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Strategies to improve the solubility and stability of stilbene antioxidants: A comparative study between cyclodextrins and bile acids

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ABSTRACT

Aiming at the development of an active food packaging, the goal of this study was to increase stilbenes (resveratrol (RV), pterostilbene (PT) and pinosylvin (PS)) aqueous solubility and stability using hydroxypropyl-cyclodextrins (HP-CDs) and bile salts. To evaluate stilbene concentration, an HPLC-DAD method was validated. Stilbene solubility was improved by the formation of inclusion complexes and micellar systems with higher solubility values obtained for the inclusion complexes with cyclodextrins. Inclusion complexes revealed a 1:1 stoichiometry for RV and PT and a 1:2 for PS. Solid state characterisation was carried out using X-ray diffraction, Fourier transform infrared spectroscopy and differential scanning calorimetry. ^{1}H NMR studies were also performed to characterise the prepared complexes. Photostability studies revealed that CDs were able to increase stilbene photostability at 4 °C. This work showed that stable stilbene solutions can be achieved using hydroxypropyl-CDs, contributing for their incorporation in several materials for the food and pharmaceutical industries.

1. Introduction

Stilbenes comprise a class of plant polyphenols that have gained intense interest for their intricate structures and diverse biological activities (Shen, Wang, & Lou, 2009). These compounds and their derivatives are of noticeable interest for areas as health and food biotechnology, drug research and development and medicine due to their potential in therapeutic or preventive applications (Shen et al., 2009). Stilbenes exist as both monomers and increasingly complex oligomers. The monomeric stilbene structure is relatively simple and characterised by two benzene rings joined by an ethylene bridge (Kasiotis, Pratsinis, Kletsas, & Haroutounian, 2013). As a result of this ethylene bridge, stilbenes can occur as *cis*- and *trans*-isomers, of which the *trans*-isomer (E) is the most common configuration (Riviere, Pawlus, & Merillon, 2012). So far, different pathways leading to *trans–cis* isomerization have been described such as double bond breakage by radicals, direct photoisomerization under solar or UV irradiation (Riviere et al., 2012) and thermal isomerization (Dugave & Demange, 2003). It is thought that *trans*-isomers are biologically more active than *cis*-isomers. However, the later are not so widely studied and only a few reports comparing the biological activity between the two isomers are available (Anisimova et al., 2011; Campos-Toimil, Elies, Alvarez, Verde, & Orallo, 2007; Rius et al., 2010).

Stilbenes are widely considered phytoalexins in several plant families for their role in plant resistance to fungal pathogens. For instance, in some plants, stilbenes are constitutively expressed and, furthermore, their synthesis can also be induced in response to a large range of biotic and abiotic stress factors (Riviere et al., 2012). Resveratrol (3,5,4'-trihydroxystilbene, $C_{14}H_{12}O_3$) pterostilbene (3,5-dimethoxy-4'-hydroxystilbene, $C_{16}H_{16}O_3$) and pinosylvin (3,5-dihydroxystilbene, $C_{14}H_{12}O_2$) are known naturally occurring stilbene phytoallexins present in the wood pulp and bark of several trees, in *Vitis vinifera* leaves and berries, in peanuts, mulberries and several plant extracts, such as tea oils and herbal remedies (Bertacche, Lorenzi, Nava, Pini, & Sinco, 2006; Lopez-Nicolas, Rodriguez-Bonilla, & Garcia-Carmona, 2009a; Lopez-Nicolas, Rodriguez-Bonilla, Mendez-Cazorla, & Garcia-Carmona, 2009b).

Over the past years, there has been an increased awareness and consumption of foods and supplements containing these stilbenes due to their perceived health benefits. For instance, resveratrol, a vastly studied stilbene, has been associated with several biological activities such as anti-oxidant, anti-inflammatory, analgesic, cardio-protective, neuro-protective, chemo-preventive, anti-ageing and antimicrobial activities (Das, Lin, Ho, & Ng, 2008; Paulo, Ferreira, Gallardo, Queiroz, & Domingues, 2010). Pterostilbene

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and pinosylvin share many of resveratrol biological activities, including anti-cancer, anti-ageing, and antimicrobial activities (Lee et al., 2005; Lin, Yue, & Ho, 2009; Macickova et al., 2010; Park et al., 2012). Despite all these established biological activities, the poor solubility of these compounds and their sensitivity to external agents such as air, light, and oxidative enzymes can constitute a serious problem for their bioavailability and, therefore prevent stilbenes from achieving their desirable activity.

Several research studies have described that the complexation of polyphenols with cyclodextrins (CDs) and micellar systems led to a marked increase in their aqueous solubility and, even improved their stability and bioactivity (Dai, Chen, & Zhou, 2008; Lu, Cheng, Hu, Zhang, & Zou, 2009).

Bile acids are bi-planar natural compounds with two functionally different molecular surfaces: a hydrophobic convex surface of steroid core and a hydrophilic concave surface (Simonovic & Momirovic, 1997). This coexistence of polar and non-polar surfaces in molecules (amphyphilic molecules) induces their self-association in micelles, above a certain bile acid concentration (critical micellar concentration (CMC)), and influences their physical–chemical properties (Atanackovic, Posa, Heinle, Gojkovic-Bukarica, & Cvejic, 2009). Micellar solutions of bile acids can solubilise poorly soluble organic substances, improving their resorption and enhancing cell permeability to substances (Atanackovic et al., 2009). Till now, various polymers and oligomers based on bile acids have been prepared for their potential biological and pharmaceutical applications as drug carrier systems, due to its ability to solubilise hydrophobic drugs, as molecular containers, non-polymeric hydrogelators and chemosensors (Atanackovic et al., 2009).

CDs are cyclic oligosaccharides derived from starch containing six (αCD), seven (βCD), eight (γCD) or more (α-1,4)-linked α-D-glucopyranose units (Tong, 2001). Due to this specific structure of hydrophobic inner cavity and hydrophilic outer surface, CDs have the almost unique ability to form inclusion complexes with various organic, inorganic, biological and pharmaceutical molecules through non-covalent interactions (Dahan, Miller, Hoffman, Amidon, & Amidon, 2010). Since natural CDs, in particular βCD, show limited aqueous solubility, new modified CDs with substitution of the hydrogen bond-forming hydroxyl groups have been developed. Examples of such modified CDs include the hydroxypropyl derivatives of βCD and γCD (i.e. HPβCD and HPγCD), the randomly methylated βCD (RMβCD) and others (Brewster & Loftsson, 2007). Up to now, all the properties of cyclodextrins or derivatives made them suitable for applications in analytical chemistry, agriculture, the pharmaceutical field, in food and toilet articles. In foods, CDs are used as molecular encapsulants to protect flavouring agents or other additives during the rigorous processing methods, extending the shelf life of the food item and preserving the product organoleptic properties. CDs are also used in the preparation of controlled release powdered flavours and confectionery items and are being used for the development of controlled-release active packaging systems (Del Valle, 2004; Koontz et al., 2010).

Aiming at the development of an active food packaging for fresh products, the goal of this study was to increase RV, PT and PS aqueous solubility using hydropropyl-beta-cyclodextrin (HP-β-CD), hydropropyl-gamma-cyclodextrin (HP-γ-CD) and bile salts (sodium cholate and sodium deoxycholate). The system that enabled the highest fold-increase in the aqueous solubility of test compounds was further characterised by Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), powder X-ray diffraction (DRX) and ^{1}H-NMR spectroscopy and its ability to improve test compounds photostability was also investigated.

2. Materials and methods

2.1. Materials

Pinosylvin and pterostilbene were purchased from Sequoia Research Products Limited (Pangbourne, U.K.). *trans*-Resveratrol, sodium cholate and sodium deoxycholate were purchased from TCI Europe N.V. (Zwijndrecht, Belgium). Since stilbenes are sensitive to light, reagents and samples were stored in the dark. Regarding the CDs tested, hydroxypropyl-β-CD (HP-β-CD; KLEPTOSE® HP, M_w = 1399 g/mol) was kindly provided by Roquette Freres S.A. (Lestrem, France) and hydroxypropyl-γ-CD (HP-γ-CD; M_w = 1580 g/mol) was purchased from Sigma–Aldrich.

2.2. HPLC analysis of stilbenes

Stilbene quantification was carried out using an Agilent 1290 Infinity LC HPLC system (Waldbronn, Germany) equipped with an Agilent 1290 Infinity Diode Array Detector (G4212A DAD). Compound separation was performed using a Zorbax 300 SB-C_{18} reversed-phase analytical column (5 μm, 4.6 × 150 mm) acquired from Soquímica (Lisbon, Portugal). The composition of mobile phase was a mixture of 0.1% (v/v) formic acid solution in purified water (18.2 M Ωcm at 25 °C) and acetonitrile (52:48, v/v). The mobile phase was filtered under vacuum (0.2 μm hydrophilic polypropylene filter) and degassed for 30 min in an ultrasonic bath before use. An isocratic flow of 1.0 mL/min was applied and the column oven was maintained at 25 °C. The wavelength defined for monitoring stilbenes was 306 nm for *trans*-stilbenes isomers. The retention times obtained for *trans*-resveratrol, *trans*-pinosylvin and *trans*-pterostilbene were 1.8, 2.7 and 4.7 min, respectively. This method was validated according to the guidelines provided by the Food and Drug Administration (FDA) (Department of Health & U.S. Department of Health & Food, 2001) and International Conference on harmonisation (ICH) (Harmonisation, 2005) and the parameters studied were linearity, intermediate, intra- and inter-day precision and accuracy.

2.3. Critical micellar concentration (CMC) determination

In order to determine sodium cholate and sodium deoxycholate CMC, two methods were used. The first method consisted of a non-invasive method based on conductivity measurements (Posa, Guzsvany, & Csanadi, 2009) and the second one was an invasive method based on Coomassie Brilliant Blue R-250 spectral shift (Courtney, Simpson, & Beachey, 1986).

2.4. Phase solubility studies of stilbene-CD complexes

CD solutions were prepared at different concentrations (0, 0.025, 0.05, 0.10, 0.20 and 0.30 M^{-1}) in PBS buffer (pH 7.0). A volume of 500 μL of each CD solution was added to an excess amount of stilbenes (20 mg). The resulting suspensions were vortexed and sonicated for 1 h in an ultrasonic bath with ice. The suspensions were kept protected from light on an orbital shaker at 25 °C, 250 rpm for 24 h. This equilibrium time was previous determined by preliminary studies. The resulting suspensions were filtered through 0.2 μm PVDF syringe filters (Millipore, MA, USA) to obtain clear solutions that were properly diluted with methanol:PBS (pH 7.4) (1:1) to the calibration range (1–100 μg/mL) and measured by HPLC after method validation.

The apparent stability constants ($K_{1:1}$ and $K_{1:2}$) were calculated according to the equations established by Higuchi and Connors (Higuchi & Connors, 1965).