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Interaction characterization of preheated soy protein isolate with cyanidin-3-*O*-glucoside and their effects on the stability of black soybean seed coat anthocyanins extracts

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Disodium hydrogen phosphate (PubChem CID: 71180)
Sodium dihydrogen phosphate (PubChem CID: 24204)
Hydrogen peroxide (PubChem CID: 784)
Hydrochloric acid (PubChem CID: 313)
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Sodium acetate (PubChem CID: 517045)
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ABSTRACT

The interactions of soy protein isolate with cyanidin-3-*O*-glucoside were investigated to study the protective effect of protein on anthocyanin's stability by UV–Vis spectrophotometry, Fourier transform infrared spectroscopy, circular dichroism and fluorescence spectroscopy. Preheat treatment and binding of cyanidin-3-*O*-glucoside effectively changed the secondary structure of soy protein isolate, with a decrease in α-helix, random coil structure and an increase in β-sheet and β-turn. The soy protein isolate preheated at 121 °C exhibited a strong binding affinity towards cyanidin-3-*O*-glucoside with strong *Ks* of $147.40 \times 10^4\,M^{-1}$ and also effectively increased the thermal and oxidation stabilities of black soybean seed coat extract *via* decreasing the degradation rate by 67% and 23%, respectively. Soy protein isolate interacted with cyanidin-3-*O*-glucoside mainly through hydrophobic interactions and static quenching process. Altogether, the results suggested that preheated soy protein isolate-cyanidin-3-*O*-glucoside interaction could effectively protect anthocyanins' stability through strong binding affinity influenced by the systematic alterations in the secondary structure.

1. Introduction

Anthocyanins are water-soluble natural pigments, belongs to the classes of flavonoids and large subgroup of polyphenols. These are commonly distributed in plants and they impart color to plants and highly consumed in human diets such as crops, vegetables, beans, fruits, and some colorful flowers (Khoo, Azlan, Tang, & Lim, 2017). Recently, several studies have demonstrated that anthocyanins possess multiple health effects such as antioxidant, anticancer, antidiabetic, antimicrobial and anti-inflammatory activities (Hribar & Ulrih, 2014; Jeng, Yi, Wu, & Sung, 2010). Black soybeans (*Glycine max* (L.) Merr.) have been widely consumed as a medicinal food in Korea, China, and Japan for hundreds of years (Xu & Chang, 2008) and their seed coat consists of high level of anthocyanins such as cyanidin, delphinidin, petunidin, and pelargonidin as 3-*O*-gluscosides, where cyanidin-3-*O*-glucoside is the most abundantly found anthocyanin in it (Kovinich, Saleem, Arnason, & Miki, 2010; Kurimoto et al., 2013). Black soybean seed coat anthocyanin extract (BSSCE) was well-known for its significant antioxidant, anti-obesity and anti-diabetic properties (Soyoung et al., 2015; Thompson, Pederick, & Santhakumar, 2016).

Anthocyanins are unstable compounds that can induce the chemical transformation and affects the performance and biological activities of anthocyanin-containing foods (Xiao & Högger, 2015; Xiao, 2018). Anthocyanins in aqueous solutions are very sensitive to the environmental factors such as temperature, pH, oxygen, light, metal ions, enzymes, ascorbic acid and sulfur dioxide (Torskangerpoll & Andersen, 2005;

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Xiong, Melton, Easteal, & Siew, 2006). Food processing (especially thermal processing) can easily results in significant losses of anthocyanins (Torskangerpoll & Andersen, 2005; Xiong et al., 2006). Recently, some stabilization strategies have been developed and investigated to improve the stability of anthocyanins *via* structural modification such as glycosylation, acylation (Wiczkowski, Szawara-Nowak, & Topolska, 2013), microencapsulation (Betz & Kulozik, 2011) and through adding some food additives such as acids, sugars, salts, hydrocolloids and various phenolic compounds (Kopjar, Jakšic, & Piližota, 2012). Though there are several stabilization strategies available to enhance the anthocyanin's stability, it has some disadvantages such as the process of microencapsulation is complicated and during emulsifying, high temperatures in thermal gelation might distress their bioactivities (Betz & Kulozik, 2011). Studies supported that altering the structure of anthocyanin could enhance their stability however it would affect their bioavailability and biological activity (Chang et al., 2014). Thus, an alternative approach is needed.

The intermolecular co-pigmentation between anthocyanins and other colourless compounds has been reported to be a successful way to stabilize the color intensity of aqueous solutions (Malaj, Simone, Quartarolo, & Russo, 2013). Few studies reported the protective effect of proteins on polyphenols (Cao, Jia, Shi, Xiao, & Chen, 2016; Tang et al., 2017). The interactions between polyphenolic substances and whey protein isolate (WPI) could decrease the protein precipitation with polyphenols and allow its use in complex food matrices (Thongkaew, Gibis, Hinrichs, & Weiss, 2014). α- and β-casein bound with malvidin-3-O-glucoside (MG), the major anthocyanin in grape skin anthocyanin extracts (GSAE) through hydrophilic and hydrophobic interactions, respectively, and the casein-anthocyanin interaction appeared to have a positive effect on the thermal, oxidation and photo stability of GSAE (He, Xu, Zeng, Qin, & Chen, 2016). Al-Hanish et al. (2016) studied the interactions of Epigallocatechin-3-gallate (EGCG) and Bovine a-lactalbumin (ALA), which revealed that the EGCG interacted with ALA *via* noncovalent interactions and it might impair the uptake of ALA by antigen-presenting cells. Conversely, ALA could serve as a suitable delivery system for EGCG. Some food processing methods, especially thermal processing can easily result in significant losses of anthocyanins (De et al., 2017; Xiong et al., 2006). To prevent the loss, pretreatment of edible proteins such as superfine grinding, micro-particulation and preheat before processing had been proved to protect anthocyanin's stability *via* altering their physicochemical properties, such as emulsifying, foaming and change in their secondary structure as well as interfacial properties (Lajnaf et al., 2017; Peng et al., 2016; Sun et al., 2015; Wang et al., 2017). However, limited reports were available on the interaction mechanism of preheated proteins and anthocyanins, especially on the protective effect of preheated soy protein isolates on cyanidin-3-*O*-glucoside.

This study aimed to investigate the interaction mechanism of preheated soy protein isolates (SPI) and cyanidin-3-*O*-glucoside through UV–Vis spectrophotometry (UV–Vis), Fourier transform infrared spectroscopy (FTIR), circular dichroism (CD) and fluorescence spectroscopy. Furthermore, the protective effect of SPI preheated at 40, 60, 80, 100 and 121 °C on BSSCE were investigated. The color stability and the degradation rate of anthocyanins in BSSCE were used to determine the thermal and oxidation stabilities of BSSCE-SPI interaction system.

2. Materials and methods

2.1. Materials and chemicals

Soy protein isolate (SPI) of food grade was purchased from Beijing Aoboxing Bio-tech Co., Ltd. (Beijing, China). BSSCE, with 12% anthocyanin content, was extracted in our lab according to the previous study (Chen, Wang, Pan, Gao, & Chen, 2018). Cyanidin-3-*O*-glucoside (97% purity) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade, purchased from local market.

2.2. Preheat treatment of SPI

The commercial soy protein isolate (SPI, 0.4 mg/mL) solutions were prepared in PBS solution (pH 7.4). The 0.4 mg/mL SPI solutions were heated in a water bath at 40 °C, 60 °C, 80 °C, 100 °C and treated with autoclave sterilization under 121 °C and 0.1 MPa for 15 min, which were referred to SPI1, SPI2, SPI3, SPI4, SPI5, respectively, and the unpreheated (without preheat treatment) was referred to SPI0. Then all the treated protein solutions were quickly cooled to room temperature. The preheated protein stock solutions and their unheated counterparts were stored separately at 4 °C until use.

2.3. Preparation of the cyanidin-3-O-glucoside-SPI and BSSCE-SPI mixtures

BSSCE stock solution (2.0 mg/mL) and cyanidin-3-*O*-glucoside stock solution (120 μM) were freshly prepared in PBS solution (pH 7.4) for each experiment. The BSSCE or cyanidin-3-*O*-glucoside and the protein mixtures were prepared by blending BSSCE or cyanidin-3-*O*-glucoside stock solutions with unheated and preheated SPI (SPI0-5) stock solutions (1:1, v/v) for further tests. Cyanidin-3-*O*-glucoside-SPI mixtures were used to analyze and elucidate their interaction mechanisms. BSSCE-SPI mixtures were used for thermal and oxidation stabilities tests.

2.4. Interaction mechanism of cyanidin-3-O-glucoside-SPI mixtures

The mixture of individual soy protein isolate (SPI0) or 0.05 mg/mL of preheated SPI 1–5 solutions (at 40, 60, 80, 100 and 121 °C) with cyanidin-3-*O*-glucoside were subjected to UV–Vis spectrophotometry, Fourier transform infrared spectroscopy (FTIR), circular dichroism (CD) and fluorescence spectroscopy analysis to elucidate the interaction mechanism of SPI and cyanidin-3-*O*-glucoside. The final concentration of cyanidin-3-*O*-glucoside and SPI mixtures were 60 μM and 0.05 mg/mL, respectively.

2.4.1. UV–Vis spectrophotometry (UV–Vis)

The UV–Vis absorption spectra of SPI in the absence and presence of cyanidin-3-*O*-glucoside in PBS (pH 7.4) were measured using a UV–visible spectrophotometer (Cary 100, Agilent Technologies, Santa Clara, CA, USA) at the wavelength range of 190–600 nm, at 297 K.

2.4.2. Fourier transform infrared spectroscopy (FTIR)

Infrared spectra of the SPI and Cyanidin-3-*O*-glucoside-SPI mixtures were recorded using a Bruker FTIR spectrophotometer (Bruker Optics, Ettlingen, Germany) according to the previous study (He, Xu, Zeng, Qin, et al., 2016). The sample solutions and the PBS (pH 7.4) blank were blended with KBr at a ratio of 1:100 (mL:g) and pressed into tablets for the measurement. Measurements were performed in the region from 400 to 4000 cm^{-1} and automatic signals gained were collected in 16 scans at a resolution of 4 cm^{-1} against a background spectrum recorded from the KBr pellet.

2.4.3. Circular dichroism spectroscopy

CD spectroscopy was measured using an MOS-450 spectropolarimeter (Bio-Logic, Claix, France) in the far-UV region (200–250 nm), at 297 K. The SPI in the absence and presence of cyanidin-3-*O*-glucoside in PBS (pH 7.4) were measured in a quartz cuvette with a path length of 0.1 cm. The values of scanning rate, spectral resolution, response, and bandwidth were set at 15 nm/min, 0.2 nm, 0.25 s and 0.5 nm, respectively.

2.4.4. Fluorescence spectroscopy

The fluorescence spectra of the SPI and Cyanidin-3-*O*-glucoside-SPI