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Composition and Stability of Anthocyanins from Purple *Solanum tuberosum* and Their Protective Influence on Cr(VI) Targeted to Bovine Serum Albumin

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Supporting Information

ABSTRACT: Anthocyanins from the purple *Solanum tuberosum* newly cultivated by the Taian Academy of Agricultural Sciences were extracted and analyzed using high-performance liquid chromatography (HPLC) and UV−vis spectroscopy. Four individual anthocyanins were detected as the major components, and the total anthocyanin content was 273.5 ± 14.3 mg of cyanidin-3-glucoside equiv/100 g of dry seeds. Results of color stability showed that the purple *S. tuberosum* anthocyanins (PSTAs) are more stable under low pH and temperatures. Heat and general food additives have fine compatibility with PSTAs; however, they are very sensitive with oxidant and reduction. The influence of PSTAs on Cr(VI) targeted to bovine serum albumin (BSA) was also studied. The quenching of BSA fluorescence caused by Cr(VI) could be delayed by PSTAs. UV−vis and circular dichroism (CD) data suggested that PSTAs can protect the secondary and tertiary structures of BSA by probably interacting with Cr(VI) in advance.

KEYWORDS: purple *Solanum tuberosum*, Cr(VI), bovine serum albumin, HPLC, fluorescence, UV−vis, circular dichroism

INTRODUCTION

Anthocyanins, a group of phenolic compounds, are widely distributed in nature. They are found in all parts of the plant but are most obvious in fruits and flowers, presenting a spectrum ranging from pink through red and violet to dark blue. Anthocyanins are utilized by the food industry as natural food colorants because of their attractive color and safety.[1,2] In recent years there has been increasing interest in anthocyanins due to their roles as antioxidants, anti-inflammatory agents, and anticancer agents.[3−8] It is reported that the antioxidant status of human beings can be improved by fruit- and vegetable-based diets.[9] In vitro studies have indicated the activity of anthocyanins as modulators of immune and inflammatory responses and as anticancer agents.[10] Thus, anthocyanins have wide potential value for application not only in food science but also in medicine and pharmacy.

The stability of colorants during exposure to light, heat, different temperatures, high or low pH, and oxidative or reductive conditions is a desired property of anthocyanins. Previous studies have shown the stability of anthocyanins extracted fruits and vegetables,[11,12] and their colorant is mainly influenced by the above factors. The raw material (purple *Solanum tuberosum* I) contains anthocyanins from a kind of purple potato (*Solanum tuberosum*) newly cultivated by the Taian Academy of Agricultural Science. Because of the novelty of the variety, there is a demand for definition of extraction conditions, composition of its primary pigment, and stability analysis of the pigment.

Serum albumin is the major component in the circulatory system (∼60% of the total) and is of essential function. Serving as the main transporter for a variety of compounds, it constitutes three domains where binding sites locate.[13] With the help of serum albumin, a wide range of endogenous and exogenous compounds in the bloodstream such as fatty acids, heme, bilirubin, metal ions, and drugs are transported to their target organs. In recent years bovine serum albumin (BSA) has been widely and long used as a model protein because of its structural homology with human serum albumin.[14]

Wide applications of chromium compounds in metal surface refinery, tanning, dyes, and pigments has made them one of the major pollutants in water. Chromium(III) oxides are only slightly water-soluble and elemental chromium(0), which does not occur freely in nature, therefore are not considered to be health hazards. However, the toxicity and carcinogenic properties of chromium-(VI) have been known for a long time. The acute toxicity of chromium(VI) is due to its strong oxidational properties. After it reaches the bloodstream, it damages the kidneys, the liver, and blood cells through oxidation reactions.[15] Genotoxicity, or rather DNA damage by chromium(VI), was proposed as the mechanism of its carcinogenicity.[16]

A previous study has shown a protective action of anthocyanin extract from red cabbage leaves against chromium toxicity.[17] Research by Olah et al. testified that plants with higher anthocyanin accumulation show higher tolerance against chromium(VI) than normal species.[18] However, there is a severe lack of studies

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concerning animals. When chromium(VI) enters a biological fluid, the first step would be association with biomacromoecules, mostly protein. Thus, studies on the binding of chromium(VI) to serum albumin and how to prevent chromium(VI) from interacting with biomacromolecules are necessary. In this work, we first prepared and analyzed the pigments from purple *S. tuberosum* by spectroscopic and chromatographic methods; then utilizing fluorescent, UV−vis, and CD spectrophotometry, we evaluated the effect of PSTAs on the interaction of BSA and Cr(VI). An overall view of the protein behavior was displayed to manifest the prevention effects of the anthocyanins. These data may have both practical and theoretical interest for the application and development of PSTAs.

EXPERIMENTAL PROCEDURES

Reagents. K_2CrO_4, NaAc, NaH_2PO_4, Na_2HPO_4, Na_2SO_4, HAc, HNO_3, H_2SO_4, HCl, NaOH, glucose, and sucrose were all purchased from Tianjin Tianda Chemical Reagent Co., Ltd. (Tianjin, China). H_2O_2 was purchased from Laiyang Chemical & Industrial Co., Ltd. (Laiyang, China). Four kinds of macroporous adsorption resins (HPD-100, HPD-200A, HPD-450, and HPD-600) were produced by Cangzhou Bon Adsorber Technology Co., Ltd. (Cangzhou, China). NaAc and HAc were made as buffer used in an acid range. NaH_2PO_4 and Na_2HPO_4 were prepared as 0.10 M PBS buffer to stabilize the pH at 7.4 for protein measurements. BSA was bought from Sinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China), dissolved in ultrapure water to form a 0.5 g/L solution, and then preserved at 4 °C for later use. All solutions were prepared with ultrapure water.

Pigment Material. By using a pedigree method and backcross method we hybridized wild species from South Africa and the local species. After generations of screening, we successfully selected a stable strain and named it Purple *Solanum tuberosum* I. This potato strain has a higher ability to resist viral disease and late blight than common varieties with both the tuber and its skin purple.

Extraction Condition Exploration. The purple potatoes were cultivated at the Taian Academy of Agriculture Sciences (Taian, China, 2008), collected at maturity in the spring. Experiments were done to select a preliminary processing method. Method 1: Fresh potatoes were cut into pieces, dried at normal temperature, and finally ground into powder. Method 2: Fresh potatoes were ground into paste, air-dried at 50 °C, and ground a second time. Method 3: Fresh potatoes were cut into pieces, air-dried at 50 °C, and ground. All of the products processed with the three methods were filtered through a 0.25 mm filter. A UV−vis spectrophotometer (UV-2450, Shimadzu, Japan) were used to record the spectra of the three extraction solutions.

Material prepared according to method 1 (0.200 g) was stirred in 20, 30, 40, or 50 mL of liquid (1, 1.5, 2, or 3 mol/L HCl or ethanol and HCl mixture 1:1 in volume) at 40, 50, 60, or 70 °C for 10 or 20 min or 0.5, 1, or 1.5 h. Altering one element and fixing the others produced four groups of experiments. The extractions were added to 250 mL volumetric flasks in sequence and then diluted with ultrapure water to the marks. The best extraction condition was determined by comparing the absorption spectra.

Anthocyanin Purification by Macroporous Resin. Four types of macroporous resin (HPD-100, HPD-200A, HPD-450, and HPD-600) were purchased from Hebei Bonherb Technology Co., Ltd. Pretreatment was done according to the instructions. For each type of resin, 2.50 g was added into a 25 mL colorimetric tube and filled with extraction solution ($A = 0.43$) to the mark. Supernatant was collected to be measured on a spectrophotometer after being oscillated for 1 h.

Two grams of resin with pigment adsorbed was added into a 25 mL colorimetric tube. Twenty milliliters of ethanol solution of 50, 60, 70, 80, or 90% with 1% HCl (v/v) was used as eluent. The supernatants were measured on a spectrophotometer after 3 h, according to which proper eluent was chosen.

One liter of pigment solution obtained at the best extraction conditions was transferred into a column (2 × 50 cm) filled with resin. The takeoff rate was controlled to ∼20 drops/min. Afterward, resin was eluted at a speed of ∼15 drops/min. The eluate was collected to a round flask (250 mL) and was evaporated using a rotary evaporator to obtain a solid substance.

HPLC Analysis of the Pigments. Anthocyanins were separated by a Waters 510 high-performance liquid chromatography (Waters, USA) using a Hypersil BDS C_{18} reversed-phase column (Elite, China), and the absorbance of the eluant was recorded using a Waters 486 tunable absorbance detector (Waters, USA). Specifically, the column was eluted at a flow rate of 1 mL/min, and the injection volume was 20 μL. Solvent A consisted of 100% HPLC grade acetonitrile, whereas solvent B was a mixture of 10% (v/v) acetic acid in ultrapure water. The solvent gradient started with a composition of 10% solvent A and ended with 25% to 15 min. Chromatography data were collected and processed using a Zheda 2000 chromatography workstation.

Determination of the Total Anthocyanin Content (TAC). As the major anthocyanins present in the material have not been determined, it is appropriate to calculate total anthocyanin content by using the absorptivity and molecular weight of cyanidin-3-glucoside (Supporting Information, Figure S1).[19] The TAC was determined utilizing the pH differential method described by Lee.[20] Ten milligram of pigment sample was transferred into a 250 mL volumetric flask and made up to the final volume with pH 1.0 HAc−NaAc buffer. Another 10 mg of the sample was made into a pH 4.5 solution in the same way. The absorbance of the solutions was measured at both 530 and 700 nm, and TAC, expressed as milligrams of cyanidin-3-glucoside equivalents per 100 g of dry potato powder, was calculated as

$$\mathrm{TAC\ (mg/100g)} = \frac{\mathrm{Abs}}{\varepsilon L} \times \mathrm{MW} \times \mathrm{DF} \times \frac{V}{G} \times 100 \qquad (1)$$

where $\mathrm{Abs} = (A_{520\ nm} - A_{700\ nm})\mathrm{pH\ 1.0} - (A_{520\ nm} - A_{700\ nm})\mathrm{pH\ 4.5}$; ε is the extinction coefficient of cyanidin-3-glucoside; L is the cell path length (1 cm); MW, the molecular weight, is 449.2 g/mol for cyanidin-3-glucoside; DF is a dilution factor; V is the final volume; and G is the dry potato powder weight. This equation could also be used in calculating the anthocyanin concentration of the stock solution.

Color Stability Analysis of the Pigment. Five diverse aspects were considered to investigate the stability of the pigment. NaAc−HAc buffer was used to adjust the pH of the pigment solution, and the stability was reflected by the absorbance monitored with a UV−vis spectrophotometer. Na_2SO_3 stock solution was added into 3.2×10^{-2} M^{-1} PSTA solutions to make the final concentration of Na_2SO_3 0, 4×10^{-3}, 8×10^{-3}, and 1.2×10^{-2} M^{-1}. For the temperature experiment, five PSTA solutions were treated at 293, 313, 333, and 353 K in a water bath. H_2O_2 of different concentrations (0, 3, 6, and 9%, v/v) was used to study the influence of oxidant. Sucrose, 0, 1.46×10^{-3}, 2.92×10^{-3}, and 4.38×10^{-3} M^{-1} (final concentration), and NaCl, 0, 8.56×10^{-3}, 1.71×10^{-2}, and 2.57×10^{-2} M^{-1} (final concentration), were added as food additives. The absorbencies of all the solutions were measured with a UV−vis spectrophotometer after incubation of 1 h.

Influence of PSTAs on BSA−Cr (VI) Interaction. Fluorescence spectra were recorded on a fluorescence spectrophotometer (F-4600, Hitachi Co. Ltd., Tokyo, Japan) equipped with a 1 cm quartz cell. The samples were excited at 278 nm with excitation and emission slit widths of 5 nm. The emission wavelength was adjusted from 290 to 410 nm with a scanning speed of 1200 nm/min.

CD measurements were operated on a J-810 spectropolarimeter (Jasco, Tokyo, Japan) under constant nitrogen flush. Data were recorded over the range of 200−250 at 0.5 nm pitch intervals with an average of

two duplications. The scanning rate was set to 200 nm/min. The spectra were measured in a temperature-controlled 1 cm path length cell.

For UV−vis measurements, spectra were gauged on the spectrophotometer using a 1 cm quartz cell. Both excitation and emission bandwidths were set to 5 nm. Data were collected in the range of 190−350 nm.

RESULTS AND DISCUSSION

Screening of the Best Extraction Condition. Spectra of the leaching liquor obtained according to the three methods are shown in Figure 1. Two typical absorption peaks of the anthocyanins, one at 270−280 nm and the other at 510−540 nm,[21] are easily observed. The leaching liquor obtained by Method 3 exhibits higher absorbance than the others, showing the best yield of anthocyanins. For the first two methods, anthocyanins are more likely to touch O_2, especially for Method 2, and they were destroyed by oxygen. A good yield of pigment could be obtained by 1.5 mol/L HCl solution at 60 °C for 20 min with the solid/liquid ratio of 1 g/150 mL (Figure 2). Elution of purple potato powder with ethanol yielded a turbid extract solution, which may be due to the formation of complexes (anthocyanins interact with other organic macromolecules). Long time at high temperature may have a destructive effect on the structure of anthocyanins and induce pigment loss. At the increasing ratio of solid/liquid, the yield reached a maximum when the elution was 30 mL but experienced a decrease afterward.

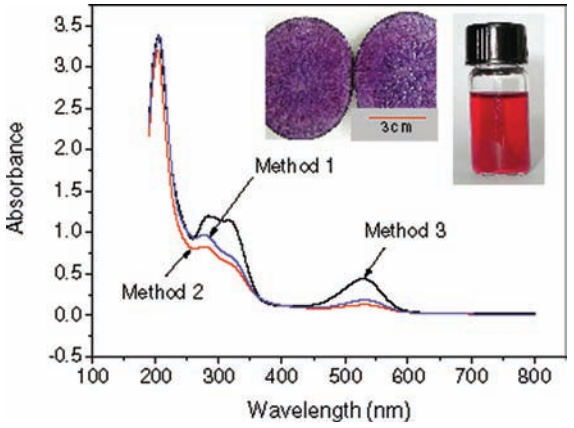

Figure 1. UV−vis spectra of PSTA extracts by three pretreatment methods. (Insets) Photographs of purple *Solanum tuberosum* tuber and the extract by method 1.

Purification, Concentration, and Separation of the Pigment Solution. The absorbencies of the pigment solutions in the presence and absence of the resins are shown in Figure 3A. HPD-600 resin possessed the worst result of adsorption efficiency, whereas HPD-200A achieved the optimal and almost all of the pigment was adsorbed. As represented in Figure 3B the elution effect increases with the concentration of ethanol and reaches the optimum at 80%. After purification and concentration, atropurpureus solids were obtained, suggesting a good separation result compared with the thick material obtained without purification. According to eq 1 the total content of anthocyanins calculated was 273.5 ± 14.3 mg in cyanidin-3-glucoside equiv/100 g of dry seeds.

Figure 4 shows that four peaks appeared in the chromatograms of PSTAs, which were detected at 520 nm. From the chromatographic and UV−vis spectral features, peaks 1, 2, 3, and 4 accounted for 24.4, 36.6, 21.9, and 17.1% of the total amount of all anthocyanins, respectively, and they were eluted after 1.27, 1.77, 1.97, and 4.71 min, respectively. The same operations were repeated on oxidative PSTAs. It is interesting to observe that the same four components were eluted after the same minutes and no new peaks were introduced with different intensities of 26.8, 32.9, 33.2, and 7.1%. These results illuminate that after

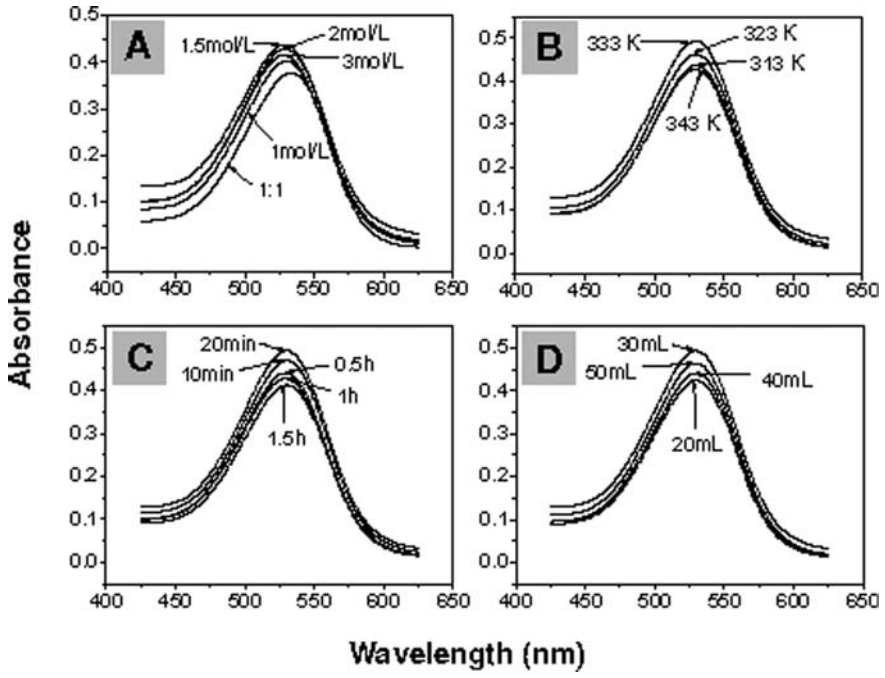

Figure 2. UV−vis detection of the extract under different extracting conditions: (A) 60 °C for 20 min with the solid/liquid ratio 1 g/150 mL; (B) 1.5 mol/L HCl solution for 20 min with the solid/liquid ratio 1 g/150 mL; (C) 1.5 mol/L HCl solution at 60 °C with the solid/liquid ratio 1 g/150 mL; (D) 1.5 mol/L HCl solution at 60 °C for 20 min with 1 g of solid.

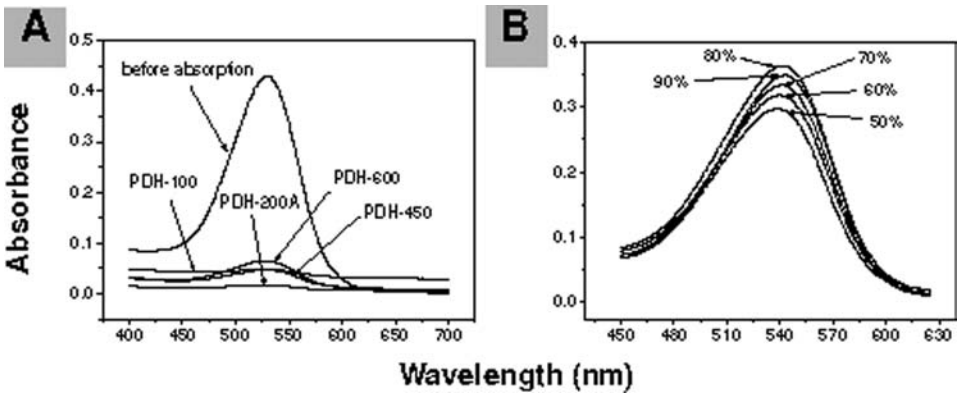

Figure 3. Adsorption and desorption data: (A) absorbance of PSTAs solutions after adsorption on diverse sorts of resin; (B) absorbance of eluates by different concentrations of ethanol. The concentration of PSTAs solutions before adsorption was 3.2×10^{-2} M^{-1}.

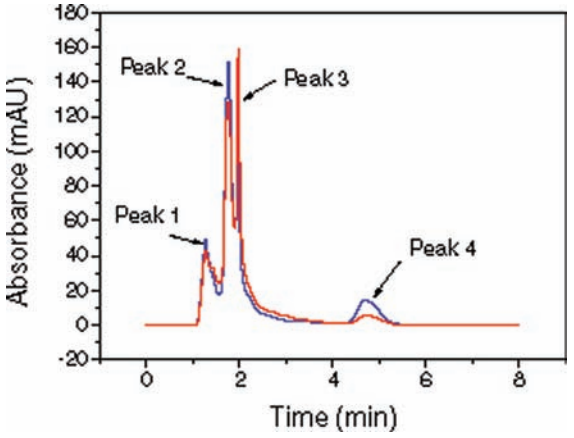

Figure 4. Overlay chromatogram of PSTAs before (blue) and after (red) being exposed in air. Detection was done at 530 nm.

oxidation the amounts of the main components were changed to various degrees.

Color Stability of PSTAs under Diverse Conditions. The stability of PSTAs in this research was investigated by examining the influence of oxidant, reductant, food additives, pH, and temperature (Figure 5). We chose H_2O_2 and Na_2SO_3 as oxidant and reductant, respectively, and it can be easily observed that they all have negative effects on the pigment. With increasing concentration of the agents the absorbance of pigment solutions decreased significantly, suggesting high sensitivity to oxidant and reductant. The addition of sucrose and salt could hardly alter the absorbance of the pigment solution, so it seems to have little influence on PSTAs. In diverse temperature and pH the absorbance of pigment solution varied a lot, which manifested a temperature- and pH-sensitive feature. Specifically, varying pH levels induce structural transformations, which affect both color quality and intensity (Figure 5).[22]

Fluorescence Quenching Study of BSA−Cr(VI) with and without PSTAs. The fluorescence intensity of BSA is attributed to its three intrinsic fluorophores, tryptophan, tyrosine, and phenylalanine, and among these tryptophan moieties contribute most (~95%). A variety of molecular interactions can result in fluorescence quenching of the intrinsic fluorophores because of molecular rearrangements, energy transfer, ground state complex formation, or collision.[23] Figure 6A shows the fluorescence spectrum of BSA in the absence and presence of Cr(VI). As a function of the increasing amount of Cr(VI), the fluorescence intensity experienced a decreasing tendency and there is a little blue shift of the emission peaks. These phenomena indicated that the microenvironment around the Trp-212 residue was changed and that a slight increase of hydrophobicity in the vicinity of the Trp-212 residue took place.[24] By adding 5×10^{-5} M PSTA solutions to each sample and fixing the other experimental conditions, fluorescence measurements were repeated. We noted that the addition of PSTAs enables weak quenching of the fluorescence, suggesting a weaker binding of Cr(VI) to BSA or a lesser amount of Cr(VI) approaches BSA. As the amount added to the system increased to 2.5×10^{-4} M, the quenching exhibited became even weaker. Because the absorbance of these samples was >0.1, this time inner filter effect (IFE), which was caused by the absorption of both excitation and emission radiation, was taken into consideration.[25] According to Gu,[26] the fluorescence of a mixture showing IFE can be calculated when absorbance effects are corrected by multiplying appropriate correction factors as shown in eq 2:

$$F_{ideal}(\lambda_{ex},\lambda_{em}) = F_{obs}(\lambda_{ex},\lambda_{em})CF_p(\lambda_{ex})CF_s(\lambda_{em}) \approx F_{obs}(\lambda_{ex},\lambda_{em})10^{(A_{em}+A_{ex})/2} \quad (2)$$

CFp is the correction factor for pIFE, which depends on the total absorbance of the sample at λ_{ex}, whereas CFs is the correction factor for sIFE, which depends on the total absorbance of the sample at λ_{em}. A_{ex} and A_{em} represent the absorbance at the fluorescence excitation and emission wavelengths, respectively. F_{obs} is the observed fluorescence.

The Stern−Volmer equation relates the quenching of fluorescence to the concentration of quencher in the relationship $F_0/F = 1 + K_{SV}[Q]$, where F and F_0 represent fluorescence intensity with and without quencher, respectively. K_{SV} is the Stern−Volmer constant, which can reflect the quenching extent, and [Q] is the quencher concentration.[27] As illustrated in the inset plots, the quenching rate, which was reflected by the slope, decreased sharply with the addition of PSTAs, and finally no obvious quenching was observed. The observed difference in the Stern−Volmer plots may be assigned to the protective effect of PSTAs. The extent of the changes suggested that PSTAs can prevent BSA molecules from being touched, and thus the structure of BSA was not easily changed. It was shown that Cr(VI) binds to BSA in electrostatic fashion.[28] Cr(VI) is redox

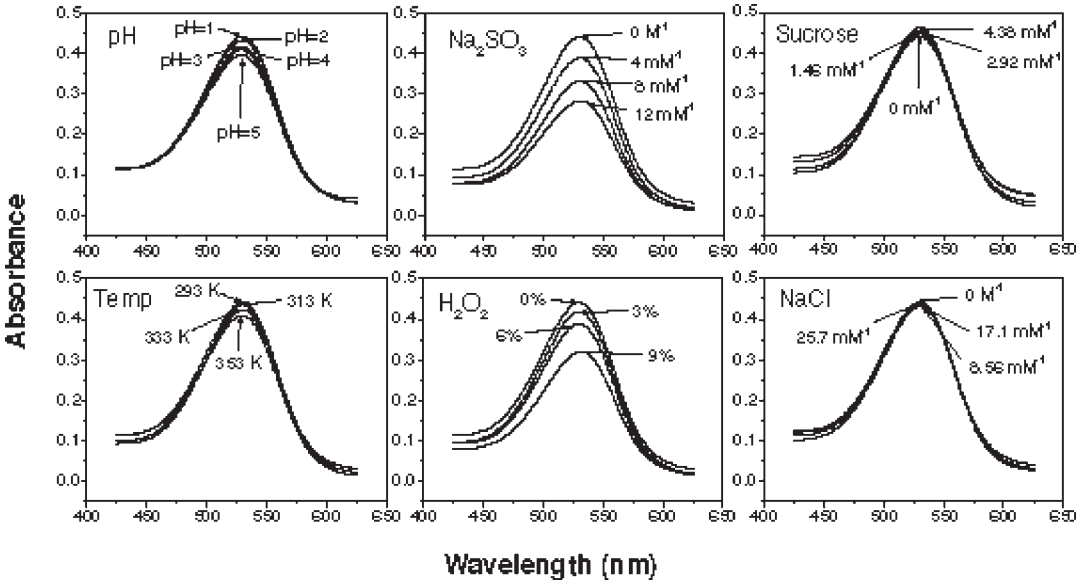

Figure 5. Color stability of PSTAs: UV−vis absorbance of PSTAs solutions at different pH values and temperatures, with different concentrations of oxidant, reductant, and food additives.

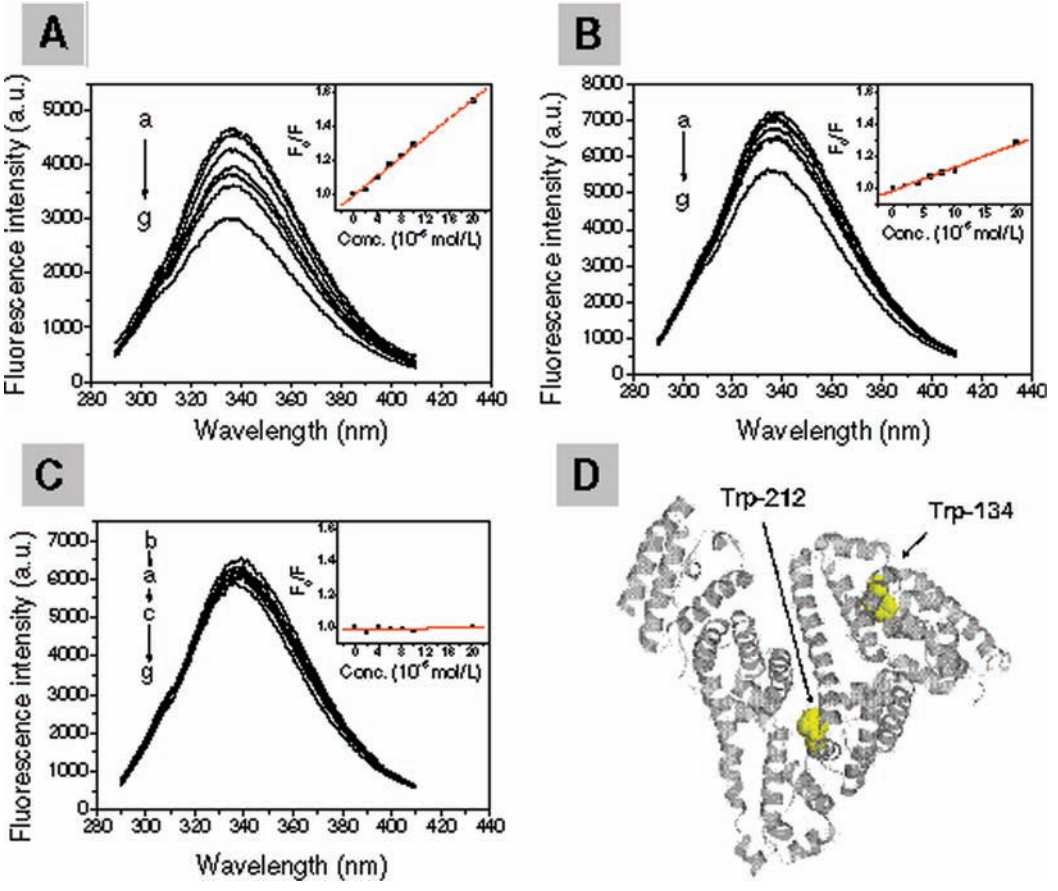

Figure 6. Fluorescence emission spectra of Cr(VI)−BSA and Cr(VI)−PSTAs−BSA systems: (A) (a) native BSA (7.5×10^{-7} M), (b−g) with Cr(VI) at 2, 4, 6, 8, 10, 20×10^{-6} M; (B) (a) BSA (7.5×10^{-7} M) and PSTAs (5×10^{-5} M), (b−g) with Cr(VI) at 2, 4, 6, 8, 10, 20×10^{-6} M; (C) (a) BSA (7.5×10^{-7} M) and PSTAs (2.5×10^{-4} M), (b−g) with Cr(VI) at 2, 4, 6, 8, 10, 20×10^{-6} M [(insets) Stern−Volmer plots of F_0/F as a function of [Cr(VI)] for the binding of Cr(VI) to BSA]; (D) structure of BSA (HSA as model) showing tryptophan residues in green. Samples were detected in 0.01 M PBS buffer (pH 7.4) at 25 °C.

active. As anthocyanins exhibit a great reductive property, it is of very possible that Cr(VI) might interact with the phenolic compounds through redox reaction prior to interacting with BSA. Moreover, few fluorescent alterations of BSA were observed

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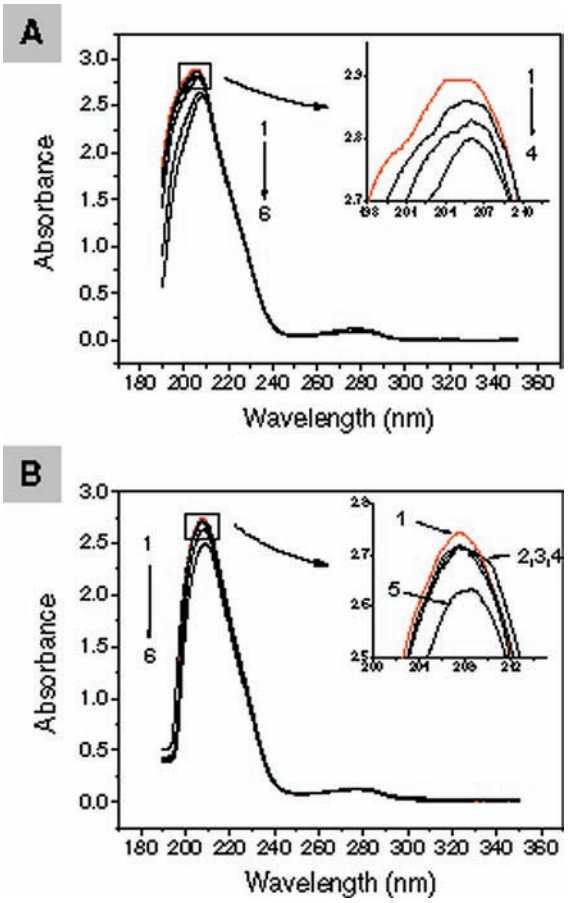

Figure 7. UV−vis absorption spectra of Cr(VI)−BSA and Cr(VI)−PSTAs−BSA systems: (A) (1) free BSA (2.25×10^{-6} M), (2−6) with Cr(VI) at 5, 10, 20, 50, 80×10^{-6} M; (B) (1) BSA (2.25×10^{-6} M) and PSTAs (2.5×10^{-4} M), (2−6) with Cr(VI) at 5, 10, 20, 50, 80×10^{-6} M. Samples were measured in 0.01 M PBS buffer (pH 7.4) at 25 °C.

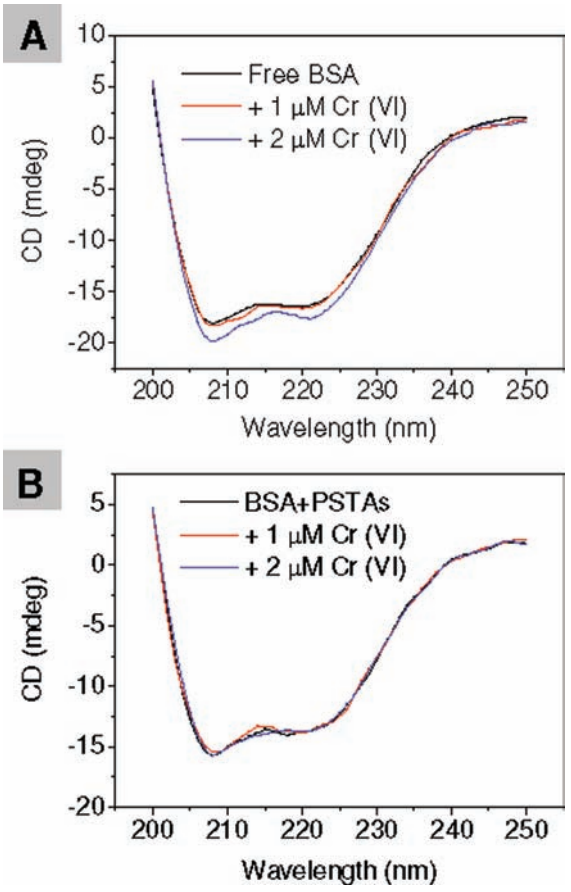

Figure 8. Circular dichroism of BSA−Cr(VI) and BSA−Cr(VI)−PSTA systems. All measurements were taken in aqueous solutions, 0.01 M PBS at 25 °C. Protein and PSTA concentrations were fixed at 1.5×10^{-7} and 1×10^{-5} M, respectively.

when PSTAs was as high as 2.5×10^{-4} M, which proved the innocuousness of the product of Cr(VI) and anthocyanins.

UV−Vis Detection of the Influence of PSTAs on BSA−Cr(VI). Moreover, we further studied the difference of UV−vis absorbance spectra of BSA−Cr(VI) system in the absence and presence of PSTAs. As shown in Figure7 there are two typical absorption peaks, a strong one at about 210 nm and a weak one at 280 nm, which were associated with polypeptide backbone structure and the three aromatic amino acid residues, Trp, Tyr, and Phe, respectively.[29] After incubation with Cr(VI) for 30 min, a significant decrease in absorbance of BSA at 210 nm can be seen as a result of complexation. With an increasing concentration of Cr(VI) the decrease became more and more evidently seen. In our previous study we concluded that the absorption at about 210 nm caused by the $\pi \rightarrow \pi^*$ transition of BSA's characteristic polypeptide backbone structure C═O is intimately related to the microenvironment of the protein backbone.[30] When Cr(VI) interacted with BSA, the protein molecules became unfolded, letting more H_2O molecules touch the main chain. At 278 nm changes of the peaks are not as distinct as at 210 nm. There is merely a rough increase, showing an increase of hydrophobicity around the aromatic amino acid residues, which is in accordance with the fluorescence result.

However, the presence of PSTAs seemed to help the BSA molecule structure avoid being altered. A fixed concentration of PSTAs was added into the BSA−Cr(VI) system, and similar measurements were taken. At light concentrations, PSTAs were capable of protecting the BSA structure, and the absorbance was stabilized to a certain extent. This phenomenon may be assigned to the fact that the reaction rate of anthocyanins−Cr(VI) is much faster than that of BSA−Cr(VI); therefore, anthocyanins could interact with Cr(VI) ahead of the binding with BSA.

CD Spectra and Protein Conformation. CD spectroscopy was used here to study the secondary structure of BSA and the conformational changes upon the addition of Cr(VI) or PSTAs. The CD spectroscopic results shown in Figure 8 and Table 1 exhibit remarkable changes on secondary structural content of BSA. The free protein has a secondary structural content of 58.2% α-helix, 10.6% β-sheet, 8.1% β-turn, and 23.2% random coil, which is consistent with the spectroscopic studies of BSA previously reported.[31,32] On interaction with Cr(VI), a major increase of α-helix from 58.2 to 59.9% and further to 64.8% was observed. This result suggests that the interaction with Cr(VI) and partial unfolding of BSA could cause changes in the secondary structural components. Several previous papers also manifested that the conformational changes occurring in BSA are attributed to the complex with ligands.[13,14,33]

To examine the effect of PSTAs on the above interaction, we added them into the system and repeated the experiment.

Table 1. Secondary Structural Fractions of BSA in BSA−Cr(VI) and BSA−Cr(VI)−PSTA Systems

		content (%)			
	system	α-helix (±2%)	β-sheet (±1%)	β-turn (±1%)	random coil (±2%)
A	free BSA	58.2	10.6	8.1	23.2
	+1 μM Cr(VI)	59.9	9.0	8.5	23.1
	+2 μM Cr(VI)	64.8	5.9	9.3	23.5
B	BSA + PSTAs	52.3	15.5	7.2	25.3
	+1 μM Cr(VI)	51.6	17.5	7.8	23.7
	+2 μM Cr(VI)	53.1	16.9	7.4	23.4

No obvious changes on secondary structural conformation of protein were observed, indicating that there is almost no BSA−Cr (VI) interaction after the addition of PSTAs. These results are quite consistent with the fluorescence and UV−vis outcomes.

In summary, we report the newly cultivated purple *S. tuberosum* from the Taian Academy of Agricultural Sciences. A good yield of pigment could be obtained by 1.5 mol/L HCl solution at 60 °C for 20 min with the solid/liquid ratio of 1 g/150 mL. Four major compounds were determined by HPLC analysis. By exploring their color stability, we concluded that PSTAs are quite sensitive to pH, oxidant, and reductant; general food additives have little influence on them. Moreover, PSTAs are stable at low temperatures. For daily cooking, potatoes are usually sautéed, fried, or steamed before being eaten, causing only a small decrement of anthocyanin content.[34,35] Despite all of this, a quick cooking is advocated and mashed purple potatoes are not recommended. On the basis of our spectroscopic data, Cr(VI) binding to BSA occurs via alteration of the secondary and tertiary structures and causes partial protein unfolding. PSTAs proved to have the function of anti-Cr(VI) and impeded the conformational destruction to protein. Anthocyanins are known to possess reductive properties due to their active site, which locates in the 1-position of the C ring, and this site is probably the key site for rapid interaction with Cr(VI) and therefore protection of the protein. Thus, the described properties of PSTAs may also be valid for anthocyanins from other anthocyanin-rich fruits and vegetables. However, as the components of PSTAs were not identified, it is still not clear which constituent(s) function(s) in the observed phenomena. Isolation, characterization, and related works utilizing HPLC, nuclear magnetic resonance (NMR), Fourier transform infrared (FTIR) spectroscopy, etc., are ongoing in our laboratory. From these angles, this exploratory research may provide positive implications for using anthocyanins as natural colorants in the food industry and helpful information on the biomedical application of anthocyanins (e.g., to be manufactured as anti-free-radical, anti-inflammatory, antiviral, and anticancer therapeutic agents). In addition, it also illustrated how and to what extent anthocyanins protect against biological oxidation.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Additional figures. This information is available free of charge via the Internet at http://pubs.acs.org.

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