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Complexation of thermally-denatured soybean protein isolate with anthocyanins and its effect on the protein structure and in vitro digestibility

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ABSTRACT

The complexation of anthocyanin-rich black rice extracts (ARBRE) with soybean protein isolate (SPI) heated at 0, 70, 85, and 100 °C and its effect on protein digestibility were studied. The structural changes of SPI during its interaction with ARBRE in all the samples were studied by Fourier transform infrared, circular dichroism, and fluorescence spectroscopy. The secondary structure changes of SPI in all the samples after complexation with ARBRE showed a significant increase in α-helix and a significant decrease in β-sheet contents. Results also showed that ARBRE quenched the SPI fluorescence (in both unheated and heated samples) via static quenching with a single binding site. The digestibility of unheated and heated SPI was improved upon complexing with ARBRE. The formation of the SPI-ARBRE complexes is beneficial for the application of soy protein-based products in foods by increasing their protein digestibility and nutritional quality.

1. Introduction

Anthocyanins are a class of water-soluble flavonoid that are found naturally as pigments in fruits, vegetables, some cereal grains, and flowers (Pineda-Vadillo et al., 2017). Anthocyanins are also effective antioxidants that play an important role in the prevention of cardiovascular diseases (Jennings et al., 2012), inflammation (Collins et al., 2016), and certain cancers (Konczak & Zhang, 2004). Meanwhile, studies have indicated that regular intake of anthocyanins or anthocyanins-rich foods can prevent type 2 diabetes mellitus and is beneficial for obesity control (Tsuda, 2012). Owing to their health-promoting effects, numerous studies on anthocyanins have been conducted over the recent years.

At present, the complexation between proteins and polyphenols has received increasing attention on changes of the physicochemical properties and subsequently the health implications of the proteins. Some researchers had found that the complexation between proteins and polyphenols can lead to changes in the structural properties of the proteins (Stojadinovic et al., 2013; Ozdal, Capanoglu, & Altay, 2013; Guimarães Drummond e Silva et al., 2017). Roy et al. (2012) found that the native structure of human and bovine serum albumin was changed after complexation with catechin and epicatechin because α-helix was transformed to β-sheet. He et al. (2016) found that the complexation between bovine β-lactoglobulin and malvidin-3-glucoside via hydrophobic interaction changed the secondary structure of the bovine β-lactoglobulin with a decrease in α-helix, β-turn, and random coil and an increase in β-sheet. Furthermore, some studies showed that the addition of polyphenols improved the digestibility of proteins by increasing the initial rate of the enzymatic reaction (Tagliazucchi, Verzelloni, & Conte, 2005). Tantoush et al. (2012) reported that the complexation of food allergens and catechin-rich extracts of green tea improved the digestibility of food allergens. However, the complexation of proteins and polyphenols would impair the digestibility of proteins due to the inhibitory effect of polyphenols on pepsins and trypsins (Stojadinovic et al., 2013). Emmambux and Taylor (2003) found that the digestibility of sorghum kafirin was decreased after complexation with sorghum condensed tannins. Similarly, Stojadinovic et al. (2013) reported that the non-covalent interactions of β-lactoglobulins and polyphenols (from tea, coffee, and cocoa) were found to cause a decrease in the digestibility of β-lactoglobulins. Therefore, the different views on the complexation between proteins and polyphenols and its effect on the protein digestibility need to be further investigated.

Soybean protein is an important source of plant protein, which is widely used in the food industries due to its functional properties and nutritional values (Moras, Rey, Vilarem, & Pontalier, 2017). Soybean protein is commercially available as soybean protein isolate (SPI) with approximately 90% protein. The functional and nutritional qualities of SPI are strongly dependent on cartain extrinsic and environmental

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parameters of the SPI, such as temperature, pH, and protein concentration. (Utsumi & Kinsella, 1985). The commercial food grade SPI is usually heated at high temperatures of approximately 100 °C and this heating process greatly increases the surface hydrophobicity of the protein (Sorgentini, Wagner, & Aiidn, 1995). However, SPI's binding performance after such treatment is relatively unexplored, especially in SPI-polyphenol interactions.

Black rice is a special cultivar of rice and it is widely consumed since the ancient time in China and in other parts of eastern countries in Asia (Hou, Qin, Zhang, Cui, & Ren, 2013). It has been regarded as a health-promoting food due to its high content of anthocyanins. Studies have shown that the supplementation of black rice anthocyanins markedly reduced oxidative stress and improved lipid profile in animal models (Ling, Cheng, & Ma, 2001; Ling, Wang, & Ma, 2002). However, anthocyanins are not chemically stable and are sensitive to degradation when exposed to harsh environmental conditions such as thermal treatment, pH, light, oxygen, enzymes, ascorbic acids, sulfur dioxide or sulfite salts, metal ions, and copigments (Tiwari, O'Donnell, Muthukumarappan, & Cullen, 2009).

The aim of this study was to investigate the effect of complexation of SPI heated at different temperatures (70, 85, and 100 °C) and black rice anthocyanins on the structure and digestibility of the SPI. The structural changes of the SPI complexes were characterized using Fourier transform infrared, circular dichroism, and fluorescence spectroscopy. The digestibility of the SPI complexes with anthocyanins was evaluated in vitro under simulated gastrointestinal conditions.

2. Materials and methods

2.1. Materials

Soybeans were purchased from Shengda Biological Technology Co., Ltd. (Harbin, China). Black rice extract powder with anthocyanin concentrations of approximately 20% was purchased from Shanxi Taiji Huaqing Biological Technology Co., Ltd. (Shanxi, China). Anthocyanin standards were purchased from Polyphenols Laboratories (Ireland, U.K.). Pepsin (from porcine stomach mucosa, 600 U/mg) and pancreatin (from bovine pancreas, 2500 U/mg) were purchased from Sigma-Aldrich (Taufkirchen, Germany). All other chemicals used were of analytical grade, unless otherwise stated.

2.2. Purification of anthocyanin-rich black rice extracts (ARBRE)

Purification of ARBRE was performed according to our previous work (Sui, Dong, & Zhou, 2014). Briefly, the ARBRE powder was dispersed in deionized (DI) water to form a known concentration of ARBRE dispersion. The solution was purified using a Sep-Pak C18 cartridge (WAT023635, Waters, USA) add loading the solution on the cartridge first….by passing 20 mL of acidified DI water (0.5% v/v, trifluoroacetic acid) through the cartridge, and then 20 mL of ethyl acetate, followed by 20 mL of methanol for extraction. The solvent in the purified ARBRE collected in methanol was evaporated using a rotary evaporator (RE-2010, Shaanxi HEB Biotechnology Co., Ltd., China) at 40 °C to remove the solvent. The purity of the obtained anthocyanin fraction was determined at 520 nm using high-performance liquid chromatography with photodiode-array detector (HPLC–DAD). The anthocyanin content of the purified ARBRE was approximately 20%.

2.3. Preparation of SPI

SPI was prepared based on our previous work (Sui et al., 2017). Briefly, soybean was ground into powder using a laboratory mill (Polymix A10, Taister Instrument Co., Ltd., Tianjin, China) and defatted in hexane at a ratio of 1:3 (w/v). The hexane was removed from the protein using a rotary evaporator. The defatted soybean (pH 8.0) was stirred for 2 h and centrifuged at 10,000*g* force for 30 min. The supernatant recovered after centrifugation was adjusted to pH 4.5 using 2 M HCl and then centrifuged at 6500*g* force for 20 min. The precipitated protein was washed with DI water three times and adjusted to pH 7.0 with 2 M NaOH. Lyophilization was performed in a freeze dryer (FD5-3, Siemon Co., Ltd., USA) and product saved until further use.

2.4. Preparation of thermally-denatured SPI and anthocyanins complex

The freeze-dried SPI was dissolved in 0.01 M phosphate buffer solution (PBS, pH 7.4) at a concentration of 1% (w/v). The powder was dispersed at room temperature for 30 min under continuous stirring. The thermal treatment was performed by heating the SPI solution in an oil bath at the temperatures of 70, 85, and 100 °C for 15 min. Once the thermal treatment was completed, the SPI solution was immediately cooled in an ice-water bath and then freeze-dried.

The preparation of the complex of thermally-denatured SPI and anthocyanins was performed according to our previous study (Sui et al., 2018) and sequentially by (1) dissolving the thermally-treated and freeze-dried SPI powder in PBS at 10 mg/mL under continuously stirring for 30 min in a water-bath at 37 °C, (2) the purified ARBRE powder was added at a ratio of 50:1 (w/w) into the heated SPI dispersion, and (3) continuously stirring for another 90 min. After complexation, the slurry was subjected to subsequent analyses.

2.5. Fourier transform infrared (FTIR) spectroscopy

Infrared spectroscopy was used to examine changes in the protein secondary structure and to infer the interaction between small molecules and proteins. This method is often used to detect the interaction between polyphenols and proteins (Jia, Gao, Hao, & Tang, 2017; Rahmelow & Hübner, 1996). Infrared spectra analysis was performed using a FTIR spectrometer (Nicolet 6700, Thermo Nicolet company, USA) equipped with deuterated triglycine sulphate (DTGS) detector and KBr beam splitter using ZnSe window. SPI, thermally-denatured SPI, and thermally-denatured SPI and anthocyanin complex solutions were freeze-dried prior to the FTIR analysis. The freeze-dried samples were blended with KBr at a ratio of 1:100 (w/w) and pressed into tablets. Spectra of the samples were recorded in the spectral range of 4000–600 cm^{-1} at a resolution of 2 cm^{-1} with a total of 100 scans. The KBr tablet was used as blank.

2.6. Circular dichroism (CD) spectroscopy

CD spectroscopy was conducted using a Jasco J-815 Circular Dichroism (CD) Spectropolarimeter (JASCO, Tokyo, Japan) in the range of 190–260 nm at 298 K. The SPI, thermally-denatured SPI, and thermal-denatured SPI and anthocyanin complex solutions were measured in a quartz curvette with a path length of 1 mm. The scanning rate used was 100 nm/min and the parameters for spectral resolution, response time, and steps were set at 0.1 nm, 1 s, and 1 nm, respectively. The percentage of the change in protein secondary structure was calculated by a CDPro software package (Bio-Logic, France).

2.7. Fluorescence spectroscopy

Fluorescence measurements were carried out using a F4500 fluorescence spectrophotometer (Hitachi, Japan). SPI was mixed with different concentrations of ARBRE in PBS (pH 7.4) at room temperature. The final concentration of SPI in the mixture was 1.0×10^{-6} M and the concentration of ARBRE ranged from 2.0×10^{-6} M to 10.0×10^{-6} M. The fluorescence spectra were recorded at excitation of 280 nm and emission from 300 to 500 nm at 298, 306, and 314 K. The fluorescence intensity at 340 nm was used. Spectra were further analyzed using an Origin 8.0 program (OriginLab, Northampton, MA, USA).

Analysis of fluorescence quenching was determined using the Stern–Volmer equation (Eq. (1)).