

# Experimental and Computational Study on the Inclusion Complexes of $\beta$ -Cyclodextrin with Selected Food Phenolic Compounds

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## Abstract

Phenolic compounds, such as caffeic acid, *trans*-ferulic acid and *p*-coumaric acid that are commonly found in food products are beneficial for human health. Cyclodextrins can form inclusion complexes with various organic compounds in which the physicochemical properties of the included organic molecules are changed. In this study, inclusion complexes of three phenolic compounds with  $\beta$ -cyclodextrin were investigated. The complexes were characterized by various analytical methods, including NMR spectroscopy, FT-IR spectroscopy, mass spectrometry, differential scanning calorimetry and scanning electron microscopy. Results showed that the phenolic compounds used in this study were able to form inclusion complexes in the hydrophobic cavity of  $\beta$ -cyclodextrin by non-covalent bonds. Their physicochemical properties were changed due to the complex formation. In addition, a computational study was performed to find factors that were responsible for binding forces between flavors and  $\beta$ -cyclodextrin. The quantum-mechanical calculations supported the results obtained from experimental studies. Thus,  $\Delta H_f$  for the complex of *p*-coumaric acid and  $\beta$ -cyclodextrin has been found as  $-11.72 \text{ kcal/mol}$ , which was about  $3 \text{ kcal/mol}$  more stable than for inclusion complexes of other flavors. A HOMO-LUMO gap for the complex of *p*-coumaric acid and  $\beta$ -cyclodextrin had the largest value ( $8.19 \text{ eV}$ ) in comparison to other complexes, which was an indication of higher stability complex.

**Keywords:** cyclodextrin, inclusion complex, modeling, phenolic compounds, quantum chemical properties

## 1. Introduction

Caffeic acid (3,4-dihydroxy-cinnamic acid, CA), *trans*-ferulic acid (4-hydroxy-3-methoxy-cinnamic acid, FA), and *p*-coumaric acid (4-hydroxy-cinnamic acid, CO) belong to the hydroxycinnamic acids of selected phenolic acids, which can be found in cereals in the free and bound forms [1]. Phenolic acids occur in fruits, vegetables or medicinal plants as secondary plant metabolites [2] and consist of one aromatic ring that has one or more hydroxyl groups [3]. For example, ferulic acid is a ubiquitous compound in nature, especially rich as an ester form in rice bran pitch, which is yielded when rice oil is produced<sup>4</sup>. Ferulic acid has antioxidant properties and therefore is important for food chemistry. Some other ferulic acid related compounds such as *p*-coumaric acid, caffeic acid are also well-known for their important properties [4-5]. The properties of these compounds depend on chemical structure, such as various biological properties, including toxicity. It is well known that the number of the hydroxyl groups in these compounds increases the

toxicity [4]. Phenolic acids are sensitive to temperature, light, and oxidation and are soluble in water [4].

Lately, another class of compounds are suggested to be added as food debittering agents – cyclodextrins [6]. For example, cyclodextrins can significantly reduce a bitter taste of phenolic compounds. Cyclodextrins (CDs) are also called as Schardinger dextrins, produced industrially by enzymatic starch degradation and composed of glucose units linked by  $\alpha$ -1,4-glycosidic bonds [6]. CDs are classified as  $\alpha$ ,  $\beta$  and  $\gamma$  with six ( $\alpha$ -CD), seven ( $\beta$ -CD) and eight ( $\gamma$ -CD) glucose units joined together, respectively [7]. The geometrical shape is truncated conical, similar to a doughnut shape with a hydrophobic cavity and a hydrophilic outer surface [8, 9]. Smaller cyclodextrins do not exist due to steric reasons [10]. All glucose units are presented in the rigid <sup>4</sup>C<sub>1</sub>-chair conformation [11]. The protons H<sup>1</sup>, H<sup>2</sup>, H<sup>4</sup> and H<sup>6</sup> are oriented towards the hydrophilic outside. The protons H<sup>3</sup> and H<sup>5</sup> are placed inside the hydrophobic cavity. All the secondary hydroxyl groups are placed on the wide rims of the torus, in contrast all primary hydroxyl groups are situated on the narrow rim. The primary hydroxyl groups can rotate freely while the rotation of the secondary hydroxyl groups is blocked because they are attached to relatively rigid chains. The complete rotation of a glucose unit around the C(1)-O-C(4) bonds is not observed due to steric reasons [12-13].

CDs are non-toxic and able to form inclusion complexes with various organic molecules in their cavity, which can be in solid, liquid and gaseous phase. The organic molecule is called as the guest; the CD is called as the host [14-15]. The inclusion complex

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formation between host and guest depends on the geometry and the size of the guest because the guest must fit into the cavity. The guest should not be too small, because this leads to large bond distances between the host and the guest. Very small molecular weight guests do not form stable complexes with cyclodextrins. Also, the guest should not have a large molecular size, because then the guest will not fit completely within the cyclodextrin cavity due to steric reasons. However, large sized compounds could form partial complexes with CDs. The guest molecules form noncovalent bonds with CDs during inclusion complex formation, which could be hydrophobic interactions, hydrogen bonds and van der Waals interactions. Furthermore, electrostatic interactions can also occur between the host and the guest in the hydrophobic cavity. The hydrogen bonds outside the cyclodextrin cavity with water molecules stabilize cyclodextrin and the steric effects have a major role for stabilization [16, 17].

There are a number of computational studies devoted to molecular modeling studies of CD conformations investigations, as well as possible inclusion complexes of them with various compounds. Thus, Zhang et al investigated inclusion complexes of one compound, cordycepin, with all three cyclodextrins ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) experimentally and computationally [18]. They showed that the most stable complex forms with  $\gamma$ -CD. In another study [19], authors investigated inclusion complexes of  $\alpha$ -,  $\beta$ -,  $\gamma$ -cyclodextrins with antipyrine compound experimentally and computationally. They applied NMR spectroscopy, semiempirical AM1 method and force field molecular dynamics (MD), where they found  $\beta$ - and  $\gamma$ -CD complexes were more stable in comparison to  $\alpha$ -CD complex. Swiech et al, investigated inclusion complexes of doxorubicin with a lipolic acid derivative of  $\beta$ -CD ( $\beta$ CDLip), where they investigated self-inclusion complex electrochemically and computationally using AMBER03 force field [20]. Pinjari et al, investigated hydrogen bonding, NMR chemical shifts and electron density topography in  $\alpha$ -,  $\beta$ -,  $\gamma$ -cyclodextrin conformers [21]. In a recent study, Zhao et al, performed a combined NMR spectroscopy and molecular docking study of inclusion complexes of isoflavones (daidzein, daidzin and puerarin) with  $\beta$ -CD [22], where they found an agreement between docking study results and experimental data.

The inclusion complexes of phenolic acids with CDs could play an important role in food industry because they are commonly found in cereals. Thus, the physicochemical properties of the included phenolic acids are changed, due to complexation. Also, such properties as solubility, durability and stability of the included phenolic acids can be improved. At the same time, volatility and toxicity of the included phenolic acids become lower, while bitter tastes and unpleasant odors are masked significantly. The phenolic compounds upon complexation are stabilized against light, UV radiation, thermal decomposition and oxidation [23-26].

In this work, we investigated inclusion complexes of three phenolic compounds (*trans*-ferulic acid, *p*-coumaric acid, caffeic acid) with  $\beta$ -cyclodextrin and analysed them by various analytical methods, including NMR spectroscopy, FT-IR spectroscopy, mass spectrometry, differential scanning

calorimetry, scanning resonance microscopy. In addition, a computational study was performed to find the main factors responsible for the interaction of  $\beta$ -cyclodextrin with the investigated flavor compounds and stability of their complexes.

## 2. Materials and Methods

### 2.1. Materials

Caffeic acid (3,4-dihydroxy-cinnamic acid, CA), *trans*-ferulic acid (4-hydroxy-3-methoxy-cinnamic acid, FA), *p*-coumaric acid (4-hydroxy-cinnamic acid, CO) and  $\beta$ -cyclodextrin ( $\beta$ -CD) were purchased from Sigma Aldrich (Fig 1A). Chemicals were of analytical grade.

#### 2.1.1. Preparation of Complexes

An equimolar mixture of flavor (3 mM) and  $\beta$ -CD (3 mM) were mixed in 50 mL solvent ( $D_2O$ ). Then, the mixture was stirred for 5h and left for 12 h in the dark at room temperature. The mixture was filtered, the solution was frozen at  $-20^\circ C$  and left for least 24 h in a freeze-drier. The resulting powder was used for characterization.

#### 2.1.2. Preparation of Physical Mixtures

Each of the flavor and cyclodextrin compounds were crushed separately in mortars and then mixed together in equimolar ratio (each 3 mM).

### 2.2. Experimental Study

#### 2.2.1. NMR Spectroscopy

For the NMR analysis the complexes were prepared as an equimolar mixture (1:1 ratio), by taking each flavor and of  $\beta$ -cyclodextrin at 5 mM in 2 mL of  $D_2O$ . All mixtures were mixed by vortexer.

400 MHz- and 500 MHz Bruker-DRX-NMR spectrometers were used for  $^1H$ -NMR analysis. The software Top Spin was used to operate the NMR-spectrometers and evaluate the measured data. All samples were prepared in  $D_2O$  and the spectra were recorded at room temperature with following measurements conditions: radiation with  $90^\circ$  pulses of 11.8  $\mu s$ , 256 as a number of repetitions and a repetition time of 13.5 s.

#### 2.2.2. Mass spectroscopy (MS)

All complexes (1 mg) were weighed and dissolved in a mixture of 0.8 mL water and 0.8 mL methanol. The spectra for all complexes were recorded on Waters' SYNAPT G2-Si instrument equipped with Electron Spray Ionizations (ESI) source and Q-ToF analyzer. MS conditions were as followed: negative ions, resolution V-mode, rate 5 $\mu$ L/min, capillary voltage 1.4 kV, cone voltage 100 V, con gas 31 L/h, desolvation temperature 250  $^\circ C$ , desolvation gas ( $N_2$ ) 402 mL/h, scan time 1.0 s and inter-scan time 0.015 s. All Data was processed with MassLynx software v4.1.

#### 2.2.3. Fourier Transform InfraRed (FT-IR) Spectroscopy

The pure CD and flavors, as well as physical mixtures and complexes were recorded between 4000 and 500  $cm^{-1}$  with 64 scans at a resolution of 4  $cm^{-1}$  by a Nicolet iS10 FT-IR spectrophotometer (Nicolet, USA). The software OMNIC was used to operate the FT-IR spectrophotometer.

#### 2.2.4. Differential Scanning Calorimetry (DSC)

The thermal behaviour of the investigated systems was studied by DSC. The complex, the physical mixture, and their pure

compounds were dried for 24 h at 110 °C. Samples were separately weight between 3 mg and 5 mg in an aluminium pan. For the measurement, DSC 6000 differential scanning calorimeter was used by Perkin Elmer. The samples were heated in an aluminium pan between 50 and 230 °C. The scanning rate was 5 °C/min and the nitrogen flow was 20 mL/min.

### 2.2.5. Scanning Electron Microscopy (SEM)

The surfaces of the samples were studied by scanning electron microscopy (JEOL JSM-6490LV, Peabody MA, USA). Samples were prepared to adhesive carbon tabs to a cylindrical aluminium mount and the excess blown off with a stream of nitrogen gas. Samples were coated with a conductive layer of gold using a Cressington 108 auto sputter coater (Ted Pella Inc., Redding CA, USA) and they were recorded at 15 kV.

### 2.3. Computational Study

Molecular modelling was carried out to further elaborate the complexation mechanism of investigated structures and  $\beta$ -cyclodextrin according to the NMR results. The structures were built using Avogadro software (Version 1.20, <http://avogadro.cc>), followed by optimization and quantum-mechanical calculation using MOPAC software package (MOPAC2012, <http://OpenMOPAC.net>).

The calculations were performed at the semi-empirical level, utilising the efficient Parameterized Model 6 (PM6) method. The semi-empirical PM6 method has been shown to be a powerful tool in the conformational study of cyclodextrin complexes and has high computational efficiency in calculating cyclodextrin systems. Thus, PM6 uses a novel parameterisation of the previously used PM3 Hamiltonian and delivers results that comparable to the Density Functional Theory (DFT) level [28]. The method is implemented in the MOPAC2012 software package. Semiempirical methods were successfully utilized in various problems [28-30].

The following molecular properties were calculated for each structure: heat of formation ( $H_f$ ), dipole moment ( $\mu$ ), total energy ( $E_T$ ), Electrostatic Surface Potential (ESP) and HOMO, LUMO energies. The values of each computational property for each inclusion complex are reported respectively in Table 2.

## 3. Results and Discussion

In this work three flavor compounds were investigated as potential food additives that can increase food nutritional properties, but which have a bitter taste which needs to be eliminated. For this purpose the complexation with  $\beta$ -cyclodextrin is suggested. We have analysed experimentally and computationally three flavor compounds involved in inclusion complexes with  $\beta$ -cyclodextrin. The structures of  $\beta$ -CD and each flavour are shown in Figure 1A.

### 3.1. NMR Analysis

First, the inclusion complex formation was studied by nuclear magnetic resonance spectrometry (NMR), to confirm the interaction of flavour compounds with  $\beta$ -CD. The  $^1\text{H}$  NMR chemical shifts for the inclusion complexes and free components were determined. In this regard a chemical shift values difference is calculated by the Eq. 1, where  $\delta_{\text{pure}}$  is the

chemical shift of the pure component and  $\delta_{\text{complex}}$  is the chemical shift of the complex.

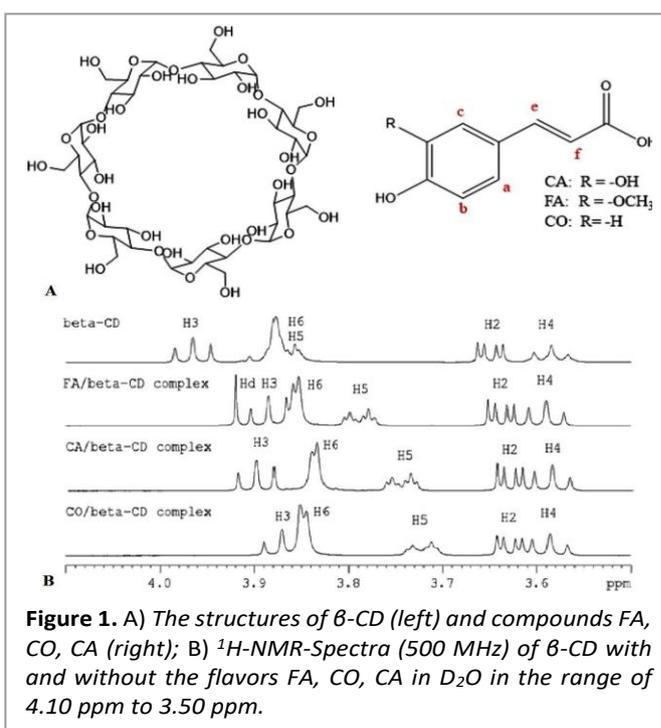
$$\Delta\delta = \delta_{\text{pure}} - \delta_{\text{complex}} \quad (1)$$

Based on the chemical shifts differences the more precise position of the intermolecular interaction between the guest and the host cyclodextrin in the complex is determined and defined the position of the guest molecule, inside or outside the cavity of cyclodextrin.

To take  $^1\text{H}$ -NMR spectra the flavors - caffeic acid, trans-ferulic acid and p-coumaric acid were complexed with  $\beta$ -CD in  $\text{D}_2\text{O}$ . For each complex, the  $^1\text{H}$ -NMR spectra and chemical shifts data are represented in Figure 1B and Table 1. The results show that the flavors - caffeic acid, trans-ferulic acid and p-coumaric acid were included completely in the cavity of  $\beta$ -CD, based on analysis of chemical shifts differences of the inner protons H3 and H5 of  $\beta$ -CD. Additionally, the H5 signal had been separated from the H6 signal and located as a single peak. The outer protons H2 and H4 had small chemical shift differences in all three  $^1\text{H}$ -NMR spectra which means that the guest protons were not located in the immediate vicinity of the outer protons H2 and H4 of  $\beta$ -CD (Table 1).

### 3.2. MS Analysis

Next, the inclusion complex formation was investigated by MS, to check in what stoichiometric proportions these compounds are interacting. In the spectrum of ferulic acid/ $\beta$ -CD complex, the characteristic peaks of the complex are at 1327.4200, 1328.4232, 1329.4259 and 1330.4286 m/z. In the spectrum of coumaric acid/ $\beta$ -CD complex, the peaks of the complex could be seen at 1297.4095, 1298.4127, 1299.4153 and 1300.4180 m/z. In the spectrum of caffeic acid/ $\beta$ -CD complex, the peaks of the complex are at 1313.4043, 1314.4076, 1315.4102 and 1316.4128 m/z. All MS spectra are shown that the three



**Figure 1.** A) The structures of  $\beta$ -CD (left) and compounds FA, CO, CA (right); B)  $^1\text{H}$ -NMR-Spectra (500 MHz) of  $\beta$ -CD with and without the flavors FA, CO, CA in  $\text{D}_2\text{O}$  in the range of 4.10 ppm to 3.50 ppm.

**Table 1.**  $^1\text{H-NMR}$  Chemical shifts ( $\delta$ , ppm) for CH protons of  $\beta$ -CD pure, flavors pure and their complexation induced shifts (CIS =  $\delta_{\text{complex}} - \delta_{\text{guest}}$ ) in  $\text{D}_2\text{O}$  at  $25^\circ\text{C}$ .\*

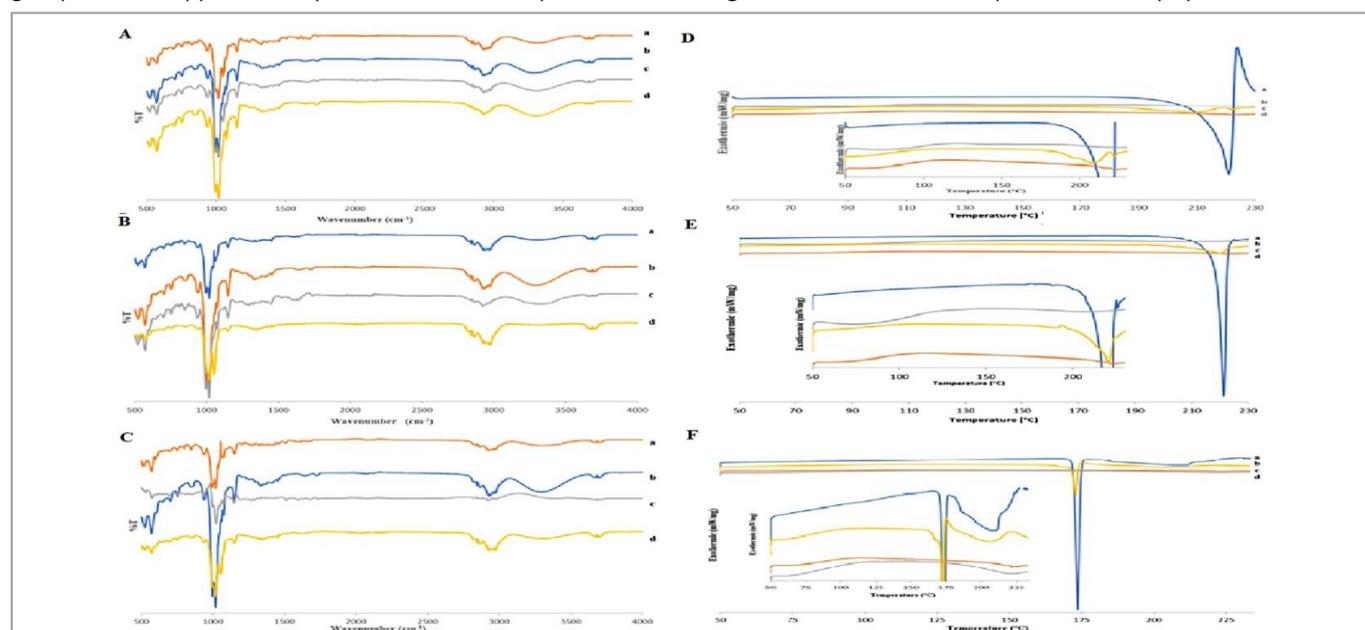
	CA			CO			FA		
	$\delta_{\text{free}}$	$\delta_{\text{complex}}$ x	$\Delta\delta$	$\delta_{\text{free}}$	$\delta_{\text{complex}}$ x	$\Delta\delta$	$\delta_{\text{free}}$	$\delta_{\text{complex}}$ x	$\Delta\delta$
H1	5.0713	5.0542	0.0171	5.0713	5.0526	0.0187	5.0713	5.0584	0.0129
H2	3.6506	3.6310	0.0196	3.6506	3.6285	0.0221	3.6506	3.6375	0.0131
H3	3.9664	3.9004	0.0660	3.9664	3.8702	0.0962	3.9664	3.8844	0.0821
H4	3.5858	3.5861	-0.0003	3.5858	3.5858	0.0000	3.5858	3.5897	-0.0039
H5	3.8621	3.7460	0.1160	3.8621	3.7216	0.1405	3.8621	3.7880	0.0741
H6	3.8786	3.8388	0.0398	3.8786	3.8479	0.0307	3.8786	3.8524	0.0262
Ha	7.1155	7.0424	0.0732	7.5721	7.5013	0.0708	7.1873	7.1168	0.0704
Hb	6.9422	6.9365	0.0057	6.9382	6.9562	-0.0180	6.9513	6.9756	-0.0244
Hc	7.1905	7.1114	0.0791	6.9382	6.9562	-0.0180	7.2940	7.1715	0.1225
Hd	7.5179	7.5521	-0.0342	7.5721	7.5013	0.0708	3.9057	3.9192	-0.0135
	6.3548								
He	1	6.26764	0.08717	7.6063	7.5949	0.0113	7.6452	7.5442	0.1010
Hf				6.3903	6.2961	0.0942	6.4025	6.2888	0.1137

complexes - coumaric acid/ $\beta$ -CD complex, ferulic acid/ $\beta$ -CD complex and caffeic acid/ $\beta$ -CD complex are formed. All MS spectra are shown in Figure 3 (A, B, C).

### 3.3. FT-IR Analysis

The FT-IR spectra for the complex systems between the flavors (FA, CO, CA) and  $\beta$ -cyclodextrin were recorded for each complex, the physical mixtures and all pure forms. All FT-IR spectra are shown in Figure 2(A,B,C). In the spectrum of  $\beta$ -CD, the significant peaks were at  $3370\text{ cm}^{-1}$  for hydroxyl groups,  $2950\text{ cm}^{-1}$  for C-H stretching vibrations;  $1019$ ,  $1176$ ,  $938$ ,  $571\text{ cm}^{-1}$ ; and  $514\text{ cm}^{-1}$  for C-O stretching vibration. In the spectra of the flavors, the prominent absorption bands of hydroxyl group were at approximately  $3345\text{ cm}^{-1}$  and the peak at  $2937$

$\text{cm}^{-1}$  is characteristic for C-H stretching vibrations. The absorption band of an aromatic conjugated carbonyl is shown at about  $1649\text{ cm}^{-1}$ . The absorption bands of an aromatic nucleus were at around  $1459$ ,  $1412$ ,  $1370$ ,  $1366$ ,  $1324$  and  $1291\text{ cm}^{-1}$ . The peak at  $1019\text{ cm}^{-1}$  is attributed to the C-O stretching vibration. The peaks at  $526$ ,  $596$ ,  $702$  and  $757\text{ cm}^{-1}$  correspond to the four hydrogen atoms on the phenyl ring of CO. The spectra of the physical mixtures were not significantly ( $p < 0.05$ ) different from the single compound. In the physical mixture between FA and  $\beta$ -CD, the peak at  $1019\text{ cm}^{-1}$  increased in intensity. Also, peak intensity at  $3345\text{ cm}^{-1}$  was decreased. On the other hand, the spectra of the complex systems have significant differences compare to that of physical mixture. For



**Figure 2.** FT-IR Spectra: **A)** (a)  $\beta$ -CD, (b) CO, (c)  $\beta$ -CD/CO physical mixture and (d)  $\beta$ -CD/CO complex. **B)** (a)  $\beta$ -CD, (b) CA, (c)  $\beta$ -CD/CA physical mixture and (d)  $\beta$ -CD/CA complex. **C)** (a)  $\beta$ -CD, (b) FA, (c)  $\beta$ -CD/FA physical mixture and (d)  $\beta$ -CD/FA complex; DSC-Diagrams **D)** (a) CA, (b)  $\beta$ -CD/CA complex (c)  $\beta$ -CD/CA physical mixture and (d)  $\beta$ -CD. **E)** (a) CO, (b)  $\beta$ -CD/CO complex, (b)  $\beta$ -CD/CO physical mixture and (d)  $\beta$ -CD. **F)** (a) FA, (b)  $\beta$ -CD/FA physical mixture, (c)  $\beta$ -CD, and (d)  $\beta$ -CD/FA complex.

example, the peak at  $1019\text{ cm}^{-1}$  increased in intensity. The peak at  $3333\text{ cm}^{-1}$  was increased in intensity and shifted slightly. All these changes demonstrated that the flavours CO, CA and FA were included in the cavity of  $\beta$ -CD.

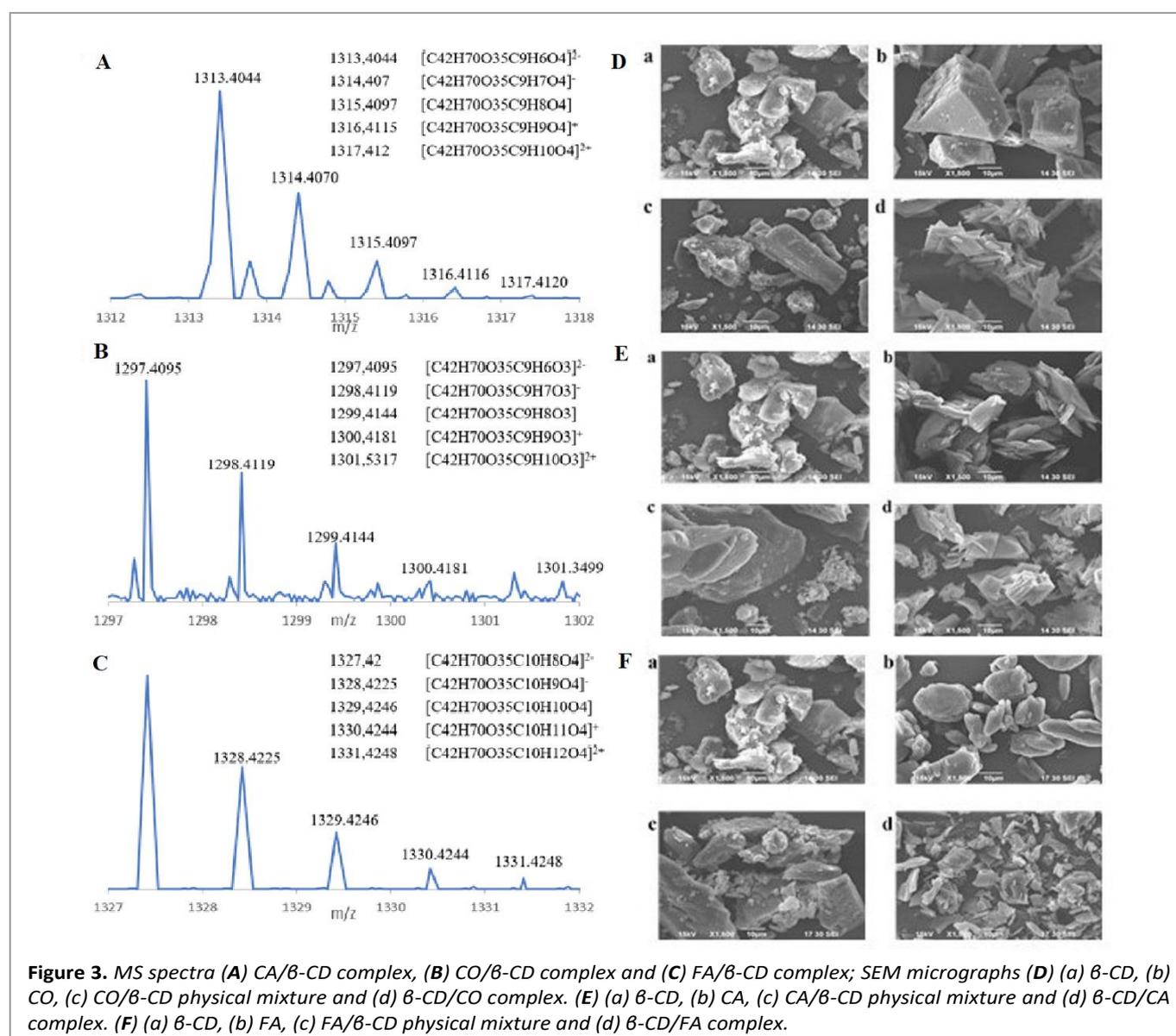
### 3.4. DSC Analysis

Further, the inclusion complex formation was studied by DSC. The thermal curves for the systems CA and  $\beta$ -CD, CO and  $\beta$ -CD, FA and  $\beta$ -CD in pure, physical mixture and complex forms were recorded. All thermal curves are shown in Figure 2 (D,E,F). The thermal curve of caffeic acid illustrates a characteristic peak at around  $221\text{ }^\circ\text{C}$  corresponding to the melting point. CO has a characteristic peak at around  $221\text{ }^\circ\text{C}$  and FA at around  $173\text{ }^\circ\text{C}$  corresponding to their melting points. In the thermal curve of  $\beta$ -CD, a wide peak at  $67\text{ }^\circ\text{C}$  is visible. All thermal curves of the physical mixtures, the characteristic peaks of each flavors were decreased significantly in intensity and the peak of  $\beta$ -CD had been shifted (CA:  $67\text{ }^\circ\text{C}$  to  $52\text{ }^\circ\text{C}$ ; CO:  $67\text{ }^\circ\text{C}$  to  $53\text{ }^\circ\text{C}$ ; FA:  $67\text{ }^\circ\text{C}$  to  $53\text{ }^\circ\text{C}$ ). However, the thermal curves of all complex systems show no similar features to pure compounds. In all complex

systems the peak at  $67\text{ }^\circ\text{C}$  of  $\beta$ -CD was shifted and increased in intensity (CA:  $67\text{ }^\circ\text{C}$  to  $79\text{ }^\circ\text{C}$ ; CO:  $67\text{ }^\circ\text{C}$  to  $80\text{ }^\circ\text{C}$ ;  $67\text{ }^\circ\text{C}$  to  $62\text{ }^\circ\text{C}$ ), whereas the characteristic peaks of each flavor had been totally disappeared. All changes in complex systems are suggested that the flavors were located inside the cavity of  $\beta$ -CD.

### 3.5. SEM Analysis

The inclusion complex formation was also studied by SEM. The SEM technique was applied to investigate the surface morphology of all three systems in pure, physical mixture and complex forms. The SEM micrograms are shown in Figure 3 (D,E,F). In pure form,  $\beta$ -CD had partially angular and partially round thick plates. The surface of pure flavors CA and CO were angular thick plates. However, the flavor FA was round thick plates. In all three-physical mixture, both compounds were mixed, and the original morphology did not change. In comparison, the particles in all images were different for the pure, physical mixture and complex forms. The particles of the complex were observed as thin crystals which are joined into

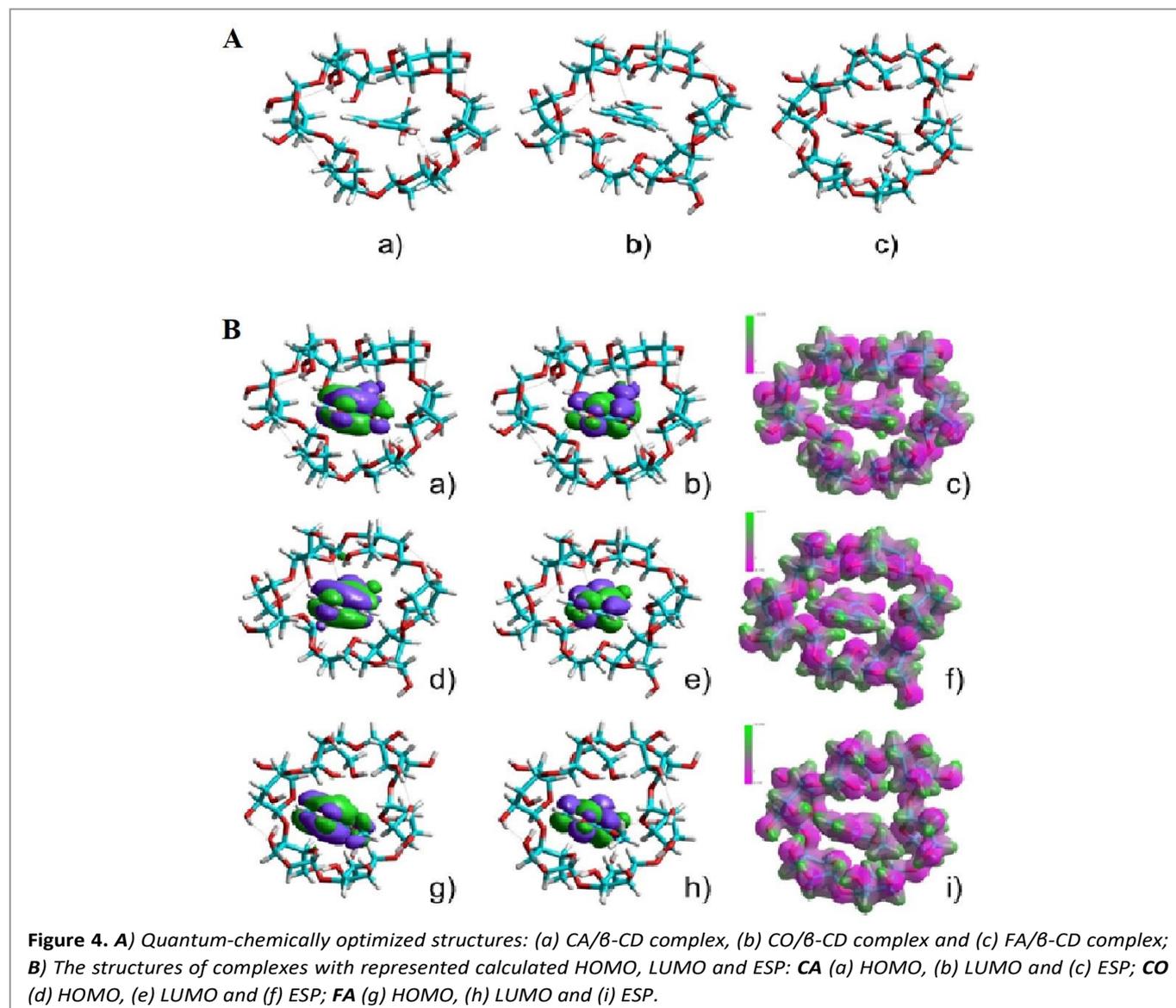


each other like blossoms. All changes illustrate that the flavors CA, CO and FA were embedded inside the cavity of  $\beta$ -CD.

### 3.6. Computational Analysis

A computational study was performed to investigate the inclusions complexes, find orientation, interaction sites and visually illustrate the formation of flavour/ $\beta$ -CD complex. The PM6 method [32] was adopted to search for the lowest energy structure of the inclusion system flavor/ $\beta$ -CD. The binding energy ( $\Delta E$ ), enthalpy changes ( $\Delta H$ ) of each structure obtained in the optimisation process were calculated by Eqs. 2-3, as follows:

more stable than CA/ $\beta$ -CD and FA/ $\beta$ -CD complexes. Thus,  $\Delta H$  for CO/ $\beta$ -CD complex is -11.72 kcal/mol, which is about 3 kcal/mol stable than for other complexes. Negative values for the  $\Delta H$  indicate that the formations of all the complexes are exothermic processes and complexes are stable. Considering the structural features of the host and guest, it becomes clear that the hydrogen bond between the hydrogen atoms of  $\beta$ -CD and the oxygen atoms of all investigated flavors strengthen the host-guest association. However, since CO molecule is less bulky than CA and FA, it has better fit within the cavity of  $\beta$ -CD, which gives better stability to the complex.



$$\Delta H_{enthalpy} = \Delta H_{flavor/CD\ inclusion\ complex} - (\Delta H_{CD} + \Delta H_{flavor}) \quad (2)$$

Typically, the more negative the enthalpy change or binding energy is, the stronger the interaction is between host and guest molecules. From the differences between binding energies or enthalpy, we can see that the CO/ $\beta$ -CD complex is

As shown in Figure 4A, all flavor molecules are almost similarly docked in the  $\beta$ -CD cavity, while CO molecule shows better stability due to a smaller size. The suitable size (approximate length of 5 Å and volume size of 517 Å<sup>3</sup> (CO), in comparison to 536 Å<sup>3</sup> and 589 Å<sup>3</sup> of volume size for CA and FA, respectively) and hydrophobic property together were suggested to provide

**Table 2.** Enthalpy of formation ( $H_f$ ),  $\Delta H$ , total energy ( $E_T$ ),  $Mv$  and frontier orbital energies ( $E_{HOMO}$ ,  $E_{LUMO}$ ) for  $\beta$ -CD complexes with flavors.\*

Complex	Calculated properties of the complex of flavor/ $\beta$ -CD						
	$E_T$	$Mv, \text{\AA}$	$H_f$	$\Delta H_f$	$E_{HOMO}$	$E_{LUMO}$	$\Delta HL$
CA- $\beta$ -CD	-16350.12	536	-1476.23	-9.81	-9.362	-1.203	8.159
CO- $\beta$ -CD	-16058.70	517	-1437.22	<b>-11.72</b>	-9.330	-1.142	<b>8.188</b>
FA- $\beta$ -CD	-16499.48	589	-1469.97	-9.28	-8.972	-0.893	8.079

\*-  $H_f$ ,  $\Delta H$  is in kcal/mol,  $E_T$ ,  $E_{HOMO}$ ,  $E_{LUMO}$  and  $\Delta HL$  are in eV

synergetic effects for the guest molecule docking into the cavity of  $\beta$ -CD (approximate inner diameter of 8.5 Å). In addition, the calculated molecular orbital energy gaps based on the higher occupied molecular orbital (HOMO) and lower unoccupied molecular orbital (LUMO) energies of the inclusion complexes confirmed the stability trend. The representation of calculated HOMO and LUMO orbitals is shown in Figure 4B. Both HOMO and LUMO orbitals are located on the flavor molecule, in each complex with the flavor compound. The energy of the HOMO and LUMO gap of the CO/ $\beta$ -CD inclusion complex has the largest value (8.19 eV) compared to that of other complexes (CA/ $\beta$ -CD and FA/ $\beta$ -CD). The results shown in Table 2 confirms that the CO/ $\beta$ -CD complex is more stable than others. In addition, calculated Electrostatic Surface Potentials (ESP) show evenly distributed charges within the complex, for all investigated systems, Figure 4B (c, f, i).

In overall, experimental studies confirmed the inclusion complexes formation for investigated flavor compounds. In addition, the computational studies confirmed the experimental findings, including the trend in chemical shifts for the NMR spectra of CD protons that are interacting with flavor molecule. main text of the article should appear here with headings as appropriate.

## Conclusions

The investigated chemical systems with experimental and computational studies show that the flavors CA, CO and FA are forming inclusion complexes with  $\beta$ -CD in a molar ratio 1:1. The chemical shifts analysis indicated that the flavors are entrapped in the CDs cavity. Analysis of MS spectra is also confirmed the complex formation. The FT-IR, DSC, SEM studies demonstrated the formation of inclusion complexes and provided data for analysis differences between the pure compounds, the physical mixture and the complex. Computational studies show that the complexes are stable with hydrogen bond interactions between investigated flavors and cyclodextrin in complexes, which support the experimental data. The computational investigation showed that the CO/ $\beta$ -CD inclusion complex is more stable, by analysis of  $\Delta H_f$  and  $\Delta HL$  energies, than other investigated complexes. Thus,  $\Delta H_f$  value for the complex of *p*-

coumaric acid and  $\beta$ -cyclodextrin has been found as -11.72 kcal/mol, which is about 3 kcal/mol more stable than for inclusion complexes of other flavors. A HOMO-LUMO gap for the complex of *p*-coumaric acid and  $\beta$ -cyclodextrin has the largest value (8.18 eV) in comparison to other complexes, which confirmed a better stability of the complex. The computational analysis confirmed the potential of PM6 quantum-chemical method for robust prediction of flavor-cyclodextrin complexes stability. The proven methodology will be used for further analysis of a large series of flavor compounds to form stable complexes with cyclodextrins.

The conclusions section should come in this section at the end of the article, before the acknowledgements.

## Conflicts of interest

There are no conflicts to declare.

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