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Multiple complexation of cyclodextrin with soy isoflavones present in an enriched fraction☆

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ABSTRACT

In the present study we evaluated the complexation of daidzein/genistein/glycitein, present in an isoflavone enriched fraction (IEF), with β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin (HPβCD). Based on the increased solubility and higher complexation efficiency, IEF and HPβCD solid complexes were prepared by kneading, freeze-drying, co-evaporation, spray-drying and microwave. The solid complexes were characterized using Fourier transformed-infrared spectroscopy, differential scanning calorimetry, X-ray diffraction, scanning electron microscopy, and nuclear magnetic resonance spectroscopy, and the isoflavone content and solubility were determined by liquid chromatography. The results suggest that the isoflavones daidzein, genistein and glycitein may be externally associated to HPβCD as well as that isoflavones/HPβCD inclusion complexes are formed through the insertion of B-ring into the cyclodextrin cavity. Except for the freeze-dried IEF/HPβCD solid complex, all complexes showed similar content and solubility. In conclusion, the three isoflavones showed to be able to simultaneously complex with HPβCD.

1. Introduction

Isoflavones are a subclass of flavonoids with a 15-carbon (C_6–C_3–C_6) backbone arranged as a 1,2-diphenylpropane skeleton. They are widely distributed in the plant kingdom, especially soybeans (*Glycine max*). Despite the main isoflavones present in soybean/soy based products are mostly in 6″-*O*-malonyl glycoside and 7-*O*-glucosides forms, only the aglycone forms (daidzein, glycitein and genistein), which are the biologically active forms, are absorbed (Setchell, 1998).

Due to its chemical structure similar to the estrogen (Kuiper et al., 1997), many biological activities have been ascribed for isoflavones, like reduced menopause related symptoms, prevention of osteoporosis and coronary heart disease, as well as prevention of prostate and breast cancer (Adlercreutz, 2002; Howes, Howes, & Knight, 2006; Yamaguchi, 2002). However, its use as a dietary supplement and pharmaceutical preparation is limited by its bitter taste, low water solubility, low stability and low bioavailability (Setchell et al., 2001; Ungar, Osundahunsi, & Shimoni, 2003).

Among conventional methods used to enhance the solubility and bioavailability of drugs with low water solubility, the preparation of inclusion complexes with cyclodextrins seems to be very promising. Cyclodextrins are macrocyclic, non-reducing maltooligosaccharides composed of glucose units linked by α-(1,4)glycosidic bonds (Szejtli, 1998). The most common cyclodextrins are the α-cyclodextrin, β-cyclodextrin (βCD), γ-cyclodextrin, which are composed of six, seven and eight glucopyranose units, respectively. Due to lack of free rotation of the bonds connecting the glucopyranose units, the cyclodextrins have a toroidal or cone shaped, where the primary hydroxyl groups are located on the narrow side of the torus while the secondary hydroxyl groups are located on the wider edge. This architecture provides the formation of an internal hydrophobic cavity, whereas the external surface is hydrophilic. The lipophilic cavity of cyclodextrin molecules provides a microenvironment into which appropriately sized non-polar moieties can enter to form

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a inclusion complex (Loftsson & Brewster, 1996). Moreover, toxicity studies have demonstrated that, when orally administered, cyclodextrins are practically non-toxic due to the lack of absorption from the gastrointestinal tract (Irie & Uekama, 1997).

Some studies have evaluated the formation of inclusion complexes of cyclodextrins with genistein and daidzein. Crupi et al. (2007) evaluated the mode of complexation between genistein and βCD, (2-hydroxypropyl)-β-cyclodextrin (HPβCD) and methyl-β-cyclodextrin by UV–vis absorption and FTIR–ATR spectroscopy. In the same year, the improvement of daidzein and genistein solubility by complexation with HPβCD at different host/guest molar ratios was also reported. (Stancanelli et al., 2007). Later, Daruhazi et al. (2008) reported the obtention of genistein/β- and γ-cyclodextrin complexes employing kneading method and Xavier et al. (2010) prepared genistein/βCD solid complexes with high drug loading by freeze-drying.

Although these studies have demonstrated the feasibility of obtaining inclusion complexes with isoflavones, none of them evaluated the simultaneous complexation of the three isoflavones with cyclodextrins, as well as the effect of different methods of complexation on solubility and complexation efficiency. Therefore, in the present study, we evaluated the complexation of an isoflavone enriched fraction (IEF) with βCD and HPβCD and prepared, by different methods, solid complexes of IEF/HPβCD, which were further characterized using Fourier transformed-infrared spectroscopy, differential scanning calorimetry, X-ray diffraction, scanning electron microscopy, and nuclear magnetic resonance spectroscopy. To the best of our knowledge, this is the first report of complexation study comprising multiple associations of molecules present in a fraction.

2. Materials and methods

2.1. Chemicals

Liquid chromatography (LC)-grade acetonitrile (Tedia, Fairfield, OH, USA), trifluoroacetic acid (Merck, Hohonbrunn, Germany), and purified water (Milli-Q^{TM} system, Millipore, Bedford, MA, USA) were used for mobile phase preparation. Daidzein (98%, HPLC purity), glycitein (97%, HPLC purity), and genistein (98%, HPLC purity) were obtained from Sigma–Aldrich (Steinheim, Germany). HPβCD and βCD were kindly supplied by Roquette Frères (Lestrem, France).

2.2. Isoflavone enriched fraction preparation

The IEF was produced from *Glycine max* dry extract. The extraction of the isoflavones was performed with ethanol 96 GL at the ratio of dry extract to ethanol of 1:20 (g/mL). The resulting mixture was heated at 60 °C for 2 h under constant agitation. The extractive solution was separated from the insoluble material by filtration and hydrolyzed with hydrochloric acid 0.5 M at 80 °C for 12 h under constant agitation. The resulting hydrolyzed solution was mixed with distilled water at the volume ratio of 1:5 (v/v) and maintained at rest for 12 h at 10 °C. The crystallized material was separated by decantation and filtration. The solid material was recrystallized in pure ethanol at 80 °C, filtrated and dried in oven at 40 °C for 24 h. The isoflavones content was determined by Liquid chromatography: 441.43 ± 3.89 mg/g of daidzein, 363.46 ± 1.04 mg/g of glycitein and 91.72 ± 0.95 mg/g of genistein.

2.3. Liquid chromatography analysis

The Liquid chromatography (LC) analysis was performed on a Shimadzu Prominence device coupled to diode array detection (DAD) instrument and an automatic injector (Kyoto, Japan). The stationary phase was a Phenomenex RP-18 column (Synergi Fusion 150 × 4.6 mm i.d.; particle size, 4 μm) guarded by a Waters precolumn (20 × 3.9 mm i.d.; particle size, 10 mm) (Milford, MA, USA). The mobile phase consisted of (A) trifluoroacetic acid 0.1% (v/v) and (B) acetonitrile:trifluoroacetic acid (100:0.01, v/v). The gradient elution was 20–25% B (0–10 min), 25–30% B (10–15 min), and 30–35% B (15–23 min). The column was washed with acetonitrile for 3 min and re-equilibrated with 20% B for 4 min before the next analysis. The flow rate was 1 mL.min^{-1} and the injection volume was 10 μL. The detection wavelength was 260 nm and the analysis was carried out at 40 °C. All the samples were solubilized in acetonitrile 50% (v/v) and sonicated for 30 min. The clear solution was filtered through a 0.45 μm PTFE membrane and injected. The method was validated over the concentration range of 0.1–10.0 μg.mL^{-1} according to ICH guidelines (ICH, 2005).

2.4. Phase-solubility study

Phase-solubility studies were performed with an IKA® stirred temperature controlled bath (IKA Werke GmbH & Co., Breisgau, Germany), which allowed an accuracy of 0.1 °C. A fixed amount of IEF exceeding its solubility was added to unbuffered aqueous solutions containing increasing concentrations of HPβCD or βCD in 2 mL glass vials. Vials were sealed to avoid changes due to evaporation and magnetically stirred for 2 days at 24.0 ± 0.01 °C or 37.0 ± 0.01 °C, protected from light to prevent any degradation of the molecules. After the equilibrium was reached, the suspensions were centrifuged for 30 min at 7000 rpm and filtered through a 0.45 μm PTFE membrane (Millipore HAWP) for direct analysis by LC. Experiments were carried out in triplicate.

The apparent stability constant (K_s), the complexation efficiency (*CE*) and the molar ratio of the isoflavones/cyclodextrins complexes were calculated based on the phase-solubility diagrams according to the following equations:

$$K_s(M^{-1}) = \text{slope}/(S_0 \times (1 - \text{slope}))$$

$$\text{CE} = \text{slope}/(1 - \text{slope})$$

$$\text{Molar ratio} = 1 + (1/\text{CE})$$

where S_0 is the intrinsic solubility of isoflavones in water (solubility of isoflavones in absence of cyclodextrin).

2.5. Preparation of isoflavone enriched fraction/(2-hydroxypropyl)-β-cyclodextrin complexes

2.5.1. Freeze-drying method

The required amount of IEF and aqueous solution of HPβCD were mixed at the molar ratio of 1:1 and stirred at 25 °C for 2 days protected from light. After this period, the dispersion filtered through a 0.45 μm PTFE membrane (Millipore HAWP), frozen at −18 °C for 24 h and then dried 48 h by lyophilization in an Edwards EF4 Modulyo freeze dryer (Edwards, UK).

2.5.2. Spray-drying method

The required amounts of IEF and HPβCD at the molar ratio of 1:1 were dissolved in ethanol 77% (v/v) at 60 °C. The resultant solution was cooled down to room temperature and dried in a Mini spray-dryer B-290 coupled to Inert loop B-295 (Büchi, Switzerland). The spray-dryer was operated under the following conditions: inlet temperature 130 °C, outlet temperature 50 °C, and flow rate of 3 mL min^{-1}.

2.5.3. Kneading/microwave method

Pre-weighed amounts of IEF and HPβCD at the molar ratio of 1:1 were grounded for 10 min in a ceramic mortar. The resultant mixture was further kneaded with ethanol 66% (v/v) for 45 min.