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Complexing of chlorogenic acid with β-cyclodextrins: Inclusion effects, antioxidative properties and potential application in grape juice

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ABSTRACT

Chlorogenic acid (CGA) is a polyphenol commonly existed in fruits and vegetables with a variety of bioactivities. To increase its stability and potential broader industrial application, CGA/cyclodextrin (CD) complexes were prepared using β-cyclodextrin (β-CD) and its derivative (2-hydroxypropyl)-β-cyclodextrin (2-HP-β-CD). Their inclusion complexation behavior and characterization were investigated by FT-IR, XRD, SEM, and ^{1}H NMR spectroscopy. The morphologic and spectral studies indicated the formation of inclusion complexes. Moreover, spatial arrangements of inclusion complexes were proposed based on ^{1}H NMR results. The antioxidant activities of the complexes were improved compared with the CGA without inclusion, and the CGA/HP-β-CD complexes showed the highest antioxidant activity. In addition, the CGA/CD complexes were added in grape juice to explore the potential on the preservation of anthocyanin and color quality. Results showed that the degradation of anthocyanins was reduced when CGA and CGA/CDs inclusions were added in grape juice due to copigmentation effect, and their copigmentation strength showed in an order of CGA/HP-β-CD complex > CGA/β-CD complex > free CGA.

1. Introduction

Chlorogenic acid (CGA) is a polyphenol derivative existed widely in fruits, vegetables, black teas, soy beans, and wheat (Wang, Wang, & Yang, 2007). Our previous work also demonstrated that the major phenolic compound in *Eucommia ulmoides Oliver* (Du-Zhong in Chinese, a traditional Chinese medicine) leaf is CGA (Shao, Hong, Liu, He, & Sun, 2011). CGA possesses well documented bioactivities such as antioxidant activity (Sato et al., 2011), antimicrobial properties (Puupponen-Pimia et al., 2001; Zhu, Zhang, & Lo, 2004), and anxiolytic activity (Bouayed, Rammal, Dicko, Younos, & Soulimani, 2007). However, CGA is easily to be oxidized and sensitive to heat and light (Chao, Wang, Zhao, Zhang, & Zhang, 2012), which restricts its application in industries.

Encapsulation of polyphenols has been proposed as an alternative to improve their stability, bioactivity and extend its application (Fang & Bhandari, 2010). Inclusion complexation with cyclodextrins (CDs) has been evidenced to be a good encapsulation technology (Davis & Brewster, 2004; Loftsson & Duchene, 2007). CDs are a series of water-soluble cyclic oligomers consisting of six to eight D-glucose monomers linked by α (1→4) glycosidic bonds, which form hydrophobic central cavities with hydrophilic external walls (Del Valle, 2004; Hamilton, Kelly, & Fogarty, 2000; Szejtli, 2004). Hence, CDs are able to interact with a variety of hydrophobic compounds to form inclusion complexes (Ambrus et al., 2011; Karathanos, Mourtzinos, Yannakopoulou, & Andrikopoulos, 2007; Zielenkiewicz, Kózbiał, Golankiewicz, & Poznánski, 2010). When CDs form inclusion complex with guest molecules, the physicochemical properties of the guest molecules could be positively modified, such as enhancing the solubility and stability of the guest molecules (Caliceti et al., 2003; Yang et al., 2010), reducing the drug bitterness and decreasing tissue irritation (Carrier, Miller, & Ahmed, 2007; Misiuk & Zalewska, 2009; Roik & Belyakova, 2011).

Among the natural CDs, β-cyclodextrin (β-CD) is particularly useful in the pharmaceutical industry because of its high encapsulation efficiency, suitable cavity dimensions, and low cost (Sancho, Gasull, Blanco, & Castro, 2011). In addition, its modified derivative of hydroxypropyl-β-cyclodextrin (HP-β-CD) is well

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studied in drug encapsulation because of its good inclusion capacity as well as high water solubility, non-toxicity and biocompatibility (Eid et al., 2011; Stella & He, 2008).

The addition of phenolic compounds to prevent anthocyanin degradation due to copigmentation effect has been widely recognized (Chen & Hrazdina, 1981; Mazza & Brouillard, 1987; Mazza & Miniati, 1993). Earlier studies also demonstrated that CGA could be applied as a copigment to enhance the color stability and intensity of cyanidin-3-glucoside (Bakowska, Kucharska, & Oszmianski, 2003). However, to our knowledge, no literature is available on utilization of inclusion complexed polyphenols as copigments of anthocyanins. Therefore, the aim of this study was to prepare inclusion complexes between CGA and two different CDs, namely β-CD and 2-HP-β-CD, to investigate their effects on preservation of anthocyanins and color quality of grape juice. The obtained complexes were characterized by FT-IR, XRD, SEM, and ^{1}H NMR spectroscopy and their physical, chemical and biological properties were compared. This study may expand the industrial applications of CGA inclusion complexes as enhanced antioxidants and provide a new method to improve the color stability of anthocyanin rich products through copigmentation with encapsulated polyphenols.

2. Materials and methods

2.1. Materials and chemicals

CGA (molecular weight = 354.31), β-CD (molecular weight = 1134.98) and 2-HP-β-CD (molecular weight = 1541.54) were purchased from Aladdin (Shanghai, China). DPPH• (2, 2-di (4-tert-octylphenyl)-1-picrylhydrazyl) free radical, ferrous sulphate ($FeSO_4$), potassium ferricyanide ($K_3Fe(CN)_6$), and ferric chloride ($FeCl_3$) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Other reagents and chemicals were of analytical reagent grade. The water used was double distilled and deionized.

Grapes were purchased from a local supermarket and stored at 4 °C before being processed into juice.

2.2. Preparation of CGA/β-CD and CGA/HP-β-CD inclusion complexes

The preparation of CGA/β-CD and CGA/HP-β-CD inclusion complexes was followed the methods of previous reports (Lu, Chen, Fu, Xiong, & Hu, 2012; Misiuk & Zalewska, 2009) with modifications. Briefly, the CGA was slowly added in aqueous solution of CDs under magnetic stirring at an approximate molar ratio of 1:1 (50 mg CGA and 162 mg β-CD, 50 mg CGA and 221 mg HP-β-CD, respectively) and temperature of 50 °C, and then stirred for another 10 h at room temperature. Subsequently, the un-dissolved CGA solids were removed by filtration, and the solution was frozen at −40 °C for 24 h and then lyophilized in a freeze dryer (ALPHA2-4LD, Martin Christ, Germany).

2.3. Physicochemical characterization

The X-ray diffraction spectra of CGA/CD complex powders were conducted on a Rigaku TTRIII Rotating Target diffractometer (Rigaku, Japan) with Cu Kα radiation (40 kV, 100 mA), at a scanning rate of 5°/min. Powder samples were mounted on a vitreous sample holder and scanned with a step size of $2\theta = 0.02°$ between $2\theta = 5°$ and 45°.

FTIR spectra of β-CD, HP-β-CD, CGA, CGA/β-CD inclusion and CGA/HP-β-CD inclusion powders were obtained with a Nicolet 6700 IR spectrometer (Thermo Fisher Scientific, MA, USA) using KBr pellets. The diffuse reflectance technique was utilized in the mid-IR (400-4000 cm^{-1}) spectral region.

Scanning electron microscopy (SEM) photographs of the samples were determined on a Quanta 200 environmental scanning electron microscopy (FEI, Hillsboro, OR, USA). The powders were previously fixed on a brass stub using double-sided adhesive tape and then were made electrically conductive by coating with a thin layer of gold (approximately 300 Å) in a vacuum for 30 s and at 30 W. The micrographs were obtained with an accelerating potential of 15 kV under low vacuum.

^{1}H NMR spectra were conducted on a Bruker Avance DRX spectrometer (Brook Company, Switzerland) at 500 MHz and 25 °C in D_2O. The chemical shifts (δ) were presented in terms of parts per million (ppm) with the HOD signals as the internal reference.

2.4. Antioxidant activities in vitro

2.4.1. DPPH radical scavenging activity assay

The DPPH radical scavenging activity of CGA and its CD complexes was determined according to the method of Shimada, Fujikawa, Yahara, and Nakamura (1992) with minor modification. All the tested samples were dissolved in ethanol to prepare various CGA concentrations of 1.5, 0.75, 0.375, 0.188 and 0.095 mg/ml, respectively. Aliquot of 0.5 ml of sample solution was mixed with 2 ml of 0.1 mM DPPH• in ethanol. The mixture was shaken vigorously and kept in dark for 30 min. The absorbance was measured at 517 nm. A control was also prepared by replacing the sample with ethanol. The scavenging activity was calculated using the following equation:

$$AU = (1 - A_s/A_0) \times 100\% \quad (1)$$

where AU is the radical-scavenging activity, A_s is the absorbance of sample (CGA and complexes) and A_0 is the absorbance of blank sample.

2.4.2. Ferric reducing power assay

The reductive potential of samples was determined following Oyaizu (1986) with minor modification. A mixture of 2.5 ml samples (0.095–1.5 mg/ml), 2.5 ml pH 6.6 phosphate buffer (0.2 mol/L) and 2.5 ml 1% (w/v) $K_3Fe(CN)_6$ were incubated at 50 °C for 20 min. The reaction was terminated by adding 2.5 ml trichloroacetic acid (10%, w/v), and the solution was mixed with 5 ml water and 1 ml 0.1% (w/v) $FeCl_3$. The mixture was shaken and its absorbance was measured at 700 nm against a blank. The increase of reaction mixture absorbance indicates an increase of reduction capability.

2.5. Copigmentation of CGA and CGA/CD complexes with grape juice anthocyanins

2.5.1. Juice preparation

Grape juice was prepared by manually pressing the fruit using cheese cloth and filtered through a filter paper (Φ12.5 cm). Juice samples were further prepared with and without addition of selected additives (CGA, CGA/HP-β-CD inclusion, CGA/β-CD inclusion). CGA was added in a pigment (anthocyanin):copigment (CGA) ratio of 1:50 (Kopjar, Jaksic, & Pilizota, 2012). After preparation, samples were kept in glass bottles and left in the dark at 4 °C for 10 days of storage.

2.5.2. Measurement of monomeric anthocyanins

Determination of monomeric anthocyanins was conducted by a pH-differential method (Giusti & Wrolstad, 2001). Sample absorbance was measured at 510 and 700 nm using a PC 2401 UV–VIS spectrophotometer (Shimadzu, Tokyo, Japan). The monomeric