Study of Flavonoid/Hydroxypropyl-β-Cyclodextrin Inclusion Complexes by UV-Vis, FT-IR, DSC, and X-Ray Diffraction Analysis

Ji-Sang Kim
Department of Food and Nutrition, Kyungnam University, Gyeongnam 51767, Korea

ABSTRACT: The objective of this study was to investigate characterization of inclusion complexes of flavonoids with hydroxypropyl-β-cyclodextrin (HP-β-CD). The inclusion complexes of flavonoids with HP-β-CD was prepared by the freeze-drying method and its characterization was investigated by different analytical techniques including ultraviolet-visible spectroscopy, Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry, and X-ray diffractionmetry. The catechin/HP-β-CD complex exhibited the highest encapsulation efficiency (83.37%), followed by epicatechin/HP-β-CD (81.51%), morin hydrate/HP-β-CD (81.38%), and quercetin/HP-β-CD (81.16%). The inclusion complexes of HP-β-CD showed a decrease in the absorption of flavonoids with a small shift (≈2 nm) of the λmax, while similar to the characteristic absorption peak of flavonoids. However, the FT-IR spectra of the flavonoid/HP-β-CD inclusion complexes did not display any features that were like the pure flavonoids, although the spectra were very similar to that of HP-β-CD. The melting point of flavonoids disappeared, and the thermal properties of HP-β-CD were altered following formation of the inclusion complex between flavonoids and HP-β-CD, resulting in a shift in the melting peak.

Keywords: flavonoids, hydroxypropyl-β-cyclodextrin, inclusion complex

INTRODUCTION

Cyclodextrins (CDs) are cyclic (α-1,4)-linked oligosaccharides of α-D-glucopyranose units. The three most studied CDs are the α-, β-, and γ-CDs containing six, seven, and eight D-glucopyranose units, respectively. CDs and its derivatives have been used extensively as the host to increase the solubility of poor water-soluble organic guest molecules by formation of inclusion complexes (Duchêne et al., 1999; Del Valle, 2004). The resulting noncovalent inclusions or host-guest complexes are of current scientific and technological interest for their peculiar physical, chemical, and biological properties. Such noncovalent associations can improve the guest water solubility, bioavailability, and stability; they can also regulate the release of the guest molecules (Szente and Szejtli, 1999; Loftsson and Duchêne, 2004). The resulting noncovalent inclusions or host-guest complexes are of current scientific and technological interest for their peculiar physical, chemical, and biological properties. Such noncovalent associations can improve the guest water solubility, bioavailability, and stability; they can also regulate the release of the guest molecules (Szente and Szejtli, 1999; Loftsson and Duchêne, 2007). The potential guest list for molecular encapsulation in CDs is quite varied and includes such compounds as straight or branched chain aliphatic, aldehydes, ketones, alcohols, organic acids, fatty acids, aromatics, gases, and polar compounds such as halogens, oxyacid, and amines (Schmid, 1989). Due to the availability of multiple reactive hydroxyl groups, the functionality of CDs is greatly increased by chemical modification. Through modification, the applications of CDs are expanded. CDs are modified through substituting various functional compounds on the primary and/or secondary face of the molecule. Modified CDs are useful as enzyme mimics because the substituted functional groups act in molecular recognition. The same property is used for targeted drug delivery and analytical chemistry as modified CDs show increased enantioselectivity over native CDs. Hydroxylpropyl-β-cyclodextrin (HP-β-CD), a hydroxyalkyl derivative, is a promising alternative to α-, β-, and γ-CDs as it displays improved water solubility and is more toxicologically benign (Gould and Scott, 2005). With these advantages, HP-β-CD has been successfully used to improve the pharmaceutical properties of active pharmaceutical ingredients (APIs) and exhibits a better potential for application than β-CD (Pérez-Abril et al., 2017).

Flavonoids are the most widely spread and diverse group of polyphenols. Flavonoids form part of a family of naturally occurring polyphenolic compounds characterized by a common benzo-γ-pyrene structure. They are one of the most important groups of compounds present in...
vegetables, especially in the genus *Citrus* (family Rutaceae) (Benavente-García et al., 1997; Benavente-García and Castillo, 2008). More than 8,000 compounds with a flavonoid structure have been identified, this large number arising from various combinations of the multiple hydroxyls, methoxyl, and O-glycoside group substituent on the basic benzo-γ-pyrone (C₆-C₃-C₆) (Hodek et al., 2002). Quantitatively, four types of flavonoids are the most common in the plant kingdom: flavanones, flavones, flavonols, and anthocyanins. These compounds not only play an important physiological and ecological role but are also of commercial interest because of their multitude of applications in the food and pharmaceutical industries (Marín et al., 2007; Benavente-García and Castillo, 2008). The flavonoid is a naturally occurring substance and is excellent in biocompatibility and has various biochemical properties such as anti-angiogenesis, anti-inflammation, antioxidant, anti-bacteria, and anti-viral. Since flavonoids exhibit advantageous properties to the living body, the natural flavonoid substance is expected to be highly effective when used as cancer and inflammation treatment drugs. However, since most flavonoids have hydrophobic ring structures, they show poor solubility in an aqueous solution due to their chemical structural characteristics and have the property of dissolving in organic solvents. Examples of organic solvents in which flavonoids are dissolved include methanol, ethanol, acetone, dimethyl sulfoxide, chloroform, hexane, and acetic acid. It is known that catechin has high solubility in methanol, quercetin has high solubility in dimethyl sulfoxide, and myricetin is highly soluble in ethanol. However, flavonoids such as catechin, epicatechin, morin, and quercetin possess relatively low solubility in water. The low solubility of the flavonoids in water often presents a problem for its medicinal applications. Hence, the development of semisynthetic, water-soluble flavonoids, i.e., hydroxymethylrutosides and inositol-2-phosphatequercetin, have been implicated for the treatment of hypertension and microbleeding (Havsteen, 2002). The EE was determined as described by Sansone et al. (2011). Samples (10 mg) of each encapsulation were dissolved in 4 mL MeOH, sonicated for 5 min and centrifuged for 10 min at 3,000 rpm. Flavonoids concentration was determined in the supernatant solutions using high performance liquid chromatography (HPLC). HPLC analysis was performed using an Agilent 1260 Infinity Quaternary LC (Hewlett Packard, Wilmington, NC, USA), equipped with a multiple wavelength operating at 280 nm. Chromatographic separations were achieved using an Agilent Zorbax RRHD SB-C18 column (2.1 mm i.d. × 100 mm, 1.8 μm particle size; Agilent Technologies, Inc., Santa Clara, CA, USA). The column temperature and flow rate were set at 30°C and 0.3 mL/min, respectively. Two solvents (solutions A and B) were used to achieve a gradient elution. Solution A was composed of water containing 0.1% formic acid, while solution B was composed of acetonitrile containing 0.1% formic acid, and the following gradient was employed: 0% B (0 min), 5% B (0.35 min), 15% B (3.5–7.1 min), 40% B (7.1–25 min), 40% B (25–26 min), 100% B (26–27 min), 100% B (27–29 min), and 0% B (29–35 min). The standards employed for analysis were CA, EC, MH, and QC. The flavonoid was identified and quantified based on analytical standard curves. EE was calculated using the following equation:

$$\text{EE} (%) = \frac{\text{ADC}}{\text{TDC}}$$

where ADC is the actual drug content and TDC is the theoretical drug content.

### MATERIALS AND METHODS

#### Chemicals and reagents

HP-β-CD, CA, EC, MH, and QC were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All chemicals used were of analytical grade and were obtained from Merck KGaA (Darmstadt, Germany), unless mentioned otherwise.

#### Preparation of the flavonoid/HP-β-CD complexes

The complex was prepared by mixing (at 1:1 M ratio) flavonoids and HP-β-CD according to the freeze-drying method described by Pralhad and Rajendrakumar (2004). A mixture of HP-β-CD (0.004 mol) and flavonoids (0.004 mol) was diluted in 50 mL water. The mixture was incubated at 30°C for 24 h at 150 rpm, and then filtered through a 0.45 μm membrane filter. The resulting solution was frozen at −80°C and then subject to lyophilization in a freeze-drier (FDU-1200, EYELA, Tokyo, Japan) for 24 h to obtain a powder.

#### Efficiency of encapsulation (EE)

The EE was determined as described by Sansone et al. (2011). Samples (10 mg) of each encapsulation were dissolved in 4 mL MeOH, sonicated for 5 min and centrifuged for 10 min at 3,000 rpm. Flavonoids concentration was determined in the supernatant solutions using high performance liquid chromatography (HPLC). HPLC analysis was performed using an Agilent 1260 Infinity Quaternary LC (Hewlett Packard, Wilmington, NC, USA), equipped with a multiple wavelength operating at 280 nm. Chromatographic separations were achieved using an Agilent Zorbax RRHD SB-C18 column (2.1 mm i.d. × 100 mm, 1.8 μm particle size; Agilent Technologies, Inc., Santa Clara, CA, USA). The column temperature and flow rate were set at 30°C and 0.3 mL/min, respectively. Two solvents (solutions A and B) were used to achieve a gradient elution. Solution A was composed of water containing 0.1% formic acid, while solution B was composed of acetonitrile containing 0.1% formic acid, and the following gradient was employed: 0% B (0 min), 5% B (0–3.5 min), 15% B (3.5–7.1 min), 40% B (7.1–25 min), 40% B (25–26 min), 100% B (26–27 min), 100% B (27–29 min), and 0% B (29–35 min). The standards employed for analysis were CA, EC, MH, and QC. The flavonoid was identified and quantified based on analytical standard curves. EE was calculated using the following equation:

$$\text{EE} (%) = \frac{\text{ADC}}{\text{TDC}}$$

where ADC is the actual drug content and TDC is the theoretical drug content.
UV-visible spectroscopy
The UV-visible absorption spectra of the flavonoids, HP-β-CD, and inclusion complexes were recorded using a Synergy HTX spectrophotometer (BioTek Instruments Inc., Winooski, VT, USA). Each sample (50 mmol) was dissolved with water at the room temperature. The aqueous solutions were scanned, respectively, in the range from 200 to 400 nm to obtain the UV-visible absorption spectra.

FT-IR
The FT-IR spectra of flavonoids, HP-β-CD, and the inclusion complexes were recorded between 5,000 and 550 cm⁻¹ (mid infrared region) on a FT-IR spectrometer (FT/IR-6300, JASCO Corporation, Tokyo, Japan) with 250 scans at a resolution of 2 cm⁻¹. Each sample was ground with spectroscopic grade potassium bromide (KBr) powder and then pressed into 1 mm pellets (2 mg of sample per 200 mg dry KBr). A blank KBr disk was used as background. FT-IR spectra were smoothed, and the baseline was corrected automatically using the spectrophotometer’s built-in software (Spectra Manager TM II, JASCO Corporation).

DSC
DSC analysis was carried out for flavonoids, HP-β-CD, and the inclusion complexes with a Q10 differential calorimeter calibrated with indium (TA Instruments, New Castle, DE, USA). All samples were previously freeze-dried. Each dried powder (2~3 mg) was heated in a crimped aluminum pan at a scanning rate of 5°C/min between 25 and 400°C temperature range under a nitrogen flow of 40 mL/min. An empty pan sealed in the same way was used as reference. Reproducibility was checked by running the sample in triplicate.

XRD
The X-ray powder diffraction patterns were obtained with a PANalytical X’Pert PRO MPD X-ray diffractometer (PANalytical Inc., Almelo, The Netherlands) using a Ni-filtered, Cu-Kα radiation, a voltage of 40 kV and a 30 mA current. Analyses were performed on the same samples prepared for DSC studies. Data were collected in the range of 2θ=5.00 to 50.00° (θ being the angle of diffraction), with the following parameters at room temperature: step width: 0.02°, step time: 0.4 s, scanning speed: 5°/min, divergence slit width: 0.2 mm, scatter slit width: 0.6 mm, and receiving slit width: 0.2 mm. Samples were freeze-dried, and then 10 mg samples were added into the slide for packing prior to X-ray scanning.

Statistical analysis
The experimental data were subjected to analysis of variance (ANOVA), and the significant differences between the mean values determined from measurements carried out in five replicate tests (i.e., P<0.05) were obtained by Duncan’s multiple range test using statistical analysis software (SPSS 20.0, IBM Inc., Armonk, NY, USA).

RESULTS AND DISCUSSION
EE
The EE value is defined as the ability of the CDs to form a complex with the hydrophobic molecule through inclusion of it (drug) in the CDs hydrophobic cavity. It is more convenient to compare the EE than the equilibrium constant (Kc) values, since EE is less sensitive to errors related to estimation of intrinsic drug solubility. The encapsulation efficiency of encapsulated CA/HP-β-CD, EC/HP-β-CD, MH/HP-β-CD, and QC/HP-β-CD are shown in Table 1. The CA/HP-β-CD complex exhibited the highest encapsulation efficiency (83.37%), followed by EC/HP-β-CD (81.51%), MH/HP-β-CD (81.38%), and QC/HP-β-CD (81.16%). Sansone et al. (2011) reported that the encapsulation efficiency of naringin and quercetin in an emulsion system with phthalate cellulose acetate ranged from 62.0% to 94.0% depending on the combinations used. The authors described the encapsulation rate of a polyphenol compound is related directly to the type of cyclodextrin used. Furthermore, some studies suggested that the encapsulation efficiency of curcumin in different cyclodextrins is variable (Tang et al., 2002; Tomren et al., 2007). Indeed, HP-β-CD immobilizes more curcumin molecules in comparison with the other tested cyclodextrins. The quercetin and myricetin affinities for cyclodextrins are also related to the type of cyclodextrin used (Tomren et al., 2007). In addition, Kim et al. (2009) reported that these groups of flavonoids can interact with CDs by directing the B-ring towards the secondary rim of the CDs or heading the A-ring towards the secondary rim of the CDs. Also, the driving forces between cyclodextrin and drugs which have been proposed to justify the complex formation are hydrogen bonds, van der Waals forces, and hydrophobic interactions, and the release of

| Sample        | Efficiency of encapsulation (unit: %) |
|---------------|--------------------------------------|
| CA/HP-β-CD    | 83.37±0.49a                          |
| EC/HP-β-CD    | 81.51±0.53b                          |
| MH/HP-β-CD    | 81.38±0.46b                          |
| QC/HP-β-CD    | 81.16±0.89b                          |

Data represent the mean values for each sample: standard deviation (n=5). Different letters (a,b) indicate a significant difference at P<0.05. HP-β-CD, hydroxypropyl-β-cyclodextrin; CA, catechin; EC, epicatechin; MH, morin hydrate; QC, quercetin.
“high-energy water” molecules from the cavity (Salústio et al., 2009). Therefore, these results suggested that the structure of flavonoids and various driving forces are important factors in the formation of the complexes of HP-β-CD and flavonoids.

**UV-visible spectroscopic analysis**

UV-visible spectroscopy analyzes the alterations of the electronic energy levels within the molecules because of electrons transfer to different orbitals. In the case of polyphenols, their spectra result from electronic transitions between π-type molecular orbitals (Anouar et al., 2012). The obtained absorption spectra for CA/HP-β-CD, EC/HP-β-CD, MH/HP-β-CD, and QC/HP-β-CD complexes are presented in Fig. 1. HP-β-CD displayed no absorbance in the UV-visible spectrum. The λmax values of CA, EC, MH, and QC were 230 nm and 280 nm, 230 nm and 280 nm, 262 nm and 376 nm, and 256 nm and 372 nm, respectively. As expected, the UV absorbance of HP-β-CD was very low and did not exhibit any appreciable peak within 200 ~ 400 nm as the molecule does not contain π-electrons (double bonds) that can absorb UV energy. Moreover, the inclusion complexes of HP-β-CD showed a decrease in the absorption of flavonoids with a small shift (≈ 2 nm) of the λmax, while being similar to the characteristic absorption peak of polyphenols. Zhao et al. (2010) reported that a blue shift of ca. 2 nm for chlorogenic acid was detected after inclusion with β-CD. However, Górnas et al. (2009) reported that a bathochromic shift of the absorption peaks of chlorogenic and caffeic acids in the presence of β-CD was observed. These results might suggest that the possibility of an interactions between flavonoids and HP-β-CD existed as a result of a partial shielding of the chromophore electrons in the HP-β-CD cavity, indicating that flavonoids are capable of forming inclusion complexes with HP-β-CD. In addition, the spectrum of all inclusion complexes features the high peaks of flavonoids, which prove to be insensitive to HP-β-CD.

**FT-IR analysis**

FT-IR is a useful technique that can be used to confirm the formation of an inclusion complex. The FT-IR spectra of the encapsulated CA/HP-β-CD, EC/HP-β-CD, MH/HP-β-CD, and QC/HP-β-CD are presented in Fig. 2. In the spectrum of CA (Fig. 2A), there were strong absorption bands in the range of 1,687 ~ 1,031 cm⁻¹. The IR spectrum of CA showed the characteristic bands at 3,401 cm⁻¹ for O-H group, 2,919 cm⁻¹ for C-H group, 1,687 cm⁻¹ for C=O group, and 1,523 cm⁻¹ for C=C group. The IR data obtained for CA agreed with previous reports (Ramos-Tejada et al., 2002). In the spectrum of EC (Fig. 2B), there were strong absorption bands in the range of 1,625 ~ 977 cm⁻¹. The IR spectrum of EC showed the characteristic bands at 3,502 ~ 3,415 cm⁻¹ for O-H group, 2,931 cm⁻¹ for C-H group, 1,625 cm⁻¹ for C=O group, and 1,521 ~ 1,442 cm⁻¹ for C=C group. In the spectrum of MH (Fig. 2C), there were strong absorption bands in the range of 1,662 ~ 1,085 cm⁻¹. The IR spectrum of MH showed the characteristic bands at 3,455 ~ 3,166 cm⁻¹ for O-H group, 2,919 cm⁻¹ for C=O group, and 1,521 ~ 1,442 cm⁻¹ for C=C group. In the spectrum of MH (Fig. 2C), there were strong absorption bands in the range of 1,662 ~ 1,085 cm⁻¹. The IR spectrum of MH showed the characteristic bands at 3,455 ~ 3,166 cm⁻¹ for O-H group, 1,662 cm⁻¹ for C=O group, and 1,509 ~ 1,457 cm⁻¹ for C=C group. The IR data ob-

---

**Fig. 1.** Ultraviolet-visible absorption spectra of (A) CA and CA/HP-β-CD, (B) EC and EC/HP-β-CD, (C) MH and MH/HP-β-CD, and (D) QC and QC/HP-β-CD. CA, catechin; EC, epicatechin; MH, morin hydrate; QC, quercetin; HP-β-CD, hydroxypropyl-β-cyclodextrin.
In the spectrum of QC (Fig. 2D), there were strong absorption bands in the range of 1,671 to 1,095 cm\(^{-1}\). The broad bands at 3,423, 3,374, and 3,288 cm\(^{-1}\) originate from the valence vibrations of O–H groups that are probably involved in the formation of intramolecular hydrogen bonds with the C=O group of the ring. The position and intensity of the two bands of C=O also indicate the existence of these bands. The intensive band at 1,671 cm\(^{-1}\) which belongs to the valence vibration of free ketone groups (C=O) is in agreement with the literature (Pralhad and Rajendrakumar, 2004). The band at 1,612 cm\(^{-1}\) is associated with C=O vibration, which is included in the formation of relatively strong hydrogen bonds with O–H groups in positions 3 and 5. In the area of deformation bands at 1,361, 1,317, and 1,243 cm\(^{-1}\), there are bands that belong to the valence C–O and deformation C–OH vibrations. The FT-IR spectrum of pure HP-β-CD illustrated intense broad absorption bands at 3,600 to 3,000 cm\(^{-1}\), which corresponded to the free stretching vibrations. The CH\(_3\) and CH vibrations were at 2,960 cm\(^{-1}\). The large band in the region 1,155 to 1,033 cm\(^{-1}\) was ascribed to the C–O stretching vibration. However, the FT-IR spectra of the flavonoid/HP-β-CD inclusion complexes did not display any features that were similar to the pure flavonoids (Fig. 2), although the...
spectra were very similar to that of HP-\β-CD. Changes in the FT-IR spectra were also observed by Qiu et al. (2014) when evaluating the encapsulation of barbigerone in HP-\β-CD. The authors observed displacement, reduction, and disappearance of the peaks relating to barbigerone in the matrix of HP-\β-CD. Therefore, these results indicated that the fundamental changes which appear in the FT-IR spectra of inclusion complexes of flavonoid with HP-\β-CD are reflected mainly in the C=O stretching spectral region. These changes suggest a similar basic complexation mechanism for guest molecules included in the torus host cavity.

**DSC analysis**

DSC was used to identify the inclusion complex between the drug and HP-\β-CD. Some evidence of inclusion complexation was obtained from thermal analysis. When guest molecules were embedded in HP-\β-CD cavities, their melting, boiling, or sublimation point generally could shift to a different temperature or disappear within the temperature range where HP-\β-CD was decomposed. The DSC curves for CA/HP-\β-CD, EC/HP-\β-CD, MH/HP-\β-CD, and QC/HP-\β-CD complexes are presented in Fig. 3. The DSC curve of CA (Fig. 3A) showed a sharp endothermic peak at 92, 162, and 212°C. The DSC curves of EC (Fig. 3B) and MH (Fig. 3C) showed sharp endothermic peaks at 239 and, 196 and 286°C, respectively. The DSC curve of QC (Fig. 3D) displayed one sharp endothermic peak at 318°C, confirming the crystalline conformation of the starting material. The DSC curve of HP-\β-CD showed a broad endothermic peak at approximately 294°C. However, the DSC curve of the inclusion complex

**Fig. 3.** Differential scanning calorimetry thermograms of (A) CA and CA/HP-\β-CD, (B) EC and EC/HP-\β-CD, (C) MH and MH/HP-\β-CD, and (D) QC and QC/HP-\β-CD. CA, catechin; EC, epicatechin; MH, morin hydrate; QC, quercetin; HP-\β-CD, hydroxypropyl-\β-cyclo-dextrin.
showed the complete disappearance of CA, EC, MH, and QC peaks. Marini et al. (1996) reported the broad endothermic peak at 294°C was related to dehydration of water molecules that bind to cyclodextrin molecules. Hedges et al. (1995) suggested that the exothermic peaks emerging after 300°C were possibly due to melting and thermal decomposition of the HP-β-CD itself. These results suggested that the complete disappearance of the endothermic peak corresponding to flavonoids, and the DSC curves showing the characteristic shape of the amorphous substances, proves the complete formation of the inclusion complex.

XRD analysis
Powder X-ray diffractometry is a useful method for the confirmation of CD complexation in powder or microcrystalline states, whereas single-crystal XRD analysis provides complete structural characterization in the crystalline state (Harata, 1998). As shown in Fig. 4, the XRD pattern of CA, EC, MH, and QC showed intense, sharp peaks due to the crystalline nature of the compound. CA had strong crystallinity peaks at 2θ of 15.3°, 16.4°, 19.1°, 22.0°, 25.4°, 25.9°, and 26.8°. EC had strong crystallinity peaks at 2θ of 18.1°, 19.5°, 22.9°, 24.8°, 26.8°, 28.3°, and 29.1°. MH had strong crystallinity peaks at 2θ of 10.4°, 14.4°, 25.6°, 26.6°, 27.4°, and 28.2°. QC had strong crystallinity peaks at 2θ of 10.7°, 13.4°, 14.4°, 17.7°, 24.3°, 25.0°, 26.0°, 27.6°, and 29.7°. In contrast, the XRD pattern of HP-β-CD revealed a broad peak in the range of 15~25° (2θ), confirming its amorphous character. The XRD pattern of the inclusion complexes displayed a large, broad background under the crystalline peaks, which was like that of the amorphous HP-β-CD. Also, the XRD patterns of the inclusion complexes showed a complete ab-

Fig. 4. Powder X-ray diffraction patterns of (A) CA and CA/HP-β-CD, (B) EC and EC/HP-β-CD, (C) MH and MH/HP-β-CD, and (D) QC and QC/HP-β-CD. CA, catechin; EC, epicatechin; MH, morin hydrate; QC, quercetin; HP-β-CD, hydroxypropyl-β-cyclodextrin.
sence of crystalline peaks, suggesting the formation of a new structure, in which flavonoids probably is included in the hydrophobic cavity of HP-β-CD. It is described that a lack of crystallinity is indicative of the formation of an inclusion complex.

In conclusion, the objective of this study was to investigate characterization of inclusion complexes of flavonoids with HP-β-CD by different analytical techniques including UV-visible spectroscopy, FT-IR, DSC, and XRD. Flavonoids was encapsulated in matrices of HP-β-CD by lyophilization. The results of UV-visible spectroscopy, FT-IR, DSC, and XRD demonstrated that flavonoid/HP-β-CD complex has different physicochemical characteristics from free flavonoid. The results showed that HP-β-CD complexation technology might be a promising strategy to improve the food application of flavonoids. Further studies should be performed to measure the release of flavonoids from the capsules, to determine if the encapsulating material increases efficiency.

**AUTHOR DISCLOSURE STATEMENT**

The author declares no conflict of interest.

**REFERENCES**

Anouar EH, Gierschner J, Duroux JL, Trouillas P. UV/visible spectra of natural polyphenols: a time-dependent density functional theory study. Food Chem. 2012. 131:79-89.

Benavente-García O, Castillo J, Marín FR, Ortuño A, Del Río JA. Uses and properties of Citrus flavonoids. J Agric Food Chem. 1997. 45:4505-4515.

Benavente-García O, Castillo J. Update on uses and properties of citrus flavonoids: new findings in antioxidant, cardiovascular, and anti-inflammatory activity. J Agric Food Chem. 2008. 56: 6185-6205.

Del Valle EMM. Cyclodextrins and their uses: a review. Process Biochem. 2004. 39:1033-1046.

Duchêne D, Wouessidjewe D, Ponchel G. Cyclodextrins and carrier systems. J Control Release. 1999. 62:263-268.

Fang Z, Bhandari B. Encapsulation of polyphenols—a review. Trends Food Sci Technol. 2010. 21:510-523.

Górnas P, Neumann G, Baczynski K, Poleswki K. Beta-cyclodextrin complexes with chlorogenic and caffeic acids from coffee brew: spectroscopic, thermodynamic and molecular modelling study. Food Chem. 2009. 114:190-196.

Gould S, Scott RC. 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD): a toxicology review. Food Chem Toxicol. 2005. 43:1451-1459.

Harata K. Structural aspects of stereodifferentiation in the solid state. Chem Rev. 1998. 98:1803-1828.

Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacol Ther. 2002. 96:67-202.

Hedges AR, Shieh WJ, Sikorski CT. Use of cyclodextrins for encapsulation in the use and treatment of food products. In: Risch SJ, Reineccius GA, editors. Encapsulation and Controlled Release of Food Ingredients. American Chemical Society, Washington, DC, USA. 1995. p 60-71.

Hodek P, Trefil P, Stiborová M, Flavonoids-potent and versatile biologically active compounds interacting with cytochromes P450. Chem Biol Interact. 2002. 139:1-21.

Kim H, Choi J, Jung S. Inclusion complexes of modified cyclodextrins with some flavonoids. J Ind Phenom Macromol Chem. 2009. 64:43. https://doi.org/10.1007/s10847-009-9534-9

Loftsson T, Duchêne D. Cyclodextrins and their pharmaceutical applications. Int J Pharm. 2007. 329:1-11.

Marín FR, Soler-Rivas C, Benavente-García O, Castillo J, Pérez-Alvarez JA. By-products from different citrus processes as a source of customized functional fibres. Food Chem. 2007. 100: 736-741.

Marini A, Berbenni V, Bruni G, Giordano F, Villa M. Dehydration of β-cyclodextrin: facts and opinions. Thermochim Acta. 1996. 279:27-33.

Panwar QK, Memon S. Synthesis, spectral characterization and antioxidant activity of Tin(II)-morin complex. Pak J Anal Environ Chem. 2012. 13:159-168.

Pérez-Abril M, Lucas-Abellán C, Castillo-Sánchez J, Pérez-Sánchez H, Céron-Carrasco JP, Fortea I, et al. Systematic investigation and molecular modelling of complexation between several groups of flavonoids and HP-β-cyclodextrins. J Funct Foods. 2017. 36:122-131.

Pralhad T, Rajendrakumar K. Study of freeze-dried quercetin-cyclodextrin binary systems by DSC, FT-IR, X-ray diffraction and SEM analysis. J Pharm Biomed Anal. 2004. 34:333-339.

Qi N, Cheng X, Wang G, Wang W, Wen J, Zhang Y, et al. Inclusion complex of barbigerone with hydroxypropyl-β-cyclodextrin: preparation and in vitro evaluation. Carbohydr Polym. 2014. 101:623-630.

Ramos-Tejada MM, Durán JDG, Ontiveros-Ortega A, Espinosa-Jimenez M, Perea-Carpio R, Chibowski E. Investigation of alumin/(+)-catechin system properties. Part I: a study of the system by FTIR-UV-Vis spectroscopy. Colloids Surf B Biointerfaces. 2002. 24:297-308.

Salustio PJ, Feio G, Figueirinhas JL, Pinto JF, Cabral Marques HM. The influence of the preparation methods on the inclusion of model drugs in a β-cyclodextrin cavity. Eur J Pharm Biopharm. 2009. 71:377-386.

Sansone F, Picerno P, Mencherini T, Vilecco F, D’Urso AM, Aquino RP, et al. Flavonoid microparticles by spray-drying: influence of enhancers of the dissolution rate on properties and stability. J Food Eng. 2011. 103:188-196.

Schmid G. Cyclodextrin glycosyltransferase production: yield enhancement by overexpression of cloned genes. Trends Biotechnol. 1989. 7:244-248.

Szent L, Szejtli J. Highly soluble cyclodextrin derivatives: chemistry, properties, and trends in development. Adv Drug Deliv Rev. 1999. 36:17-28.

Tang B, Ma L, Wang HY, Zhang GY. Study on the supramolecular interaction of curcumin and β-cyclodextrin by spectrophotometry and its analytical application. J Agric Food Chem. 2002. 50:1355-1361.

Tomren MA, Måsson M, Loftsson T, Tornesen HH. Studies on curcumin and curcuminoids XXXI. Symmetric and asymmetric curcuminoids: stability, activity and complexation with cyclodextrin. Int J Pharm. 2007. 338:27-34.

Zhao M, Wang H, Yang B, Tao H. Identification of cyclodextrin inclusion complex of chlorogenic acid and its antimicrobial activity. Food Chem. 2010. 120:1138-1142.