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Efficient three-dimensional fluorescence measurements for characterization of binding properties in some plants

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ABSTRACT

The main aim of this research was to characterize some plants and to determine their similarities and differences, using spectroscopic methods. The interactions of soluble polyphenols of different plants with human serum albumin (HSA) were investigated by 3D-fluorescence. The obtained fluorescence results allow to classify the investigated plants according to their binding properties. The HSA-binding capacities of these plants were the highest for asparagus and correlated with their antioxidant properties. To our knowledge this is the first report showing differences and similarities in these plants, using spectroscopic techniques. The fluorescence spectral methods, which were applied as a powerful tool showing the quenching properties of intrinsic fluorophores in protein molecules in the presence of some plant polyphenols, can contribute in pharmaceutical and food applications.

1. Introduction

Polyphenols are important in human physiology. As micro-components with chemopreventive and antioxidant properties they play a preventive role in cancer and coronary artery disease. The form and the level of plant polyphenols in the human body determine their efficiency. Increased fruit and vegetable consumption is an effective strategy to increase antioxidant intake and to decrease oxidative stress and may lead to reduced risk of developing chronic diseases, such as cancer and cardiovascular disease [1–3]. Flavonoids are common polyphenolic compounds widely distributed in fruits and vegetables. These compounds are relatively hydrophobic molecules, suggesting the role of blood transport proteins in their delivery to tissues.

Determination of polyphenol binding to main protein in blood human serum albumin is one of the main properties which are important in pharmacological studies. The binding of structurally different flavonoids to human serum albumin was investigated by fluorescence spectroscopy using the quenching of the albumin fluorescence, and the enhancement of the flavonoid fluorescence [4,5]. There are a number of such publications in the field of protein polyphenol interactions, including our recent studies [6–9]. The binding of the main substances in green tea and their Cu (II) complexes showed that the gallate moiety of the polyphenols plays a crucial role in determining the mode of interaction with HSA [10]. In another study of the binding of several polyphenols such as catechins [(−)-epigallocatechin-3-gallate, (−)-epigallocatechin, (−)-epicatechin-3-gallate], flavones (kaempferol, kaempferol-3-glucoside, quercetin, naringenin) and hydroxycinnamic acids (rosmarinic acid, caffeic acid, *p*-coumaric acid) to bovine serum albumin (BSA) similar results were obtained with (−)-epicatechin-3-gallate showed the highest Stern-Volmer modified quenching constant, followed by (−)-epigallocatechin-3-gallate [11]. Non-

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covalent interactions between (sup)-lactoglobulin and polyphenol extracts of teas, coffee and cocoa were studied by fluorescence at pH values of the gastrointestinal tract [12]. The interactions between resveratrol and albumin were compared with those already published, involving curcumin, genistein, quercetin and other well-known food-containing polyphenols [13]. Quercetin and kaempferol showed the strongest equilibrium binding affinities to HSA in comparison with luteolin and resveratrol [14]. Specific interactions with protein amino acids were evidenced with luteolin, which has a variety of pharmacological properties [15]. In spite of the shown above numerous references, it is a lack of information about the use of fluorescence in the characterization of important plants. Therefore, the objective of the study was to determine the binding and antioxidant properties of some green vegetables using fluorescence measurements. Such information is reported for the first time.

2. Materials and methods

2.1. Materials and instrumentation

Trolox (6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid); 2,2′-azobis-2-methyl-propanimidamide; $FeCl_3 \times 6H_2O$; Folin–Ciocalteu reagent (FCR); Tris, tris (hydroxymethy1)amino-methane; lanthanum (III) chloride heptahydrate; $CuCl_2 \times 2H_2O$; and 2,9-dimethyl-1,10-phenanthroline (neocuproine), potassium persulfate, catechin, gallic acid and human serum albumin were obtained from Sigma Chemical Co., St. Louis, MO, USA. 2, 4, 6-tripyridyl-*s*-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. All reagents were of analytical grade. Deionised and distilled water were used throughout.

2.2. Plant samples

Conventionally grown in the greenhouse *Asparagus officialis* L. cv. UC157 was compared with lotus plant (*Nelumbo nucifera Gaertn.*), cucumber (*Cucumis sativus* L.), green pepper (*Capsicum annuum* L.), and green leaves lettuce (*Lactuca sativa*) which were purchased at the local market, Gwangju city, Republic of Korea. The samples were chosen from the same area, grown in similar conditions and were immediately processed: their edible parts were prepared manually without using steel knives. The prepared vegetables were weighed, chopped and homogenized under liquid nitrogen in a high-speed blender (Hamilton Beach Silex professional model) for 1 min. A weighed portion (50–100 g) was then lyophilized for 48 h (Virtis model 10–324), and the dry weight (DW) was determined. The samples were ground to pass through a 0.5 mm sieve and stored at −20 °C until the bioactive substances were analyzed.

2.3. Determination of bioactive compounds and total antioxidant capacities (TACs)

Polyphenols were extracted with methanol and ethanol (concentration 20 mg/mL) during 1 h in a cooled ultrasonic bath, which are the best solvents for polyphenol extraction. Total polyphenols (mg gallic acid equivalents (GAE)/g DW) were determined by Folin-Ciocalteu method using spectrophotometer (Hewlett-Packard, model 8452A, Rockvile, USA) and measuring obtained absorbance after the complex reaction at wavelength of 750 nm [16]. Flavonoids, extracted with 5% $NaNO_2$, 10% $AlCl_3 \times H_2O$ and 1 M NaOH, were measured at 510 nm. Total flavanols were estimated using the *p*-dimethylaminocinnamaldehyde method, and the absorbance was measured at 640 nm [17]. The extracts of condensed tannins (procyanidins) with 4% vanillin solution in MeOH were measured at 500 nm. (+)Catechin served as a standard for flavonoids, flavanols and tannins as previously was described in details [6,7]. Anthocyanins were determined by the measuring of plant absorbances of extracts (1 g of the defatted sample was extracted with 1 mL of acetonitrile containing 4% acetic acid) at 510 nm and 700 nm in buffers at pH 1.0 and 4.5, and calculated using following equation: $A = [(A_{510} - A_{700})\ pH1.0 - (A_{510} - A_{700})_{pH4.5}]$ with a molar extinction coefficient of cyanidin-3-glucoside of 29,600. Results were expressed as mg of cyanidin-3-glucoside equivalent (CGE) per 100 g DW [18]. Chlorophylls a and b, and total carotenoids (xanthophylls + carotenes) were extracted with 100% acetone and determined spectrophotometrically at different absorbances (nm) such as at 661.6, 644.8, and 470, respectively [19]. Total ascorbic acid was determined by CUPRAC assay in water extract (100 mg of lyophilized sample and 5 mL of water). The absorbance of the formed bis (Nc)-copper (I) chelate was measured at 450 nm [20].

TACs were determined using the following methods: 2, 2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) method. ABTS radical cation was generated by the interaction of ABTS (7 mM/L) and $K_2S_2O_8$ (2.45 mM/L). This solution was diluted with methanol and measured at 734 nm [21].

Ferric-reducing/antioxidant power (FRAP): FRAP reagent (2.5 mL of a 10 mmol ferric-tripiridyltriazine solution in 40 mmol HCl plus 2.5 mL of 20 mmol $FeCl_3 \times H_2O$ and 25 mL of 0.3 mol/L acetate buffer, pH 3.6) of 900 μL was mixed with 90 μL of distilled water and 30 μL of plant extract samples as the appropriate reagent blank and measured at 595 nm [22].

Cupric reducing antioxidant capacity (CUPRAC): To the mixture of 1 mL of copper (II)-neocuproine and NH_4Ac buffer solution, acidified and non acidified methanol plant extracts (or standard) solution (x, in mL) and H_2O [(1.1 − x) mL] were added to make the final volume of 4.1 mL and the absorbance was measured at 450 nm [23].

2.4. Fluorometric measurements

Two (2D-FL) and three dimensional (3D-FL) fluorescence measurements for plant extracts at a concentration of 0.01 mg/mL were recorded on a model FP-6500, Jasco spectrofluorometer, serial N261332, Japan. The 2D-FL was taken at emission wavelengths from 310 to 500 nm; and at excitation of 295 nm. The 3D-FL spectra were collected with subsequent scanning emission spectra from 250 to 500 nm at 1.0 nm increments by varying the excitation wavelength from 200 to 350 nm at 10 nm increments. Catechin was used as a standard. All solutions for protein interaction were prepared in 0.05 mol/L Tris–HCl buffer (pH 7.4), containing 0.1 mol/L NaCl [4,6–9].

2.5. Statistical analysis

To verify the statistical significance, means ± SD of five independent measurements were calculated. One-way analysis on variance (ANOVA) for statistical evaluation of results was used, following by Duncan's new multiple range tests to assess differences between group's means. P values of <0.05 were considered to be significant.

3. Results and discussion

The bioactive compounds and their antioxidant activities of the studied vegetables are given in Table 1. All antioxidant indices were higher in methanol extracts than in ethanol. These results are in agreement with our [6–8,24,25] and other reports [26–34]. The results which were presented in our previous reports with the values of red and green pepper [6,7], asparagus and lotus [8], lettuce [24], and cucumber [25] slightly differ from the values shown in Table 1 for these plants. This can be explained by