchased from Sigma-Aldrich, St. Louis, MO, USA). Finally, 2-propanol (ACS reagent, Reag. Ph. Eur.) used for FTIR cleaning was obtained from Merck & Co., Inc., Rahway, NJ, USA.

Gas chromatography-mass spectrometry (GC-MS)

The FA profile of the hazelnut oil was determined by GC-MS, after derivatization to FAMEs. Derivatization was performed by quantitative transesterification in a 100 mL one-neck flask equipped with reflux condenser. 5 mL of BF_3·MeOH 20% and ~100 mg of hazelnut oil were used for derivatization. The mixture was refluxed at least 30 min, until no oil remains. Then, 2 mL of hexane was added and continued for another 15 min for completing the transesterification. The organic layer was separated in the neck region by adding enough saturated sodium chloride solution. The organic layer was transferred into a GC vial with ~0.5 g of anhydrous sodium sulfate and stored at 4 °C until GC-MS analysis. GC-MS analysis was performed on a GC Hewlett Packard 6890 Series equipment, coupled with a Hewlett Packard 5973 Mass Selective Detector. The following GC conditions were used: Zebron 5-MS column (30 m length, 0.25 mm i.d., 0.25 µm film thickness), temperature program from 50-300 °C (heating rate 6 °C/min), injector temperature 300 °C, detector temperature 300 °C, carrier gas He (99.9999% purity), injected sample volume 2 µL, delay time 4 min. The MS conditions were: energy source EI 70 eV, temperature 150 °C, scan range 50-300 amu, scan rate 1/s. RI values were determined using C_8-C_{20} alkane standard mixture and a RI *vs.* RT correlation equation of $RI = 672.792 + 73.268 \cdot RT - 3.287 \cdot RT^2 + 0.148 \cdot RT^3 - 0.00201 \cdot RT^4$ [16]. On the other hand, the identification of the main FAMEs from the derivatized hazelnut oil was

591 performed by comparing the experimental RI values with those for the FAME standard
592 mixture. Moreover, the experimental MS spectra were compared with those from the
593 NIST/EPA/NIH Mass Spectral Library 2.0 (2011). Acquisition and handling of the GC-MS
594 data were performed using the Enhanced MSD ChemStation D .02.00.275 (Agilent
595 Technologies, Santa Clara, CA, USA), while the MS identification was performed with the
596 NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library 2.0
597 (Gaithersburg, MD, USA). Determinations were performed in duplicate and the main findings
598 reveal a high oleic acid relative content (as methyl ester) of 69.91(±4.14) % at a RI of 2096.4.
599 The other important FAs, as methyl esters, were palmitoleic, palmitic, linoleic,
600 elaidic/vaccenic, and stearic acids with concentrations of 0.13, 7.54, 15.51, 2.85 and 2.73%,
601 respectively (a total of 98.68% identified FAMEs in the hazelnut oil).
602
603 ***Synthesis of ternary complexes by kneading method***
604 The synthesis of β-CD/hazelnut oil/flavonoid glycoside or flavonolignan ternary complexes
605 was performed using the kneading method, which is the most appropriate for such type of
606 complexes [13, 14, 44]. In this study, two β-CD:hazelnut oil:flavonoid molar ratios of 1:1:1
607 and 3:1:1 were used. Particularly, 1322(±5) or 3959(±10) mg of β-CD hydrate (for 1:1:1 and
608 3:1:1 molar ratios, respectively), 909(±5) mg hazelnut oil, 613(±3) mg hesperidin, 628(±5)
609 mg naringin hydrate, 656(±5) mg rutin hydrate and 488(±1) mg silymarin were weighted,
610 taking into account the water content and purity of compounds. The mean molar mass for the
611 hazelnut oil of M = 900 g/mol was determined as triolein, according to GC-MS data and a
612 purity of ~97% [27, 67]. The following ternary complexes were obtained: β-CD/hazelnut
613 oil/hesperidin at 1:1:1 and 3:1:1 molar ratios (codes "X1H" and "X3H"), β-CD/hazelnut
614 oil/naringin at 1:1:1 and 3:1:1 molar ratios (codes "X1N" and "X3N"), β-CD/hazelnut
615 oil/rutin at 1:1:1 and 3:1:1 molar ratios (codes "X1R" and "X3R") and β-CD/hazelnut

oil/silymarin at 1:1:1 and 3:1:1 molar ratios (codes "X1S" and "X3S"). The amounts of β-CD, hazelnut oil and flavonoid, corresponding to 1:1:1 or 3:1:1 were mixed in a preheated mortar at 60 °C. Then, 4 mL water and 1 mL ethanol for 1:1:1 complexes or 6 mL water and 1.5 mL ethanol for 3:1:1 complexes were added. The mixture was kneaded for at least 30 min, until a viscous paste is obtained. The mortar temperature decreases to the room temperature during kneading. The wet complex was dried until constant mass at room temperature in the dark. The dried complex was then grinded in the same mortar, recovered and weighted. The recovering yield was determined as the percent ratio of the recovered dried complex and the sum of starting compounds. Two samples were obtained for the 1:1:1 ternary complexes and single samples for the 3:1:1 ternary complexes.

Fourier-transform infrared spectroscopy (FTIR)

FTIR analysis of the ternary complexes and the starting compounds was performed using a Bruker Vertex 70 FTIR equipment (Bruker Optik GmbH, Ettlingen, Germany), equipped with an ATR (single-reflection Platinum diamond attenuated total reflectance) system. The following FTIR conditions were set up: acquisition range 4000-400 cm^{-1}, resolution 4 cm^{-1}, number of scans 128, sample mass 10-20 mg, spectrum range for the DLaTGS detector 12000-250 cm^{-1} and sensibility $D^* > 2108\ cm \cdot Hz^{1/2} \cdot W^{-1}$. OPUS ver. 7.2 software (Bruker Optik GmbH 2012, Ettlingen, Germany) was used for the acquisition and handling of the FTIR. All determinations were performed as triplicates for the starting compounds and as duplicates for the ternary complexes.

Thermal analyses

Thermal and oxidative stability of complexes can be evaluated through thermal analyses. TG-DTG and DSC techniques were used for both complexes and starting compounds. TG-DTG

641 analysis was performed on a Netzsch TG 209F1 Libra equipment, while DSC analysis was
642 conducted on a Netzsch 204 F1 Phoenix apparatus (both from Netzsch Group, Selb,
643 Germany). The TG-DTG and DSC conditions were similar: temperature program of 25-500
644 °C, with a heating rate of 10 °C/min, nitrogen purge and protection flow of 40 mL/min, the
645 data acquisition and handling by Netzsch Proteus-Thermal Analysis ver. 6.1 software
646 (Netzsch Group, Selb, Germany). Only representative ternary complexes were evaluated by
647 thermal analyses.
648
649 ***Statistical analysis and principal component analysis (PCA)***
650 Means (± standard deviations, SD) of the values were obtained for the multiplicate
651 determinations using Basic Statistics&Tables and One-way ANOVA modules in Statistica 7.1
652 software (StatSoft, Inc., Tulsa, OK, USA). PCA for the FTIR data was performed with the
653 Principal Components & Classification Analysis module from the above-mentioned package.
654 The discrimination between samples was based on the scores plot, while the importance of
655 variables to the classification was based on the loadings plot in PCA analysis. Both FTIR
656 wavenumber (WN) and intensity (I) of the specific bands identified in all analyzed samples
657 were used as input data. PCA was performed with both FTIR variable types (both WN and I)
658 or as separated variable types (only WN or only I). PCA analysis was based on correlations, a
659 compute variances as SS/(N-1), with centered factor coordinates of the variables (or principal
660 components, coded as "PC"). All significant PCA results are presented in the Supporting
661 Information File 1 (Figures and Tables).
662
663 **Supporting Information File 1:** Thermal analysis, FTIR and FTIR-PCA data for ternary
664 complexes
665

Acknowledgements

Authors want to thank Simona Funar-Timofei ("Coriolan Drăgulescu" Institute of Chemistry, Romanian Academy) for the help with Statistica 7.1 software and to the "Program to increase performance and innovation in doctoral and postdoctoral research excellence - PROINVENT", Contract No. 62487/03.06.2022, POCU/993/6/13, SMIS Code 153299 for support.

Funding. This research received no external funding.

References

[1] Kurkov, S. V.; Loftsson, T. *Int. J. Pharm.* **2013,** *453*, 167-180. 10.1016/j.ijpharm.2012.06.055.

[2] Duchêne, D.; Bochot, A. *Int. J. Pharm.* **2016,** *514*, 58-72. http://dx.doi.org/10.1016/j.ijpharm.2016.07.030.

[3] Crini, G. *Chem. Rev.* **2014,** *114*, 10940-10975. https://doi.org/10.1021/cr500081p.

[4] Medeleanu, M. A.; Hădărugă, D. I.; Muntean, C. V.; Popescu, G.; Rada, M.; Hegheş, A.; Zippenfening, S. E.; Lucan-(Banciu), C. A.; Velciov, A. B.; Bandur, G. N.; Hădărugă, N. G.; Riviş, M. *Carbohydr. Polym.* **2021,** *265*, 118079. https://doi.org/10.1016/j.carbpol.2021.118079.

[5] Del-Valle, E. M. M. *Proc. Biochem.* **2004,** *39*, 1033-1046. https://doi.org/10.1016/S0032-9592(03)00258-9.

[6] Hădărugă, N. G.; Bandur, G. N.; David, I.; Hădărugă, D. I. *Environ. Chem. Lett.* **2019,** *17*, 349-373. https://doi.org/10.1007/s10311-018-0806-8.

[7] Carrier, R. L.; Miller, L. A.; Ahmed, I. *J. Control. Rel.* **2007,** *123*, 78-99. https://doi.org/10.1016/j.jconrel.2007.07.018.

691 [8] Szente, L.; Fenyvesi, E. *Struct. Chem.* **2017,** *28*, 479-492.
692 https://doi.org/10.1007/s11224-016-0884-9.
693 [9] Paramita, V.; Novia, S. F.; Ariyanto, H. D.; Pramudono, B.; Yoshii, H.; Kusumayanti,
694 H.; Amalia, R. *Mat. Today: Proc.* **2022,** *63*, S312-S317.
695 https://doi.org/10.1016/j.matpr.2022.03.156.
696 [10] Hădărugă, N. G.; Hădărugă, D. I.; Păunescu, V.; Tatu, C.; Ordodi, V. L.; Bandur, G.;
697 Lupea, A. X. *Food Chem.* **2006,** *99*, 500-508.
698 http://dx.doi.org/10.1016/j.foodchem.2005.08.012.
699 [11] Hădărugă, D. I.; (Mitroi), C. L. B.; Gruia, A. T.; Păunescu, V.; Bandur, G. N.;
700 Hădărugă, N. G. *Food Chem.* **2017,** *236*, 49-58.
701 https://doi.org/10.1016/j.foodchem.2017.03.093.
702 [12] Hădărugă, D. I.; Ünlüsayin, M.; Gruia, A. T.; Birău-(Mitroi), C.; Rusu, G.; Hădărugă,
703 N. G. *Beilstein J. Org. Chem.* **2016,** *12*, 179-191. http://dx.doi.org/10.3762/bjoc.12.20.
704 [13] Hădărugă, N. G.; Szakal, R. N.; Chirilă, C. A.; Lukinich-Gruia, A. T.; Păunescu, V.;
705 Muntean, C.; Rusu, G.; Bujancă, G.; Hădărugă, D. I. *Food Chem.* **2020,** *303*, Art. 125419.
706 https://doi.org/10.1016/j.foodchem.2019.125419.
707 [14] Ünlüsayin, M.; Hădărugă, N. G.; Rusu, G.; Gruia, A. T.; Păunescu, V.; Hădărugă, D.
708 I. *LWT - Food Sci. Technol.* **2016,** *68*, 135-144. http://dx.doi.org/10.1016/j.lwt.2015.12.017.
709 [15] Lee, C.-M.; Kim, M.-H.; Na, H.-S.; Kim, J.; Lee, K.-Y. *Biotechnol. Bioproc. Eng.*
710 **2013,** *18*, 507-513. https://doi.org/10.1007/s12257-012-0752-4.
711 [16] Hădărugă, N. G.; Chirilă, C. A.; Szakal, R. N.; Gălan, I. M.; Simandi, M. D.; Bujancă,
712 G. S.; David, I.; Riviş, A.; Stanciu, S. M.; Hădărugă, D. I. *Foods* **2022,** *11*, 3632.
713 https://doi.org/10.3390/foods11223632.

714 [17] David, I.; Orboi, M. D.; Simandi, M. D.; Chirilă, C. A.; Megyesi, C. I.; Rădulescu, L.;
715 Lukinich-Gruia, A. T.; Muntean, C.; Hădărugă, D. I.; Hădărugă, N. G. *PLoS ONE* **2019,** *14*,
716 (11), e0225474. https://doi.org/10.1371/journal.pone.0225474.
717 [18] Adel, A. M.; Ibrahim, A. A.; El-Shafei, A. M.; Al-Shemy, M. T. *Food Pack. Shelf Life*
718 **2019,** *20*, 100307. https://doi.org/10.1016/j.fpsl.2019.100307.
719 [19] Azzi, J.; Danjou, P.-E.; Landy, D.; Ruellan, S.; Auezova, L.; Greige-Gerges, H.;
720 Fourmentin, S. *Beilstein J. Org. Chem.* **2017,** *13*, 835-844. https://doi.org/10.3762/bjoc.13.84.
721 [20] Kfoury, M.; Landy, D.; Ruellan, S.; Auezova, L.; Greige-Gerges, H.; Fourmentin, S.
722 *Food Chem.* **2017,** *236*, 41-48. http://dx.doi.org/10.1016/j.foodchem.2016.12.086.
723 [21] Hădărugă, D. I.; Hădărugă, N. G.; Costescu, C. I.; David, I.; Gruia, A. T. *Beilstein J.*
724 *Org. Chem.* **2014,** *10*, 2809-2820. https://doi.org/10.3762/bjoc.10.298.
725 [22] Hădărugă, N. G.; Hădărugă, D. I.; Isengard, H.-D. *Food Chem.* **2012,** *132*, 1741-1748.
726 http://dx.doi.org/10.1016/j.foodchem.2011.11.003.
727 [23] Menezes, P. P.; Serafini, M. R.; Quintans-Júnior, L. J.; Silva, G. F.; Oliveira, J. F.;
728 Carvalho, F. M. S.; Souza, J. C. C.; Matos, J. R.; Alves, P. B.; Matos, I. L.; Hădărugă, D. I.;
729 Araújo, A. A. S. *J. Therm. An. Calorim.* **2014,** *115*, 2429-2437.
730 https://doi.org/10.1007/s10973-013-3367-x.
731 [24] Yoshikiyo, K.; Yoshioka, Y.; Oe, Y. N. S.; Kawahara, H.; Kurata, K.; Shimizu, H.;
732 Yamamoto, T. *Food Chem.* **2019,** *294*, 56-59.
733 https://doi.org/10.1016/j.foodchem.2019.04.093.
734 [25] Alasalvar, C.; Amaral, J. S.; Satır, G.; Shahidi, F. *Food Chem.* **2009,** *113*, 919-925.
735 https://doi.org/10.1016/j.foodchem.2008.08.019.
736 [26] Ciemniewska-Żytkiewicz, H.; Verardo, V.; Pasini, F.; Bryś, J.; Koczoń, P.; Caboni,
737 M. F. *Food Chem.* **2015,** *168*, 615-622. http://dx.doi.org/10.1016/j.foodchem.2014.07.107.

738 [27] Köksal, A. İ.; Artik, N.; Şimşek, A.; Güneş, N. *Food Chem.* **2006,** *99*, 509-515.
739 https://doi.org/10.1016/j.foodchem.2005.08.013.
740 [28] Başyiğit, Z. Ö. *Res. Eng. Struct. Mat.* **2019,** *5*, (1), 33-41.
741 http://dx.doi.org/10.17515/resm2018.84ma1226.
742 [29] Hădărugă, N. G.; Hădărugă, D. I., Hydroxycinnamic acids. In *Handbook of Food*
743 *Bioactive Ingredients: Properties and Applications*, Jafari, S. M.; Rashidinejad, A.; Simal-
744 Gandara, J., Eds. Springer Nature Switzerland AG: Cham, 2022; pp 1-51.
745 https://doi.org/10.1007/978-3-030-81404-5_3-1.
746 [30] Pedro, A. C.; Maurer, J. B. B.; Zawadzki-Baggio, S. F.; Ávila, S.; Maciel, G. M.;
747 Haminiuk, C. W. I. *Ind. Crop. Prod.* **2018,** *112*, 90-97.
748 https://doi.org/10.1016/j.indcrop.2017.10.052.
749 [31] Guitard, R.; Paul, J.-F.; Nardello-Rataj, V.; Aubry, J.-M. *Food Chem.* **2016,** *213*, 284-
750 295. http://dx.doi.org/10.1016/j.foodchem.2016.06.038.
751 [32] Iqbal, R.; Mehmood, Z.; Baig, A.; Khalid, N. *Food Bioprod. Proc.* **2020,** *123*, 304-
752 311. https://doi.org/10.1016/j.fbp.2020.07.013.
753 [33] Roedig-Penman, A.; Gordon, M. H. *J. Am. Oil Chem. Soc.* **1998,** *75*, (2), 169-180.
754 [34] Tańska, M.; Mikołajczak, N.; Konopka, I. *Food Chem.* **2018,** *240*, 679-685.
755 http://dx.doi.org/10.1016/j.foodchem.2017.08.007.
756 [35] Viskupicova, J.; Danihelova, M.; Ondrejovic, M.; Liptaj, T.; Sturdik, E. *Food Chem.*
757 **2010,** *123*, 45-50. http://dx.doi.org/10.1016/j.foodchem.2010.03.125.
758 [36] Ficarra, R.; Tommasini, S.; Raneri, D.; Calabrò, M. L.; Di-Bella, M. R.; Rustichelli,
759 C.; Gamberini, M. C.; Ficarra, P. *J. Pharm. Biomed. An.* **2002,** *29*, 1005-1014.
760 [37] Hădărugă, D. I.; Hădărugă, N. G.; Bandur, G. N.; Isengard, H.-D. *Food Chem.* **2012,**
761 *132*, 1651-1659. http://dx.doi.org/10.1016/j.foodchem.2011.06.004.

762 [38] Liu, B.; Li, W.; Nguyen, T. A.; Zhao, J. *Food Chem.* **2012,** *134*, 926-932.
763 http://dx.doi.org/10.1016/j.foodchem.2012.02.207.
764 [39] Nguyen, T. A.; Liu, B.; Zhao, J.; Thomas, D. S.; Hook, J. M. *Food Chem.* **2013,** *136*,
765 186-192. http://dx.doi.org/10.1016/j.foodchem.2012.07.104.
766 [40] Pham, T. L.; Usacheva, T. R.; Kuz'mina, I. A.; Nguyen, T. N.; Thai, H.; Volkova, M.
767 A.; Le, H. K.; Nguyen, T. D.; Volynkin, V. A.; Tran, D. L. *J. Mol. Liq.* **2020,** *318*, 114308.
768 https://doi.org/10.1016/j.molliq.2020.114308.
769 [41] Pinhoa, E.; Grootveld, M.; Soares, G.; Henriques, M. *Carbohydr. Polym.* **2014,** *101*,
770 121-135. http://dx.doi.org/10.1016/j.carbpol.2013.08.078.
771 [42] Purewal, S. S.; Sandhu, K. S. *Sci. Horticult.* **2021,** *276*, 109750.
772 https://doi.org/10.1016/j.scienta.2020.109750.
773 [43] You, G.-J.; Sun, L.-L.; Cao, X.-X.; Li, H.-H.; Wang, M.; Liu, Y.-N.; Ren, X.-L. *LWT -*
774 *Food Sci. Technol.* **2018,** *94*, 172-177. https://doi.org/10.1016/j.lwt.2018.04.035.
775 [44] Anwer, M. K.; Jamil, S.; Ansari, M. J.; Al-Shdefat, R.; Ali, B. E.; Ganaie, M. A.;
776 Abdel-Kader, M. S.; Shakeel, F. *J. Mol. Liq.* **2014,** *199*, 35-41.
777 http://dx.doi.org/10.1016/j.molliq.2014.08.012.
778 [45] Chantasart, D.; Rakkaew, P. *J. Drug Deliv. Sci. Technol.* **2019,** *52*, 73-82.
779 https://doi.org/10.1016/j.jddst.2019.04.011.
780 [46] Chaudhari, P.; Birangal, S.; Mavlankar, N.; Pal, A.; Mallela, L. S.; Roy, S.; Kodoth,
781 A. K.; Ghate, V.; Nampoothiri, M.; Lewis, S. A. *Carbohydr. Polym.* **2022,** *297*, 120007.
782 https://doi.org/10.1016/j.carbpol.2022.120007.
783 [47] Cirri, M.; Maestrelli, F.; Mennini, N.; Mura, P. *J. Pharm. Biomed. An.* **2009,** *50*, 683-
784 689. https://doi.org/10.1016/j.jpba.2008.11.003.
785 [48] Cirri, M.; Maestrelli, F.; Mennini, N.; Mura, P. *J. Pharm. Biomed. An.* **2009,** *50*, 690-
786 694. https://doi.org/10.1016/j.jpba.2008.11.002.

787 [49] Jadhav, P.; Petkar, B.; Pore, Y.; Kulkarni, A.; Burade, K. *Carbohydr. Polym.* **2013,**
788 *98*, 1317-1325. http://dx.doi.org/10.1016/j.carbpol.2013.07.070.
789 [50] Li, J.; Gao, H.; Ye, Z.; Deng, J.; Ouyang, D. *Carbohydr. Polym.* **2022,** *275*, 118712.
790 https://doi.org/10.1016/j.carbpol.2021.118712.
791 [51] Liu, N.; Higashi, K.; Ueda, K.; Moribe, K. *Int. J. Pharm.* **2017,** *531*, 543-549.
792 http://dx.doi.org/10.1016/j.ijpharm.2017.04.049.
793 [52] Mura, P.; Bettinetti, G. P.; Cirri, M.; Maestrelli, F.; Sorrenti, M.; Catenacci, L. *Eur. J.*
794 *Pharm. Biopharm.* **2005,** *59*, 99-106. http://dx.doi.org/10.1016/j.ejpb.2004.05.005.
795 [53] Wang, D.; Li, H.; Gu, J.; Guo, T.; Yang, S.; Guo, Z.; Zhang, X.; Zhu, W.; Zhang, J. *J.*
796 *Pharm. Biomed. An.* **2013,** *83*, 141-148. http://dx.doi.org/10.1016/j.jpba.2013.05.001.
797 [54] Iordănescu, O. A.; Băla, M.; Iuga, A. C.; Gligor-(Pane), D.; Dascălu, I.; Bujancă, G.
798 S.; David, I.; Hădărugă, N. G.; Hădărugă, D. I. *Plants* **2021,** *10*, 1957.
799 https://doi.org/10.3390/plants10091957.
800 [55] Petroman, C.; Popescu, G.; Szakal, R.-N.; Păunescu, V.; Drăghia, L. P.; Bujancă, G.
801 S.; Chirilă, C. A.; Hădărugă, D. I.; Văduva, L.; Hădărugă, N. G.; Petroman, I. *Foods* **2021,** *10*,
802 242. https://doi.org/10.3390/foods10020242.
803 [56] Santos, C. I. A. V.; Teijeiro, C.; Ribeiro, A. C. F.; Rodrigues, D. F. S. L.; Romero, C.
804 M.; Esteso, M. A. *J. Mol. Liq.* **2016,** *223*, 209-216.
805 http://dx.doi.org/10.1016/j.molliq.2016.08.035.
806 [57] Margolis, S. A.; Huang, P. H.; Hădărugă, N. G.; Hădărugă, D. I., Water determination.
807 In *Encyclopedia of Analytical Science*, 3, Ed. Elsevier: Oxford, 2019; Vol. 10, pp 382-390.
808 https://doi.org/10.1016/B978-0-12-409547-2.14505-6.
809 [58] Ge, X.; He, J.; Qi, F.; Yang, Y.; Huang, Z.; Lu, R.; Huang, L. *Spectrochim. Acta Part*
810 *A* **2011,** *81*, 397-403. https://doi.org/10.1016/j.saa.2011.06.028.

[59] Chen, L.-F.; Shen, Q.; Shen, J.-P.; Shi, D.-T.; Chen, T.; Yu, H.-R. *Coll. Surf. A: Physicochem. Eng. Aspects* **2012,** *411*, 69-73. http://dx.doi.org/10.1016/j.colsurfa.2012.07.003.

[60] Sim, S. F.; Ting, W. *Talanta* **2012,** *88*, 537-543. https://doi.org/10.1016/j.talanta.2011.11.030.

[61] Tsirigotis-Maniecka, M.; Gancarz, R.; Wilk, K. A. *Coll. Surf. A* **2017,** *532*, 48-56. http://dx.doi.org/10.1016/j.colsurfa.2017.07.001.

[62] Puri, M.; Kaur, A.; Schwarz, W. H.; Singh, S.; Kennedy, J. F. *Int. J. Biol. Macromol.* **2011,** *48*, 58-62. https://doi.org/10.1016/j.ijbiomac.2010.09.012.

[63] Gera, S.; Pooladanda, V.; Godugu, C.; Challa, V. S.; Wankar, J.; Dodoala, S.; Sampathi, S. *Pharm. Develop. Technol.* **2020,** *25*, (8), 971-988. https://doi.org/10.1080/10837450.2020.1765378.

[64] Qian, B.-J.; Wu, C.-F.; Lu, M.-M.; Xu, W.; Jing, P. *Food Chem.* **2017,** *232*, 545-551. http://dx.doi.org/10.1016/j.foodchem.2017.04.010.

[65] Wu, W.; Zu, Y.; Wang, L.; Wang, L.; Li, Y.; Liu, Y.; Wu, M.; Zhao, X.; Zhang, X. *RSC Advances* **2017,** *7*, 54379-54390. https://doi.org/10.1039/c7ra10242a.

[66] Pooja, D.; Bikkina, D. J. B.; Kulhari, H.; Nikhila, N.; Chinde, S.; Raghavendra, Y. M.; Sreedhar, B.; Tiwari, A. K. *Int. J. Biol. Macromol.* **2014,** *69*, 267-273. http://dx.doi.org/10.1016/j.ijbiomac.2014.05.035.

[67] Oliveira, I.; Sousa, A.; Morais, J. S.; Ferreira, I. C. F. R.; Bento, A.; Estevinho, L.; Pereira, J. A. *Food Chem. Toxicol.* **2008,** *46*, 1801-1807. https://doi.org/10.1016/j.fct.2008.01.026.