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Antioxidant and antiproliferative properties of 3-deoxyanthocyanidins

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ABSTRACT

The study of the antioxidant properties of six deoxyanthocyanidins (deoxypeonidin, deoxymalvidin, luteolinidin, apigeninidin, guaiacylcatechinpyrylium and syringylcatechinpyrylium) and an anthocyanin (cyanidin-3-glucoside) was carried out. The aim was to evaluate the relationship between the structure and the antioxidant properties of individual deoxyanthocyanidins, compared to a common anthocyanin derivative, cyanidin-3-glucoside. The ability of these compounds to inhibit lipid peroxidation in a liposome membrane system was examined by monitoring oxygen consumption and the antiradical and reducing capacities were determined using the DPPH and FRAP assay, respectively. The results showed that all the compounds tested presented antioxidant properties. Cyanidin-3-glucoside presented higher antiradical and reducing activities in the DPPH and FRAP assay, although in the liposome model, the guaiacylcatechinpyrylium was more effective inhibiting lipid peroxyl radicals.

Additionally, the anti-proliferative effects of deoxyanthocyanidins, have been evaluated against two cancer cell lines from stomach (AGS, MKN-28) and one colon cancer cell (Caco-2), and compared with the effect of the respective anthocyanins.

Considering the antiproliferative activity, all compounds were active against Caco-2 cell line, being the ones with glucose moiety and oaklin Scp the most active.

Deoxyanthocyanidins, and in particular, guaiacylcatechinpyrylium may be regarded as potential food colorants.

1. Introduction

Deoxyanthocyanidins are considered the chemical ancestors of anthocyanins, the ubiquitous water-soluble pigments that are found in flowers and fruits and are responsible for their impressive blue and purple colors (Sweeny & Iacobucci, 1977). Anthocyanins are becoming increasingly important not only as food colorants, but also as antioxidants. There are reports of therapeutic benefits including vasoprotective and anti-inflammatory properties, anti-cancer and chemoprotective properties, as well as anti-neoplastic properties (Kamei et al., 1995; Lietti & Forni, 1976). Anthocyanins are therefore considered to contribute significantly to the beneficial effects of consuming fruits and vegetables (Wang et al., 2012). Consequently, there has been a rising demand for natural sources of food colorants with nutraceutical benefits (Boyd, 2000) and alternative sources of natural anthocyanins are becoming increasingly important (Azevedo et al., 2010; Fernandes, Faria, Calhau, de Freitas, & Mateus, 2014).

Oppositely to anthocyanins, 3-deoxyanthocyanidins pigments do possess neither a glucose group nor a hydroxyl group in the C-ring 3-position of the flavylium core (Pina, Melo, Laia, Parola, & Lima, 2012). This unique feature provides the 3-deoxyanthocyanins different physico-chemical and biochemical properties, comparing to anthocyanins. Indeed, they are much more stable in slightly acidic solutions than anthocyanins and anthocyanidins (Dangles & Elhajji, 1994; Iacobucci & Sweeny, 1983; Khalil, Baltenweck-Guyot, Ocampo-Torres, & Albrecht, 2010; Sousa et al., 2013) have greater resistance to color bleaching by sulfur dioxide (Ojwang, 2007) and were also recently demonstrated to be more cytotoxic to cancer cells than their anthocyanidin analogs (Shih et al., 2007), which points to the potential advantage of this type of compounds as viable commercial food colorants and justifies the research developed in the chemistry of 3-deoxyanthocyanins.

Despite their interesting color features and health benefits, the antioxidant properties of these anthocyanidin derivatives have only been briefly studied, mostly in plant extracts (Awika, Rooney, & Waniska, 2005; Cardoso et al., 2014; Kayode et al., 2011). Only recently attempts have been made to analyze the antioxidant properties of pure 3-deoxyanthocyanidin compounds (Carbonneau et al., 2014).

Most of the methods used to analyze the antioxidant properties of anthocyanins focus on the different mechanisms of the

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antioxidant defense system, such as scavenging of oxygen and hydroxyl radicals, reductions of lipid peroxyl radicals, inhibition of lipid peroxidation or chelation of metal ions (Halliwell, Aeschbach, Loliger, & Aruoma, 1995; Halliwell & Whiteman, 2004). On the other hand, the use of LDL or liposomes has become a more promising method for the assessment of antioxidant properties relevant to human nutrition (Storm & Crommelin, 1998), since they allow the study of the protection of a specific substrate by an antioxidant in a model biological membrane or lipoprotein.

The aim of this work was thus to screen several 3-deoxyanthocyanidins for their antioxidant properties, comparing the results with a common anthocyanin (cyanidin-3-glucoside). DPPH and FRAP assays were performed to evaluate their antiradical and reducing properties, respectively. Furthermore, the activity against lipid peroxidation was also tested using soybean phosphatidylcholine liposomes as a membrane model system. The extension of membrane lipid oxidation was followed by measuring the oxygen consumption. Additionally, the anti-proliferative properties of several 3-deoxyanthocyanidins was also evaluated against two cancer cell lines from stomach (AGS, MKN-28) and one colon cancer cell (Caco-2), and compared with the effect of the respective anthocyanins.

2. Materials and methods

2.1. Reagents

2-Cloro-3',4'-dihydroxyacetophenone, 2-bromo-4'-hydroxyacetophenone, 2,4,6-trihydroxybenzaldehyde, hexafluorophosphoric acid (65% in water), zinc powder, acetic acid, (+)-catechin, coniferaldehyde, sinapaldehyde, DPPH, AAPH, $FeCl_3$, DMSO, Hepes, Trolox, soybean L-α-phosphatidylcholine and NaCl were purchased from Sigma–Aldrich (Madrid, Spain). 2,4,6-Tripyridyl-*s*-triazine (TPTZ) and phloroglucinol was purchased from Fluka (Madrid, Spain).

2.2. Synthesis of deoxyanthocyanidins

Luteolinidin and apigeninidin were obtained through the chemical synthesis between 2,4,6-trihydroxybenzaldehyde and 3,4-dihydroxyacetophenone and 4-hydroxyacetophenone, respectively, in acetic acid in the presence of aqueous hexafluorophosphoric acid according to the procedure described elsewhere (Kueny-Stotz, Isorez, Chassaing, & Brouillard, 2007; Mora-Soumille, Al Bittar, Rosa, & Dangles, 2013).

The synthesis of the compounds 3-deoxypeonidin and 3-deoxymalvidin was followed according to the procedure described in the literature (Sousa, Mateus, & de Freitas, 2012).

The pigments guaiacylcatechin-pyrylium (GCP) and syringylcatechin-pyrylium (SCP) were obtained through the chemical synthesis between catechin and coniferaldehyde or sinapaldehyde, respectively, according to the procedure described elsewhere (Sousa et al., 2013).

2.3. Cyanidin-3-glucoside extraction

Cyanidin-3-*O*-glucoside (Cy-glc) was extracted and purified in the laboratory from blackberries (*Rubus fruticosus*) by semipreparative HPLC with a C_{18} reversed phase column, as described elsewhere (Azevedo et al., 2010).

2.4. Radical scavenging assay (DPPH)

Radical scavenging assays were performed using DPPH (2,2-diphenyl-1-picrylhydrazyl) as a free radical, according to the method described in the literature with some modifications (Bondet, BrandWilliams, & Berset, 1997). DPPH reacted with the tested antioxidant compound reacted and decreased the absorbance measured at 515 nm, indicating the potential scavenging of the compounds. All the pigments tested absorbed at 515 nm, thus previous control assays were performed with all the compounds in order to subtract their contribution at this wavelength. The assays were conducted in a microplate reader of 96 wells (Biotek Powerwave XS with software KC4). The scavenging reaction was carried out on the plate wells with a temperature of 25 °C. A solution of 60 μM DPPH was previously prepared in methanol. 270 μL of this solution was added in each well together with 30 μL of antioxidant. The compounds tested were at a final concentration of 10 μM. The decrease in absorbance was measured at 515 nm, at $t = 0$ and every 5 min, for 20 min. For the final results, the 0–20 min reaction time range was used. Antiradical activity was expressed as μM Trolox equivalents. The antiradical activity was calculated from the equation determined from a linear regression after plotting known solutions of Trolox at different concentrations.

2.5. Ferric reducing antioxidant power (FRAP)

The FRAP assay was performed following the method described in the literature (Benzie & Strain, 1996) with some modifications. This method consists in the reduction of ferric tripyridyltriazine complex $[Fe(III)\text{-}(TPTZ)_2]^{3+}$ to ferrous tripyridyltriazine complex $[Fe(II)\text{-}(TPTZ)_2]^{2+}$ by an antioxidant. The resulting product has an absorption maximum at 593 nm, which can be measured by spectrophotometry. Its formation will thus reflect the reductive capacity of the antioxidant. The reaction was performed in a microplate reader of 96 wells (Biotek Powerwave XS with software KC4). The reaction was carried out on the plate wells with a temperature of 37 °C. FRAP reagent (10 vol of 300 mM acetate buffer, pH 3.6 + 1 vol of 10 mM TPTZ in 40 mM HCl + 1 vol of 20 mM $FeCl_3$) was diluted to one-third with acetate buffer. 270 μL of this solution was added in each well together with 30 μL of compound. The blank assay was performed using 270 μL of FRAP reagent and 30 μL of methanol. The antioxidant compounds were dissolved in methanol and used in a final concentration of 10 μM. The absorbance at 593 nm was measured in time 0 and 4 min. The results were expressed as Trolox equivalents.

2.6. Liposome preparation

Liposomes were prepared by evaporation to dryness of L-μ-phosphatidylcholine (PC) from soybean solution in chloroform with a stream of argon; the film was then left under vacuum for 3 h to remove all traces of the organic solvent. The resulting dried lipid film was dispersed with Hepes buffer (10 mM Hepes, 0.1 M NaCl, pH 7.4) and the resulting mixture was shaken above the phase transition temperature to produce multilamellar liposomes (MLV). Frozen and thawed MLVs were obtained by repeating the following cycle five times: freezing the vesicles in liquid nitrogen and thawing the sample in a water bath at 37 °C. Lipid suspensions were equilibrated at 37 °C for 30 min and extruded 10 times through polycarbonate filters of 100 nm pore size in a 10 mL stainless steel extruder to form large unilamellar vesicles (LUV) (Rodrigues, Gameiro, Reis, Lima, & de Castro, 2001).

2.7. Oxygen consumption

The thermal degradation of the azo compound AAPH generated peroxyl radicals at a constant rate, which induced lipid peroxidation of soybean LUVs. The reaction was conducted in the presence and absence of antioxidants and followed by measuring the rate of