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Chimonanthus praecox extract/cyclodextrin inclusion complexes: Selective inclusion, enhancement of antioxidant activity and thermal stability

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ABSTRACT

In this research, the inclusion complexes of *Chimonanthus Praecox* extract (CPE) with cyclodextrins (CDs) were prepared. The samples before and after encapsulation were analyzed by UHPLC-QTOF-MS and the variation in the contents of each identified bioactive compound were visualized in the heat maps. It was found that β-CD have selective inclusion capacity to flavonoids. Moreover, encapsulation with CDs could significantly improve the antioxidant activity and thermal stability of CPE, enabling application of *Chimonanthus Praecox* extract as natural antioxidants and/or food additive especially when expected to be thermally processed. Therefore, encapsulation with CDs was a promising way in further application of bioactive compounds in plants. Additionally, this study gives new insight into the inclusion behavior between the complicated guests and different hosts, which provided specific guidance on the choice of bioactive guests with appropriate hosts.

1. Introduction

Chimonanthus praecox, famous for its fragrant flowers, is a significant industrial crop native to China, Japan, and some regions of Europe and America (Wang et al., 2011; Kozomara et al., 2008). The species received much attention in folk medicine that is used as treatments for coughs and rheumatic arthritis. Nowadays, the products of *Chimonanthus praecox* such as fresh cut flower and volatile oil have been widely used in ornamental and cosmetic industries. Especially, in our previous study (Zhang et al., 2016), comprehensive phytochemical profile of *Chimonanthus praecox* extracts (CPE) has been studied and many bioactive compounds were identified. It is well known that plant bioactive compounds, gaining growing interest recently, have shown the health benefits with antioxidative, anticarcinogenic, antihypertensive, and so on (Belščak-Cvitanović et al., 2011). However, the use of these compounds is restricted since they have limited water solubility and low stability against environmental factors such as temperature, pH and light (Pinho et al., 2014). To circumvent these drawbacks, encapsulation with cyclodextrins has been proven to be a promising approach (Liu et al., 2016).

Cyclodextrins (CDs) are a family of cyclic oligosaccharides with six, seven, or eight D-glucose units linked by α-1,4-glucose bonds, which are called α, β, or γ-CDs. They possess a toroidal shape with the primary hydroxyl groups at the narrow side and the secondary hydroxyl groups at the wide side. CDs show several advantages compared to other ring molecules: they are water-soluble, non-toxic, commercially available compounds with low price and can be functionalized by a wide variety of synthetic methods. Most importantly, CDs, as widely used supramolecular macrocyclic host molecules with a hydrophobic cavity, are known to form inclusion complexes with a great deal of inorganic/organic/biological guest molecules and ions in both aqueous solution and the solid state (Chen and Liu, 2010), which play an important role in supramolecular chemistry, analytical science, food industry, and so forth. Among them, β-CDs are being widely used in these fields due to its suitable cavity size, and γ-CDs are more appropriate to moderate or larger size guest molecules in some cases, while α-CDs are limited to complex with some small molecules or long alkyl chains (Simoes et al., 2015).

Therefore, the goal of this work was to encapsulate CPE with β-CD and γ-CD; analyze and visualize the changes in content of each identified chemical compound in CPE before and after encapsulation; determine the selective inclusion characters to flavonoids;

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and investigate the enhancement of antioxidant activity and thermal stability of CPE. To the best of our knowledge, there is no previous study on the protection of bioactive compounds in *Chimonanthus Praecox*. In addition, results here may contribute to a further application of *Chimonanthus praecox* in the production of functional bioactive components.

2. Materials and methods

2.1. Reagents and chemicals

β-Cyclodextrin, γ-Cyclodextrin, Formic acid, Rutin trihydrate, Quercetin, Quercitrin, Kaempferol, Hyperoside, Quercitrin, and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (Shanghai, China). UHPLC grade acetonitrile was purchased from Fisher Scientific (Shanghai, China). Other reagents were purchased from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Preparation of plant materials

Plant materials (*Chimonanthus praecox* flowers) were collected from Research Base of *Chimonanthus praecox*, Shanghai, China in January 2016. Identification were supervised by Dr. Du Yongqin (Research Institute of *Chimonanthus praecox*) and deposited in Shanghai Key Laboratory of Functional Materials Chemistry, East China University of Science and Technology, Shanghai, China. The petal samples were then dried at 40 °C and sieved through a 40-mesh sieve.

5 g of powdered samples were mixed with 25-fold methanol and sonicated three times for 1 h. The *Chimonanthus praecox* extracts (CPE) were then dried by an R-201 rotary evaporator (Senco, Shanghai, China) at 40 °C and stored in the dark at −20 °C for further use.

2.3. Preparation of inclusion complexes

In the encapsulation experiment, two inclusion complexes with β-CD and γ-CD were prepared according to the method described by Ding (Ding et al., 2013) and Kalogeropoulos (Kalogeropoulos et al., 2010) with some modifications. The CPE (100 mg) was dissolved into 5 mL methanol, and suspended in 50 mL aqueous solution containing 375 mg β-CD (molar ratio based on the hypothesis that the extract was quercetin, M_r of quercetin and β-CD ≈ 1:3). Then the solution was stirred for 5 h in the dark at 60 °C and subsequently filtered through 0.45 mm PVDF filter (Anaqua chemical supply, Houston, USA). After this process, the water soluble filtrate was frozen and freeze-dried. The obtained CPE/β-CD inclusion complex powder was stored under nitrogen at −20 °C until use.

The CPE/γ-CD inclusion complex was prepared with the same method mentioned above, except that the same CPE methanol solution was suspended in 50 mL aqueous solution containing 429 mg γ-CD (molar ratio based on the hypothesis that the extract was quercetin, M_r of quercetin and γ-CD ≈ 1:3).

2.4. Fourier transform infrared spectroscopy

FT-TR spectra were obtained in the frequency range between 4000 and 500 cm^{-1} using a Nicolet 6700 FT-IR spectrophotometer. Each sample was ground with spectroscopic grade potassium bromide (KBr) powder and then pressed into a 1 mm pellet. The IR spectra of inclusion complexes were analyzed and compared with the spectra of CDs, CPE alone and their physical mixtures.

2.5. Dissociation of inclusion complexes

The inclusion complexes were dissociated to determine the guest according to the method reported by Chen (Chen et al., 2006) with some modifications. The inclusion complex (50 mg) was dispersed into methanol (100 mL). Then the dissociation was aided by sonication for 30 min. This process was repeated for six times. After sonication, the suspension liquid was filtered through 0.22 μm PTFE filter (Anaqua chemical supply, Houston, USA) and dried, then stored at −20 °C. The obtained guests are defined as β-CPE and γ-CPE for short, respectively.

2.6. UHPLC-ESI-QTOF-MS analysis

Analyses were performed by an Agilent 1290 Infinity UHPLC system (Santa Clara, CA, USA). Separation was achieved on an Agilent Extend C18 UHPLC reversed-phase column (2.1 × 50 mm, 1.8 μm). The mobile phases consisted of solvent (A) 0.1% formic acid in water (v/v) and solvent (B) acetonitrile. A linear gradient program at a flow rate of 0.25 mL/min was used as follows: 0 min, 5% B; 0–5 min, 5–10% B; 5–13 min, 10–30% B; 13–18 min, 30–95% B. The injection volume was 1 μL, and the column temperature was set to 40 °C. The concentrations of samples were 12 mg/mL.

The QTOF-MS was operated in positive mode using an Agilent 6530 ultrahigh definition Q-TOF mass spectrometer (Santa Clara, CA, USA), equipped with an electrospray ionization (ESI) source. The optimized MS spectrometric parameters were as followed: gas temperature 350 °C, flow rate 10 min/L, nebulizer 30 psig, VCap 3500 V, and Fragmentor 150 V.

2.7. Determination of total flavonoid contents

The total flavonoid contents were determined according to a colorimetric method described by Lin (Lin and Tang, 2007) and modified in our laboratory. Appropriately sample (5 mL in 70% ethanol solution) was reacted with 1 mL $NaNO_2$ solution (5%) for 6 min, followed by reaction with 1 mL $Al(NO_3)_3$ solution (10%) for another 6 min. Then, 10 mL NaOH solution (4%) were added. The absorbance at 510 nm was measured 15 min later on UV2550 Spectrophotometer and compared to that of rutin standards. The total flavonoid contents were expressed in mg rutin equivalent (RE)/g CPE, β-CPE and γ-CPE.

2.8. In vitro antioxidant capacity

A modified DPPH radical scavenging assay (Brahmi et al., 2013) was carried out for determining the antioxidant capacity of CPE and inclusion complexes. Briefly, 1.5 mL of samples in methanol or ultrapure water with different concentrations were added to 1.5 mL of DPPH methanolic solution (0.1 mM). The absorbance of the mixture against methanol or ultrapure water as blank were determined at 517 nm after reacting for 30 min in the dark. Methanol or ultrapure water was used as the negative control. The results were given by IC_{50} value, which was defined as the sample concentration obtaining a 50% scavenging capacity. Thus, the lower the value, the higher antioxidant activity. IC_{50} were calculated from the graph of the DPPH•-scavenging percentage against the sample concentration and converted to the amount of guest.

2.9. Thermal studies

The enhancement in thermal stability of the CPE by inclusion with CDs was evaluated on NETZSCH- STA409PC synthesized thermogravimetry analyzer by thermogravimetric analysis (TGA). Sample (10 mg) measurements were carried out in alumina pans