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Conformational and processing properties of a high-active ingredient involving soy protein isolates bound with anthocyanins and its application in cake baking

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Abstract

Soybean protein is of plant origin and is commonly appropriate for improving the processing characteristics of foods. This study aimed to explore a novel functional ingredient that contained soybean protein isolate (SPI) and blueberry anthocyanins (BANs). The spatial conformation and secondary structure of SPI-BANs complexes were analyzed using circular dichroism and fluorescence spectroscopy, the processing properties were investigated as well as the retention of antioxidant activity during thermal treatments. Results showed that the contents of free sulfhydryl and free amino groups in complexes increased to 3.50 and 1.19 folds than those of SPI, respectively, while the surface hydrophobicity decreased by 74.23%. Compared with SPI, the BANs-modified SPI had a smaller particle size of 29.12 nm and a lower zeta-potential of -8.73 mV and on the other hand, the complexes possessed higher solubility (83.08%) and foaming and emulsifying properties (115.08% and 54.03 m^2/g). After fortification with SPI-BANs, the baking loss rate and adhesiveness of chiffon cake were reduced by 10.82% and improved to 0.24 N.mm, respectively. The high antioxidant activities of SPI-BANs under heat led to the cake's bioactivities largely enhanced by 1.99 ~ 12.71 folds, being 345.19 μg Trolox/g for the DPPH radical scavenging activity. This study developed the functional food ingredients as antioxidants and a substitute for animal-based proteins in bakery products, which was safe and sustainable by using the dietary components from plant resources.

Keywords Soy protein isolate, Molecular binding, Food ingredient, Chiffon cake, Antioxidant activity

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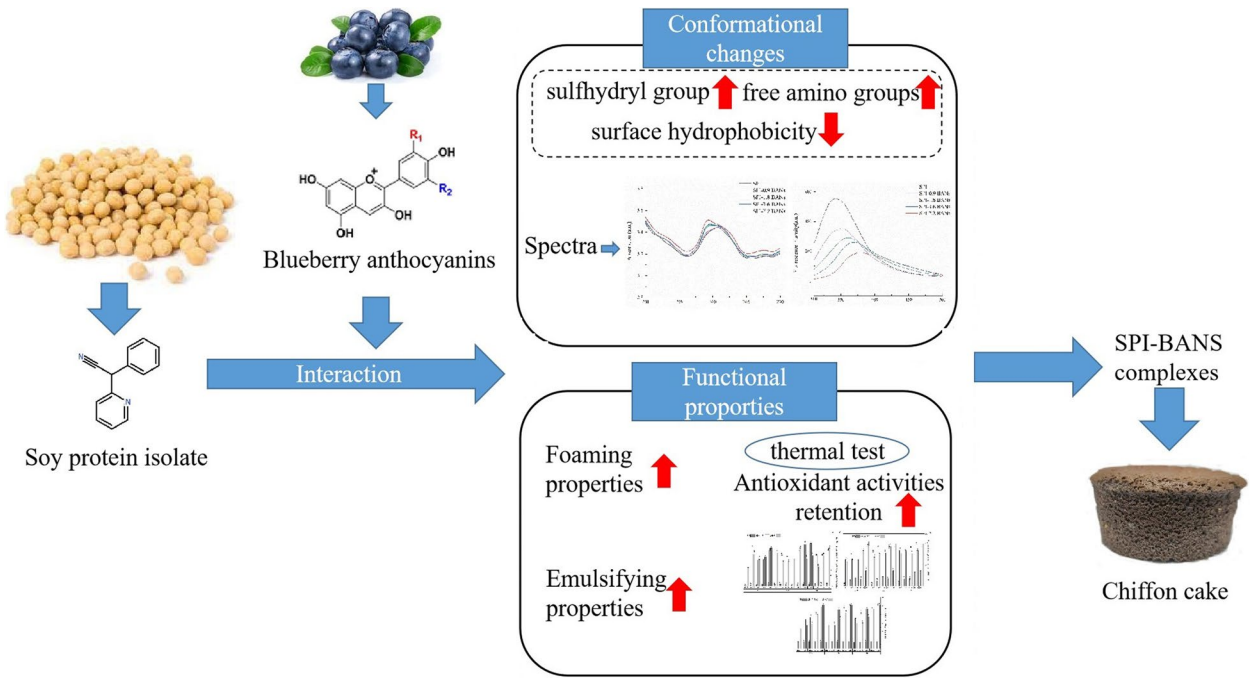
Graphical Abstract



Introduction

Soy protein isolate (SPI) is extensively used in diet and industrial production due to its great functional properties, good nutrient benefits and low cost. Although SPI has been widely used in food manufacturing, it usually has low biological activities and easily deteriorated when influenced by the processing conditions (Wang et al., 2021; Li et al., 2021). As all know, multiple studies have linked synthetic antioxidants like butylhydroxytoluene and butylated hydroxyanisole commonly applied in food processing to an increased risk of cancer and liver damage (Sun et al., 2018). Natural substances with antioxidant activity have been sought to replace synthetic antioxidants. Nowadays, there is an increasing interest in enhancing the antioxidant activity of proteins by combining them with antioxidant-active molecules, such as polyphenolics.

Anthocyanins are found in a wide range of natural plants and provide beneficial properties, including antioxidant and anticancer activities, and anti-inflammation, according to numerous researchers (Liu et al., 2015; Huang et al., 2018; Yao et al., 2021; Wu et al., 2022a). The use of anthocyanins in food, medicine and cosmetics has dramatically increased in the last few years (Rose et al., 2018; Jiang et al., 2020; Yao et al., 2021). The non-covalent association can be readily formed for the phenolic-protein complexation in numerous food systems through hydrophobic interaction, hydrogen bonds, electrostatic attraction, and van der Waals forces, the first two of which are recognized as the most important driving forces (Maqsood et al., 2013; Cao & Xiong, 2017).

It was found that the interaction of proteins with phenolic compounds led to the changes in conformational structure and aggregation state of macromolecules, which were related to the nutritional and biochemical characteristics of proteins. Ye et al. (2021) clarified that the soy protein isolates had increased foaming capacity and foaming stability, i.e., 28.33% and 14.22%, respectively due to their interaction with rutin. Jauregi et al. (2021) reported that the nanocomplexation of resveratrol with beta-lactoglobulin largely increased the solubility of whey protein (Jauregi et al., 2021). Moreover, Wan et al. (2014) investigated the soy protein-resveratrol complex and confirmed that it was used as an effective emulsifier with an increased emulsion's oxidative stability.

Baked products, especially chiffon cake are consumed all over the world. It has a unique and desirable texture that is attributed to the firm yet well-aerated porous structure, achieved by generating and retaining a sufficient amount of air cells in the cake batter during baking (Sahi & Alava, 2003). Traditionally, the egg white protein is used to stabilize foams, forming a porous structure

with finely distributed gas cells (Wilderjans et al., 2013). Due to the increasing awareness of balanced dietary nutrition and low-carbon lifestyle, it is meaningful to find and develop plant-based alternatives, i.e., soy protein isolates to egg protein for cake production without compromising its structure. Some previous studies have focused on the utilization of legumes (soy, lupin, black rice, etc.) as a partial egg replacement for the enhancement of texture in cake (Lin et al., 2017; Mau et al., 2017; Aslan & Bilgiçli, 2021; Liang et al., 2024). However, little research has been concerned about the bioactivity of cake products and not even a study developed plant-based protein as a multifunctional ingredient that could be conveniently applied in a wide range of baking products.

Therefore, this study brought insight into the alterations of conformation and physicochemical properties of soy protein isolates modified by blueberry anthocyanins (BANs). The different antioxidant activities of SPI-BANs complexes under thermal treatments were particularly analyzed. After being fortified with SPI-BANs, the basic, textural and actual bioactive properties of chiffon cake were also investigated. The purpose of the current study was to develop a novel kind of ingredients by molecular binding in response to the requirement of multifunctional proteins, i.e., SPI-BANs complexes, which could not only act as the thermal-stable antioxidants but also be applied as the replacement for animal-based proteins and the modifying agents for the improvement in cake's baking quality.

Materials and methods

Materials

Defatted soybean powder was acquired from Xinlong Asia Soybean Food Co., Ltd. (Longkou, China). Blueberry cultivar 'Brightwell' was supplied by Nanjing Youwei Organic Food Co., Ltd. 1-anililo-naphthalene-8-sulfonate (ANS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), O-phthaldialdehyde (OPA) and serine were obtained from Aladdin Co., Ltd (Shanghai, China). α-Amylase (S10003, 50 U/mg), porcine pepsin (S10030, potency 1:3000) and pancreatic enzyme (S10031, potency 1:4000) were from Yuanye Biotechnology Co., Ltd. (Shanghai, China). Other reagents of analytical grade were bought from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

Preparation of SPI and bans

According to the methods described in our previous study (Liu et al., 2021), the blueberry was smashed using a homogenizer and the anthocyanins in the slurry were extracted and purified by acid ethanol and AB-8 macroporous resins, respectively. The anthocyanin rich extracts were lyophilized with a purity of 19.1-fold that of the freeze-dried blueberry fruits. Moreover, the SPI was isolated according to the method of Sui et al. (2018). Defatted soy flour was dispersed in distilled water at a ratio of 1:15 (g/mL), and the pH was adjusted to 7.8 using 2 mol/L NaOH. The above solution was continuously stirred for 2 h at 25℃ and centrifuged at 10,000 *g* for 20 min at 4 °C. The pH of the supernatant was adjusted to 4.5 and then centrifuged at 10,000 *g* for 10 min at 4 °C. The precipitates were washed with deionized water and neutralized by 2 mol/L NaOH. The prepared SPI was lyophilized for future use, and the extraction efficiency and purity of SPI extracts were calculated as 42% and 91%, respectively.

Preparation of SPI-bans complexes

The SPI and BANs extracts were dispersed in citric acid buffer solutions (0.1 M) with pH values of 6.0 and 3.0, respectively. The SPI (1 mg/mL) and BANs at different concentrations of 0, 0.9, 1.8, 3.6, and 7.2 mg/mL were combined and mixed under a magnetic stirring at 25 °C for 30 min (Hei-PLATE, Heidolph, Schwabach, Germany) (Yang et al., 2020; Rosales et al., 2021). After standing overnight in the dark for the formation of SPI-BANs complexes in a sterilized container, the pH of the composite solution was measured as 5.5 by a pH meter (testo 206-ph2, testo AG, Berlin, Germany). The complexes prepared were accordingly denoted as SPI, SPI-BANs-1, SPI-BANs-2, SPI-BANs-3, and SPI-BANs-4. The determination of anthocyanins binding ratio was then performed according to the method by Sui et al. (2018). The prepared SPI-BANs complex particles dispersion was dialyzed in a dialysis bag with a molecular weight cut-off of 8 ~ 10 kDa to remove the free and unbound anthocyanins. The binding ratios of BANs complexed with soybean protein isolates were calculated to be 67.29 ~ 73.52%.

Preparation of chiffon cake

The chiffon cake composed of batter and foam was made according to the procedure reported by Mau et al. (2017) and data of sensory evaluation in our preliminary single factor experiments. The batter cake contained low-gluten flour (65 g), sucrose (10 g), maize oil (40 g), egg yolk (75 g) and milk (30 g), and the foam part contained egg white (135 g) and sucrose (50 g). Due to the protein content in the egg white at approximately 10% (Abeyrathne et al., 2013), SPI (10 mg/mL) was incubated with blueberry anthocyanins (18 mg/mL) in a flask in the dark for 12 h at 25 °C and the SPI-BANs complexes were used to substitute 20% of egg white, by weight ratio (the percentage had been previously optimized).

Determination of sulfhydryl (SH) group content

The free SH group content of SPI-BANs complexes was determined as indicated by Meng and Li (2021). A 40 mg of lyophilized samples were dissolved in 4 mL of Tris-Glycine buffer (0.086 mol/L Tris, 0.09 mol/L Gly, 4 mmol/L Na_2EDTA, pH 8.0), and the mixture was centrifuged at 10,000 g for 20 min at 4 °C. Afterward, the supernatant was mixed with 30 μL of Ellman's reagent solution. The absorbance was read at 412 nm, and then, the content of free SH group was calculated using the following equation:

$$SH\ (\mu\text{mol}/g) = (73.53 \times A_{412})/\ C \quad (1)$$

where 73.53 emanated from $10^6/(1.36\times10^4)$ and $1.36\times10^4\ M^{-1}cm^{-1}$ was the molar absorption coefficient; A_{412} was the absorbance measured at 412 nm (1 cm path length of cuvette); and C represented the protein concentration of samples (mg/mL).

Determination of free amino group content

The content of free amino groups was examined following the method of Wu et al. (2018). A 200 μL of solution prepared with SPI-BANs lyophilized complexes (10 mg/mL) was added with OPA reagent (4 mL) and the mixture was kept in the dark for 2 min at 35 °C. The absorbance at 340 nm was measured, and the free amino content was estimated using the L-serine standard curve.

Determination of surface hydrophobicity

The surface hydrophobicity was measured by the method reported by Ge et al. (2021). A 1 mL of SPI-BANs solution prepared using the lyophilizates of complexes (1 mg/mL) was kept in the dark for 15 min after adding 10 μL 1-anililo-naphthalene-8-sulfonate solution (ANS, 8 mmol/L). The relative fluorescence intensity was examined by a Synergy Neo2 microplate reader (BioTek, Vermont, USA) with $\lambda_{ex}=390$ nm and $\lambda_{em}=468$ nm. The surface hydrophobicity was then determined from the initial slope of linear regression analysis in the plot of intensity against protein concentration.

Determination of particle size and zeta-potential

The particle size and zeta-potential of SPI-BANs complexes were determined by a laser diffraction instrument (Zetasizer Nano ZSE, Malvern Instrument Co., Ltd., Worcestershire, UK). The refractive indexes for protein molecules and dispersion medium were 1.46 and 1.33, respectively (Chen et al., 2016).

Circular dichroism (CD) measurement

The CD spectra were determined for lyophilized complexes (10 mg/mL) at wavelengths ranging from 190 nm to 250 nm at 25 °C by a CD spectropolarimeter (Jasco 1500, Jasco Corp., Tokyo, Japan). The scan rate and band width were set as 200 nm/min and 1.0 nm, respectively (Makori et al., 2021).

Fluorescence spectroscopy measurement

The fluorescence intensity of SPI-BANs complexes was analyzed by a fluorescence spectrometer (Hitachi F-4600, Japan). The protein concentration of lyophilized complexes should be uniformly adjusted to 0.2 mg/mL. The intrinsic fluorescence was determined at $\lambda_{ex}=280$ nm and $\lambda_{em}=300\sim500$ nm with slit widths of 5 nm at a scanning rate of 1200 nm/min (Salah & Xu, 2020).

Determination of solubility

The solubility of complexes was determined by the method of Zhong & Xiong (2020), which was calculated according to Eq. (2) as follows:

$$Solubility\ (\%) = \frac{soluble\ protein\ content\ (\text{g})}{total\ protein\ content\ (g)} \times 100\% \quad (2)$$

Determination of foaming property

The approach of Sui et al. (2018) was used to determine the foaming property of complexes, with some changes. A 10 mL of sample solution (10 mg/mL) was shaken vigorously for 1 min (10000 rpm) and the foam volume of the sample was recorded at 0 and 15 min, respectively. The foam expansion and foam stability were calculated using the equations:

$$Foam\ expansion\ (\%) = \frac{\text{volume at 0 min}}{\text{initial volume}} \times 100\% \quad (3)$$

$$Foam\ stability\ (\%) = \frac{\text{volume at 15 min}}{initial\ volume} \times 100\% \quad (4)$$

Determination of emulsifying property

According to Meng and Li (2021), the complexes were used to examine the emulsifying properties including emulsifying activity index (EAI) and emulsion stability index (ESI). The mixture of peanut oil and sample (1 mg/mL) was at a ratio of 1:3 (V/V) and homogenized for 1 min (10000 rpm) by a disperser (UltraTurrax T25, IKA Labortechnik, Germany). A 50 μL of each emulsion was pipetted out at 0 min and 30 min and added into 5 mL of 1 mg/mL sodium dodecyl sulfate (SDS). The absorbance was measured at 500 nm and the indexes were calculated following the formulas:

$$\mathrm{EAI}/(\mathrm{m}^2/g) = 2 \times 2.303 \times \frac{\mathrm{A}_0 \times \mathrm{N}}{\mathrm{C} \times \mathrm{V} \times 1000} \quad (5)$$

$$\mathrm{ESI}/\min = \frac{\mathrm{A}_0}{\mathrm{A}_0\text{-}\mathrm{A}_{30}} \times 30 \quad (6)$$

where A_0 and A_{30} were the absorbances of dispersion at 0 and 30 min, respectively; N indicated the dilution factor; V denoted the oil volume fraction; and C denoted the protein concentration (g/mL).

Determination of antioxidant activity

The methods of measuring the DPPH free radical scavenging ability, ferric reducing antioxidant power (FRAP) and reducing power were referred to Wu et al. 2019, 2022b with slight modifications. Based on the results of the tests above, we chose the BANs with proper concentration (1.8 mg/mL) to interact with SPI (1 mg/mL) for the investigation of complexes' antioxidant activity at different treating temperatures (70, 85 and 100 °C) for different durations (30, 60, 90 and 120 min) in a BHS-4 constant-temperature water bath (Huzhou, Zhejiang, China). As for cake samples, they were air-dried at 60 °C and ground to pass a 0.5 mm mesh sieve. A 2.5 g of powder was extracted with 25 mL of 75% ethanol at 35 °C for 2 h and then extracted by ultrasound for 30 min. After centrifugation, the obtained supernatant was also used to determine the antioxidant activity.

Determination of textural property

According to Pasukamonset et al. (2018), the moisture content and baking loss of cakes were first determined, and the texture profile analysis (TPA) of cakes was performed using TMS-Touch System (Food Technology Co., Sterling, VA, USA) with a 36 mm diameter cylindrical probe, 50% compression and a test speed of 120 mm/min. The TPA was evaluated when the cakes were cooled to room temperature after 24 h of storage. The sample dimensions H × W × D = 20 mm × 50 mm × 50 mm were extracted from the center of each cake avoiding large air voids and analyzed immediately to prevent excess moisture loss. The measurement of the chiffon cake was shown as follows:

$$\textit{Baking loss rate}\ (\%) = \frac{\mathrm{W}_{\mathrm{cb}}\text{-}\mathrm{W}_{\mathrm{c}}}{\mathrm{W}_{\mathrm{cb}}} \times 100 \quad (7)$$

where W_{cb} was the weight of the cake batter before baking, and W_c was the weight of the cake after baking.

Statistical analysis

All the data were expressed as mean ± standard deviation (SD) of three independent experiments. Origin 2019 (OriginLab Corp., USA) was applied to plot the figures. One-way analysis of variance (ANOVA) and Duncan's multiple comparison tests were used to determine the significant differences between means ($P < 0.05$) by using IBM SPSS Version 21.0 (SPSS Inc., USA).

Results and discussion

Conformational analysis of complexes

Free sulfhydryl content, free amino group content and surface hydrophobicity

The free sulfhydryl (SH) groups in proteins play an important role in several chemical reactions in food systems due to their high chemical activity and contribute to protein polymerization by generating disulfide bonds. In Table 1, the content of free SH groups of proteins increased gradually from 21.61 to 75.69 μmol/g, increasing by 2.5-fold with augmenting BANs concentrations ($P < 0.05$). Zhang et al. (2010) had a similar result that the free SH groups of gluten protein became more after being treated with tannic acid. This phenomenon might be associated with polyphenols' reducing capability of impeding the conversion of SH groups to form the S-S bonds. On the other side, these results were also explained by the exposure of free SH groups that previously buried inside the PSI as the result of the BANs-induced conformational change of the protein. However, there were other studies stating that the free SH content decreased due to the reaction between phenolic compounds and SH groups (Cao et al., 2016). This discrepancy could be attributed to the different kinds of polyphenols and pH conditions used in various studies. As reported, the decrement of SH groups and formation of S-S bonds are two of the significant markers of protein oxidation (Cao & Xiong, 2015). The SH groups are susceptible to free radicals and are readily converted to intra- and intermolecular S-S bonds (Jongberg et al., 2011). Moreover, the type of SH groups is vulnerable to phenolic compounds, which were reported able to protect against protein oxidation by preventing the conversion of SH to S-S (Cheng et al., 2020). The plant

Table 1 Molecular interactions between SPI and BANs

Samples	Free sulfhydryl content (μmol/g)	Free amino group content (μg/mL)	Surface hydrophobicity
SPI	21.61 ± 0.25^{e}	199.42 ± 5.65^{c}	3637.5 ± 16.26^{a}
SPI-BANs-1	27.27 ± 0.15^{d}	202.91 ± 3.93^{c}	1949.67 ± 31.11^{b}
SPI-BANs-2	38.61 ± 0.27^{c}	221.93 ± 2.12^{b}	1246.85 ± 56.63^{c}
SPI-BANs-3	57.27 ± 0.41^{b}	221.45 ± 5.41^{b}	1092.95 ± 107.12^{cd}
SPI-BANs-4	75.69 ± 0.15^{a}	236.92 ± 0.75^{a}	937.34 ± 57.38^{d}

Means with different letters within a column differed significantly ($P < 0.05$). SPI, SPI-BANs-1, SPI-BANs-2, SPI-BANs-3, and SPI-BANs-4 represented the soybean protein isolates (1 mg/mL) complexed with blueberry anthocyanins at different concentrations of 0, 0.9, 1.8, 3.6, and 7.2 mg/mL, respectively

polyphenols also affect the functional capability of proteins as well as the texture of final protein-based products (Cao & Xiong, 2017).

The free amino group content in SPI was increased from 199.4 to 236.9 μg/mL with increasing BANs levels ($P < 0.05$) (Table 1), mainly due to the conformational changes of protein that led to the exposure of previously buried free amino groups upon molecular binding. On the contrary, some studies found that the hydroxyl groups of phenolics were quite reactive, consequently interacting with the free amino groups and reducing the groups' content (Sui et al., 2018; Meng & Li, 2021). In this study, the increased content of free amino groups could primarily be caused by the changes in the spatial structure of the protein.

The number of hydrophobic groups in macromolecular proteins is generally measured using surface hydrophobicity, which influences the protein interfacial behavior, such as foaming to stabilize air bubbles (Jiang et al., 2018). As the BANs concentration increased from 0.9 to 7.2 mg/mL, the surface hydrophobicity of SPI decreased from 1949.67 to 937.34 ($P < 0.05$) (Table 1). Consistently, it was demonstrated that after phenolic chemical modification, the casein, whey and soy proteins turned out to be dramatically less hydrophobic (Jiang et al., 2018; Meng & Li, 2021). These results could be explained by a shift in protein structure by the non-covalent interaction between BANs and hydrophobic amino acids of SPI, which exposed certain buried hydrophilic areas. Ge et al. (2021) also indicated that the unfolding of proteins buried the hydrophobic amino acids. Moreover, the decrease in surface hydrophobicity might be resulted from the competition of BANs and fluorescence probe ANS for binding sites on the surface of SPI that subsequently weakened the binding of ANS with SPI.

Particle size and zeta-potential

In Fig. 1, the particle size of SPI-BANs complexes ranged from 23.96 to 29.12 nm, becoming smaller than that of SPI (61.75 nm) with increasing concentrations of BANs. The absolute zeta-potential value of complexes increased accordingly from −3.61 to -8.73 mV ($P < 0.05$). This occurrence corroborated well with the particle size results, suggesting that the increase in negative charge provided a stronger interparticle repulsion that helped to keep the particles away from each other, thus resulting in better uniform protein dispersions and smaller particle sizes (Huang et al., 2017). Consistently, Ju et al. (2020) showed that the complexation between SPI and anthocyanins (ACN) led to an increase in the absolute zeta potential of SPI-ACN nanoparticles. As reported by Wei et al. (2015), the above results might also be attributed to the fact that the isoelectric points of proteins were lowered by the molecular complexation with polyphenols.

Circular dichroism, fluorescence spectroscopy

The secondary structures of SPI-BANs complexes were probed by CD spectroscopy (Fig. 2A). The binding with BANs at concentrations of 1.8 ~ 7.2 mg/mL increased the β-sheet contents of SPI from 59.11 to 85.23%, however, and decreased the α-helix and random coil contents from 8.28% to 24.38% to be 0.13% and 15.40%, respectively. These results were consistent with those reported by Liu et al. (2016), who showed that after interaction between lactoferrin and chlorogenic acid or (-)-epigallocatechin-3-gallate, the β-sheet content increased and α-helix decreased. According to Zhong & Xiong (2020) and Yong et al. (2006), the β-sheets were stabilized by interchain hydrogen bonds between polypeptide chains and the increased β-sheet content indicated that BANs caused the exposure of hydrophilic groups inside the protein, decreasing the hydrophobicity and altering its conformation. The results above were consistent with our results of surface hydrophobicity.

The functional groups of tryptophanyl and tyrosyl residues were linked to sample fluorescence stimulated at 280 nm, making a sensitive indication of protein structural changes (Sahu et al., 2008). As shown in Fig. 2B, the fluorescence intensity of the complex fell considerably from 556.75 to 187.42 a.u., indicating that the molecules, i.e., SPI and BANs, interacted and the observed decrease was due to protein's quenching caused by anthocyanins (Attaribo et al., 2020). Moreover, as the BANs concentration increased, the maximum emission wavelength of SPI changed from 342 nm to 377 nm with bathochromic (red) shifts maximum to be 35 nm, which could be explained based on the exposure of previously buried hydrophilic domains near the aromatic amino acids, i.e., tyrosine (Tyr) and tryptophan (Trp) upon BANs-induced conformational changes in the SPI molecules (Fei et al., 2014). Hence, the spatial structure of SPI could be altered and be unfolded by the addition of BANs.

Physicochemical characteristics of complexes

Solubility, foaming and emulsifying properties

The solubility of complexes was shown augmented with the increase of BANs concentration in Fig. 3A. Similarly, Zhong & Xiong (2020) reported that the solubility of mung bean globulin was significantly increased due to the interaction of globulins with phenolic compounds. When the BANs concentration was of 0.9 ~ 7.2 mg/mL, the solubility of SPI-BANs complex was improved to arrive at 1.49 ~ 2.75 folds that of SPI, varying from 45.01 to 83.08% ($P < 0.05$). Therefore, some processing properties of SPI might be improved due to the interaction

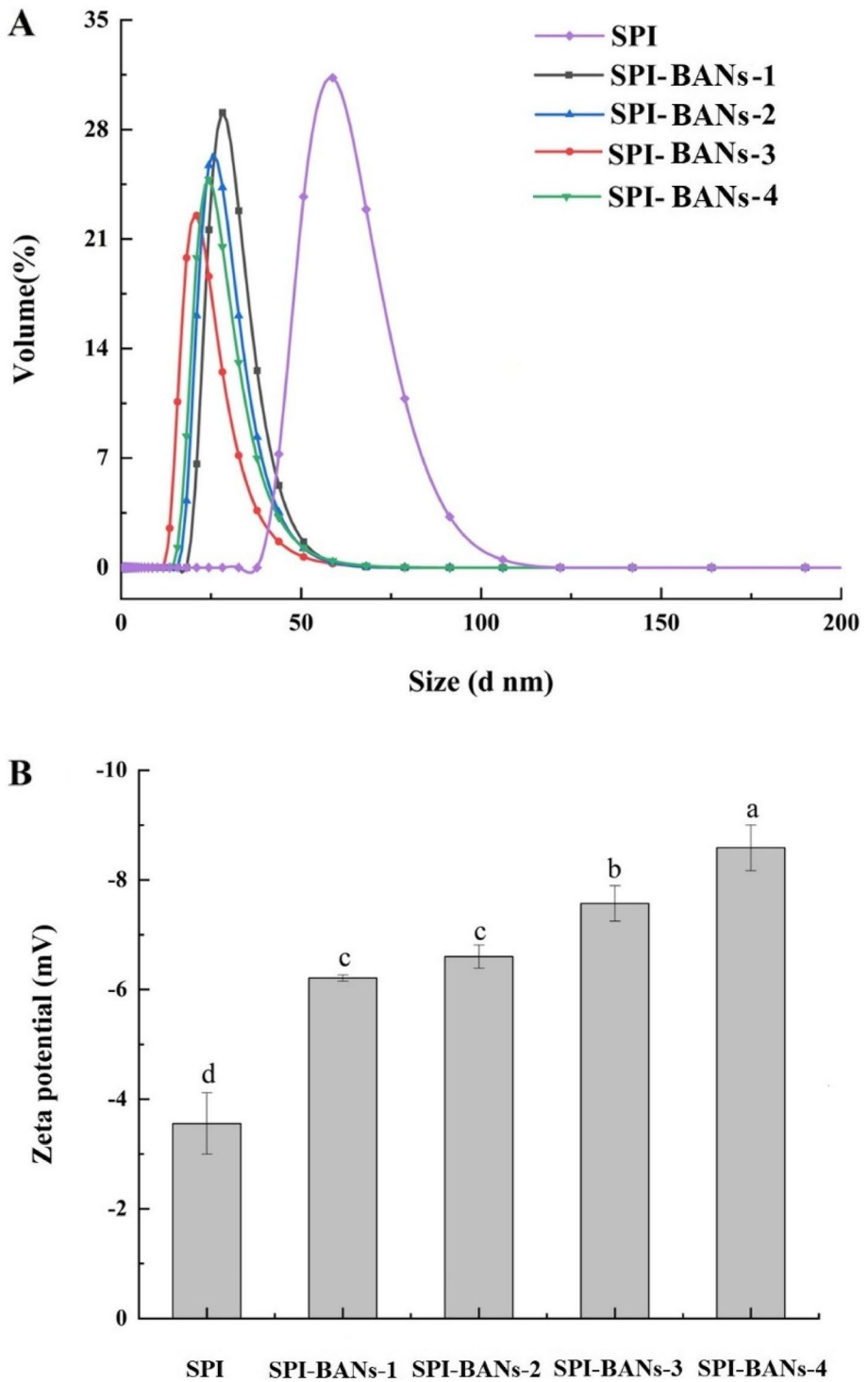


Fig. 1 The particle size (**A**) and absolute zeta-potential value (**B**) of SPI-BANs complexes. Different letters indicated the significant differences among different samples ($P < 0.05$). SPI, SPI-BANs-1, SPI-BANs-2, SPI-BANs-3, and SPI-BANs-4 represented the soybean protein isolates (1 mg/mL) complexed with blueberry anthocyanins at different concentrations of 0, 0.9, 1.8, 3.6, and 7.2 mg/mL, respectively

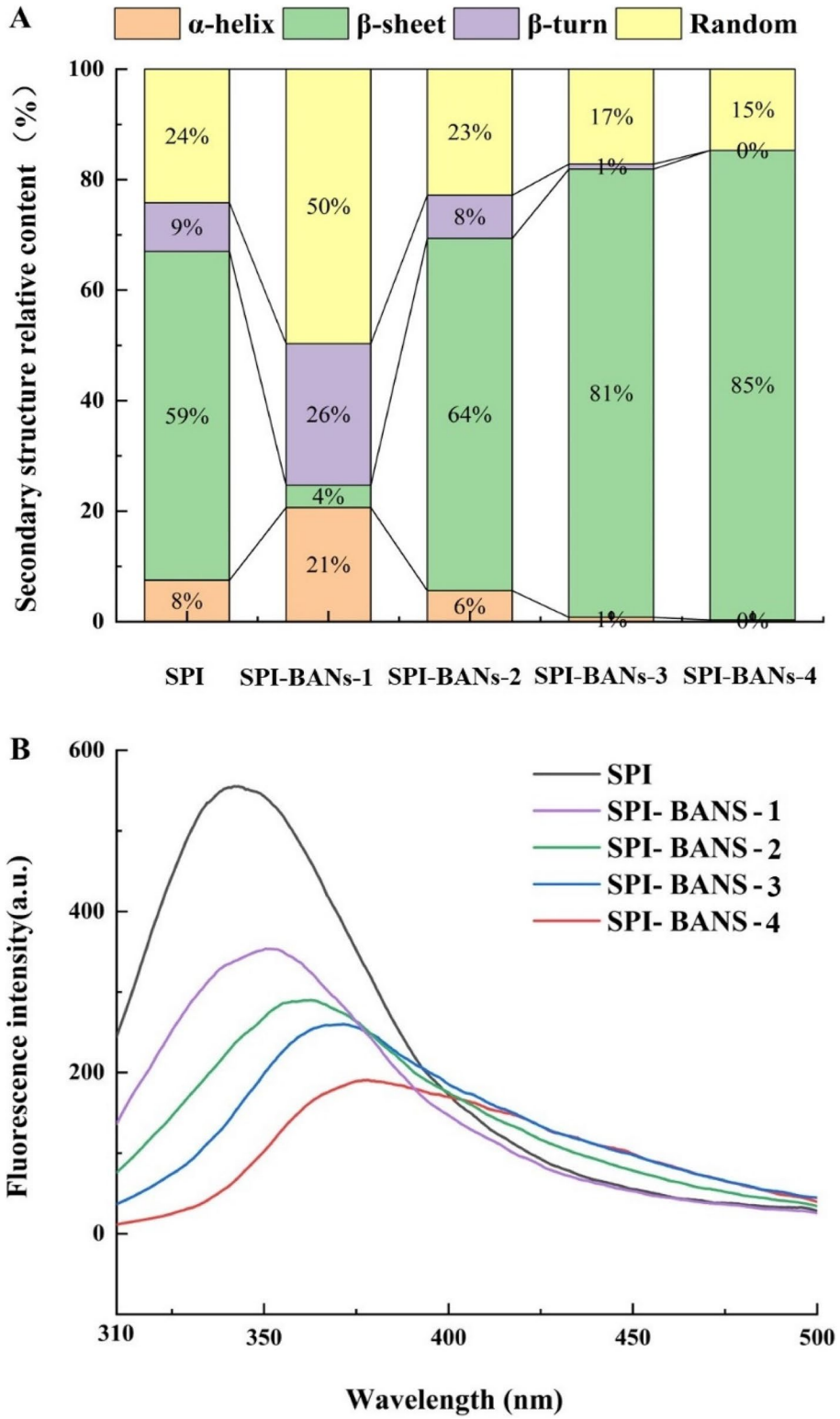


Fig. 2 The secondary structure (**A**) and fluorescence spectra (**B**) of SPI-BANs complexes. SPI, SPI-BANs-1, SPI-BANs-2, SPI-BANs-3, and SPI-BANs-4 represented the soybean protein isolates (1 mg/mL) complexed with blueberry anthocyanins at different concentrations of 0, 0.9, 1.8, 3.6, and 7.2 mg/mL, respectively

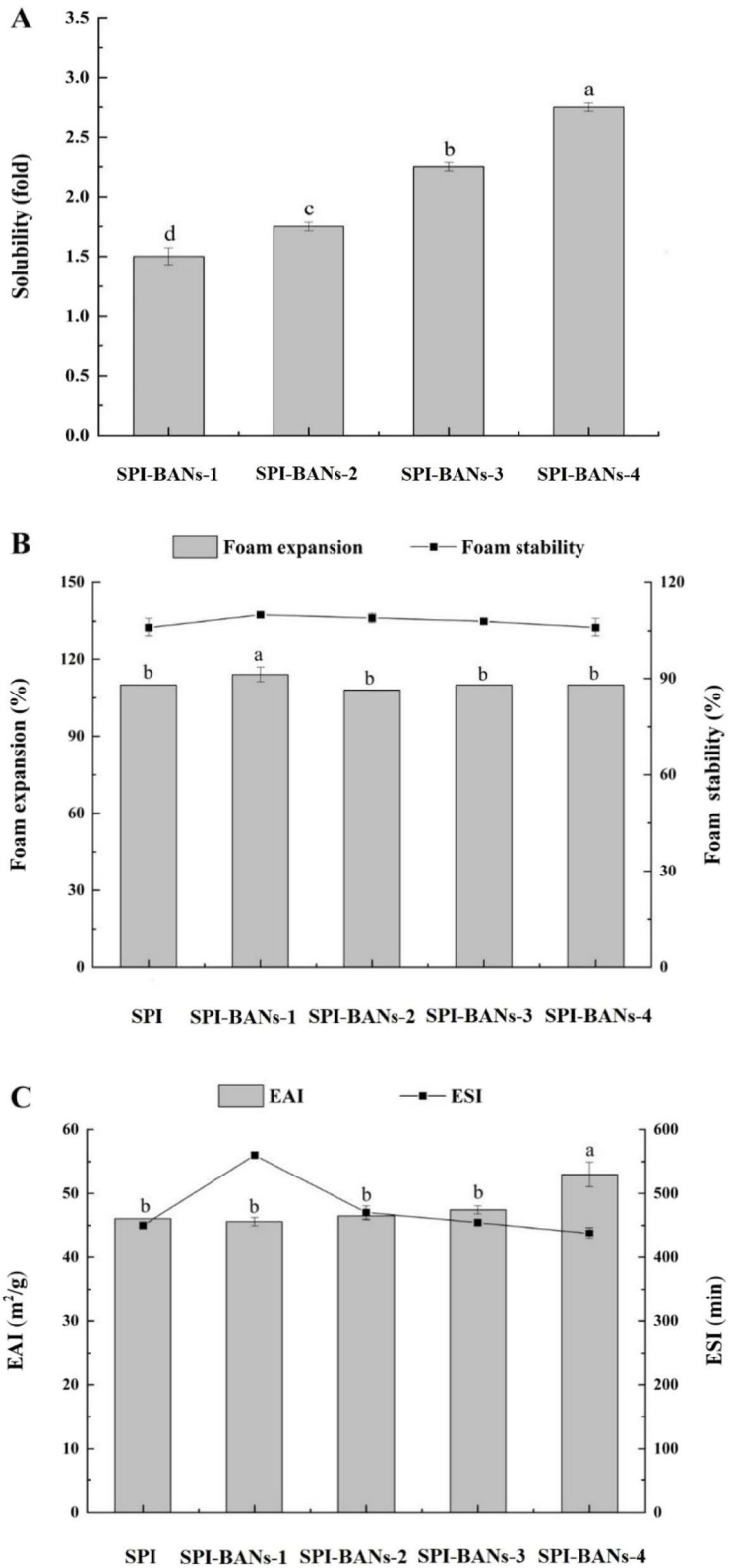


Fig. 3 The solubility (**A**), foam expansion (FE) and foam stability (FS) (**B**), emulsifying activity index (EAI) and emulsion stability index (ESI) (**C**) of SPI-BANs complexes. Different letters indicated the significant differences among different samples ($P < 0.05$). SPI, SPI-BANs-1, SPI-BANs-2, SPI-BANs-3, and SPI-BANs-4 represented the soybean protein isolates (1 mg/mL) complexed with blueberry anthocyanins at different concentrations of 0, 0.9, 1.8, 3.6, and 7.2 mg/mL, respectively

between soy protein and BANs, because the solubility was closely related to the protein's interfacial function (Yan et al., 2020).

As for the protein foaming property, it is caused by the rapid diffusion of partially unfolded proteins at the air-water interface, which reduces the surface tension of air bubbles (Wu et al., 2010). The foam expansion (FE) and foam stability (FS) of SPI-BANs complexes were shown in Fig. 3B. Compared with SPI, the protein ingredients complexed with BANs (0.9 mg/mL) had significantly higher FE and FS values, respectively being 115.08% and 109.71%, which were 4.55% and 4.49% more than those of SPI (Ps < 0.05). It was indicated that the protein-phenolic molecular interaction was capable of effectively increasing the expansion and stability of protein film. Similarly, Stubenrauch et al. (2017) suggested that hydrogen bonds between neighbouring molecules at the surface enhanced their foam stability. The presence of active hydroxyl or carboxyl groups also resulted in more films with a certain mechanical strength (Ye et al., 2021).

Additionally, the emulsifying activity and emulsion stability are two important indices for evaluating the emulsifying properties of food proteins. The EAI and ESI values of SPI-BANs complexes were presented in Fig. 3C. The addition of BANs to SPI could improve the protein's EAI and ESI values, changing from 46.11 to 54.03 m^2/g and from 447.21 to 552.09 min, respectively (Ps < 0.05). These results followed the trend of the zeta-potential as demonstrated above. The binding of BANs to SPI led to the surface charge of protein to increase its emulsification (Sui et al., 2018). The polyphenols induced a multilayer adsorptive film on the surface of proteins, which enhanced the protein's ability to reduce oil-water interfacial tension and then increased the EAI (Zhong & Xiong, 2020).

Retention of antioxidant activity of complexes

Although anthocyanins are known as strong antioxidants, their antioxidant activity diminishes as they degrade in the course of heating and digestion. The different assays in this study were carried out to investigate the antioxidant activity of SPI-BANs complexes. As shown in Fig. 4A, the DPPH radical scavenging ability of single BANs before processing was at higher levels, being 2.71 and 2.50 folds those of the complexes and single SPI, respectively (Ps < 0.05). This result was consistent with those reported by Guo et al. (2020) and Yan et al. (2020) that the polyphenols combined with proteins to provide the proteins some biological activities. Interestingly, a prominent lower DPPH radical scavenging activity of SPI-BANs complexes was shown compared to BANs, because the anthocyanins' activity was impaired due to its non-covalent interaction with SPI. It was also illustrated that the antioxidant activity of BANs decreased gradually with time during the heating process. However, the DPPH radical scavenging abilities of complexes fell from 37.59 to 34.24%, 32.79% and 25.46%, respectively at the heating temperatures of 70 ℃, 85 ℃ and 100 ℃ for 120 min (Ps < 0.05). The retention rates of the above radical scavenging abilities of complexes were 67.73%~91.09%, higher than those of BANs (58.94%~76.13%) and SPI (7.86%~29.98).

According to Shahidi and Zhong (2015), a variety of assays that monitor the antioxidant activity can be explained by different mechanisms, including hydrogen atom transfer (HAT), single electron transfer (ET), reducing power, and metal chelation, among others. The other activities, i.e., ferric reducing antioxidant power (FRAP) and reducing power of SPI-BANs complexes were indicated to possess similar tendencies to DPPH radical scavenging ability (Fig. 4B and C). The assay reaction of FRAP involves the reduction of Fe^{3+}—TPTZ to Fe^{2+}—TPTZ through single-electron transfer (SET) with an antioxidant compound (Dragsted et al., 2004). This is a colorimetric assay expressing the result as micromolar Fe^{2+} equivalents or relative to an antioxidant standard (Antolovich et al., 2002). The assay of reducing power is based on the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form in the presence of reductants (antioxidants) (Lin et al., 2006). The Fe^{2+} is monitored by measuring the formation of Perl's Prussian blue at 700 nm. The hydrogen-donating ability of antioxidants may also contribute to an increase in the reducing activity (Yang et al., 2000).

As for native SPI, the inclusion of their short peptides and carboxy terminal aromatic tyrosine residues was most likely responsible for its radical scavenging ability (Wang & Wang, 2015). Both the amino groups of peptides and the hydroxyl groups of BANs with antioxidant capacity might be covered when the SPI-BANs complexes were generated through molecular interactions (hydrogen bond/hydrophobic interaction), reducing the antioxidant activity of complexes (Yin et al., 2020). Furthermore, the heating process affected the binding of hydrophobic substances by causing conformational changes in proteins and exposing previously concealed hydrophobic regions (Kulmyrzaev et al., 2005). As for the FRAP, after the heat treatments at different temperatures, i.e., 70 ℃, 85 ℃, and 100 ℃ for 120 min, the SPI-BANs complexes performed significantly better activities than the single BANs, which were 242.21, 395.67, and 405.11 μmol Fe^{2+}/mL, respectively (Fig. 4B). The FRAP of anthocyanin-rich extracts could be enhanced by 26.17%~47.94% due to the

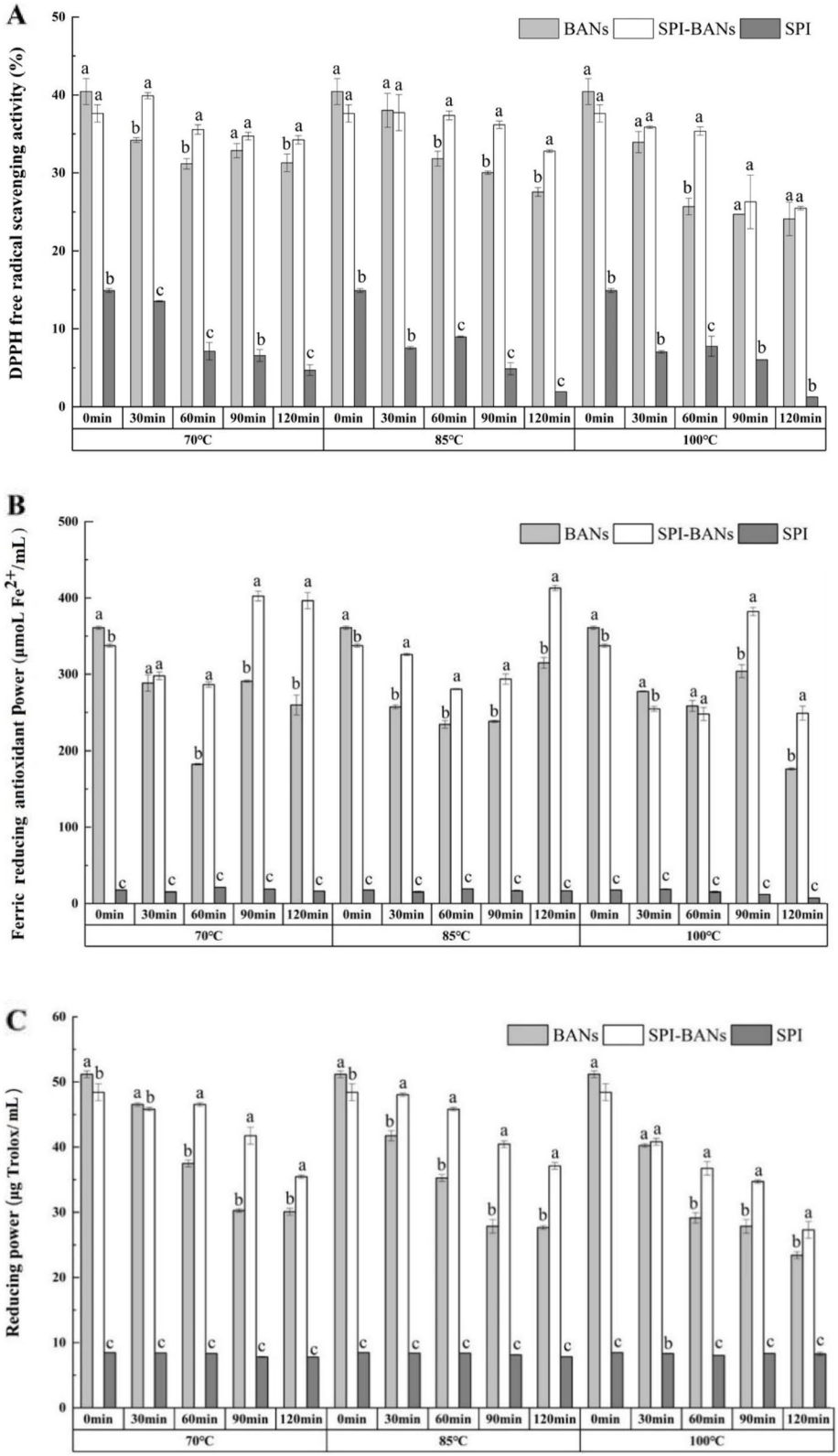


Fig. 4 The remained DPPH free radical scavenging activity (**A**), ferric reducing antioxidant power (FRAP) (**B**) and reducing power (**C**) of SPI-BANs complexes. Different letters indicated the significant differences among different samples ($P < 0.05$). SPI and SPI-BANs represented the soybean protein isolates (1 mg/mL) complexed with blueberry anthocyanins at concentrations of 0 and 1.8 mg/mL, respectively; BANs represented blueberry anthocyanins (1.8 mg/mL)

Table 2 Basic and textural properties of different cakes

	Moisture (%)	Baking loss rate (%)	Hardness (*N*)	Adhesiveness (*N*.mm)	Cohesiveness	Springiness	Gumminess (*N*)	Chewiness (mj)
C-Control	35.28 ± 0.24^{b}	30.14 ± 0.81^{a}	8.67 ± 0.71^{a}	0.21 ± 0.02^{b}	0.63 ± 0.01^{a}	14.98 ± 0.31^{a}	5.66 ± 0.42^{a}	84.95 ± 7.77^{a}
C-SPI	35.70 ± 0.59^{b}	29.25 ± 0.30^{a}	4.71 ± 0.41^{b}	0.28 ± 0.03^{a}	0.65 ± 0.01^{a}	12.39 ± 1.11^{b}	3.16 ± 0.26^{b}	39.06 ± 0.61^{b}
C-SPI-BANs	39.25 ± 0.21^{a}	26.88 ± 1.23^{b}	5.12 ± 0.29^{b}	0.24 ± 0.01^{a}	0.64 ± 0.01^{a}	11.89 ± 0.55^{b}	3.42 ± 0.20^{b}	40.70 ± 3.19^{b}

Means with different letters within each column represented the significant difference ($P < 0.05$). C-Control represented the chiffon cake without soybean protein isolates nor blueberry anthocyanins; C-SPI and C-SPI-BANs represented the chiffon cakes with soybean protein isolates and those complexed with blueberry anthocyanins as partial replacements for egg white, respectively

molecular binding with SPI. It was interesting to note that the FRAP of BANs seemed to increase after prolonged exposure during 90 ~ 120 min to the relatively low temperatures compared to the 60 min incubation. This phenomenon could be explained by the dissociation of anthocyanins with other molecules conjugated, particularly the polysaccharides and lipids in the BANs extracts that also performed the FRAP. However, a disadvantage of FRAP assay lies in that it cannot detect species that act by radical quenching, i.e., the SH group-contained antioxidants like glutathione and proteins (Dragsted et al., 2004; Antolovich et al., 2002). As displayed in Fig. 4C, the variation tendencies of reducing power were in accordance with those of DPPH radical scavenging activity. The reducing power of single BANs and SPI-BANs complexes decreased gradually with heating time, arriving at 23.50 ~ 30.02 and 28.25 ~ 37.67 μmol/mL after 120 min, respectively. Compared with BANs (46.60%~58.25%), the retention rates of composite particles for this activity were at higher levels ranging from 57.73 to 76.29%. These results demonstrated that the loss of different antioxidant activities for SPI-BANs complexes could be avoided during the thermal processing and were expected to be used as protein-based functional ingredients for practical application.

Application of SPI-bans complexes in chiffon cake

Basic property

Based on the analysis of the physicochemical characteristics of the SPI-BANs complexes, soybean proteins were given much better processing properties in conjunction with blueberry anthocyanins, such as solubility, foamability and emulsibility. Meanwhile, retention rates of biological activities of the complex particles were generally enhanced under heat treatments, compared with the SPI and BANs. The novel ingredients, i.e., SPI-BANs complexes, could be therefore considered as antioxidants and additionally to substitute the animal-derived proteins in food processing, achieving to play multifunctional roles by a natural, safe and sustainable strategy.

As shown in Table 2, the moisture of chiffon cake containing BPI-BANs complexes (C-SPI-BANs) was 39.25%, which was significantly higher than that of the control without soybean proteins (C-Control) and cake containing soy protein uncoupled with anthocyanins (C-SPI), respectively (Ps < 0.05). There also existed a statistically significant difference in baking loss rate detected between the C-SPI-BANs and the other two types of cakes, that of the C-SPI-BANs being the lowest at 26.88% (Ps < 0.05). The baking loss is crucial for structural transformation in cakes and is commonly contributed by the gas that escapes during the baking process (Mau et al., 2017). These results suggested that the addition of SPI-BANs complexes could make the chiffon cake softer and its production more efficient and cost-saving.

Textural property

When the SPI-BANs complexes were added, the appearance of baked chiffon cake was observed in dark brown, considerably different from the C-Control and C-SPI in yellow (Fig. 5). Additionally, the interior part of the cake fortified with SPI-BANs complexes contained evenly distributed pores which were elliptical and large, while the control cake had thinly scattered and unevenly distributed pores. The above color change might be associated with anthocyanin pigments undergoing oxidation reaction. Moreover, the elliptical pores were probably formed upon the coalescence of the neighbor spherical batter bubbles and the large ones were generated by the increased coalescence of local air bubbles before starch gelatinization during cake baking (Pasukamonset et al., 2018).

Table 2 also indicated that the hardness and chewiness of baked cake were decreased by partial substitution of egg white with SPI-BANs complexes (Ps < 0.05). This phenomenon might be related to the great foamability of a complex that formed interfacial film at the boundary of gas cells, conferring some stability and making the cake soft. Chewiness is used to describe the energy required for a food to be masticated to the stable situation of swallow. Besides, the cohesiveness that indicates the ability of the product to stick to itself was not significantly affected by the addition of SPI and SPI-BANs complexes. These results were consistent with the findings of Mau et al.

Fig. 5 The observation of the appearance and inner core of different chiffon cakes. C-Control represented the chiffon cake without soybean protein isolates nor blueberry anthocyanins; C-SPI and C-SPI-BANs represented the chiffon cakes with soybean protein isolates and those complexed with blueberry anthocyanins as partial replacements for egg white, respectively

(2017) that the cake with black rice powder had a similar cohesiveness to the control. Adhesiveness is defined as the negative force area between the first and second bites by TPA system, denoting the property of attraction or sticking together between surfaces of materials. In this study, the adhesiveness of SPI- and SPI-BANs-added cakes was determined to be more adhesive than the control. Springiness can quantify the elasticity of material by detecting the recovery extent between the first and second compressions, and the gumminess is calculated by hardness multiplied by cohesiveness. These two indexes of baked cake containing SPI-BANs complexes were both at lower levels than those of the control (Ps < 0.05), indicating that the fortified product might become softer and fluffier to better satisfy the customers' demand for the chiffon cake.

Antioxidant activity

As presented in Fig. 6, the DPPH radical scavenging activity, ferric reducing antioxidant power, and reducing power of chiffon cakes were determined in descending order as C-SPI-BANs > C-SPI > C-Control. Detailly, the C-SPI-BANs possessed the DPPH radical scavenging activity of 345.19 μg Trolox/g, which were 12.71 and 8.07 folds more than those of the C-Control and C-SPI (Ps < 0.05), respectively. The FRAP value of C-SPI-BANs attained 275.06 μmol Fe^{2+}/g and was augmented by 4.73-~7.05-fold due to the substitution of egg white with SPI-BANs during chiffon cake manufacturing (Ps < 0.05). As for the reducing power, that of the SPI-BANs fortified cakes was 500.16 μg Trolox/g, being 2.99 and 3.73 folds those of the C-Control and C-SPI, respectively (Ps < 0.05). It was demonstrated that the SPI-BANs complexes could greatly enhance the antioxidant activity of baked chiffon cakes, in agreement with the results of heat-induced retention rates of bioactivity of complexes. Moreover, the binding of BANs with SPI helped to enhance the thermal stability of BANs compounds, and thus the DPPH radical scavenging activity, ferric reducing antioxidant power and reducing power of SPI-BANs were maintained higher than those of BANs (Fig. 4). Mau et al. (2017) also found that the addition of black rice powder involving phenolic compounds (3.74 mg gallic acid equivalence/g) exerted an increase in antioxidant activity of chiffon cakes. Therefore, as a natural food ingredient, the soybean protein isolate combined with blueberry anthocyanins could be expected to have multifunctional performances, i.e., better foamability, better solubility and better antioxidant activity, and could be further applied to develop the chiffon cake to be a functional food with high acceptance by consumers in a more low-carbon and environmentally-friendly way.

Conclusion

In the present study, it was demonstrated that the physicochemical properties of soybean protein isolates were improved upon the combination with anthocyanins, including the solubility, foaming and emulsifying properties and antioxidant activity, due to the alterations in the conformational structures of proteins. The molecular binding also led to a better thermal stability of anthocyanins, which provided the SPI-BANs complexes high retention rate of bioactivity during the heating process. The fortification of SPI-BANs complexes in the chiffon cake reduced the dosage of egg white, decreased the baking loss rate and improved the textural property. On the other hand, the baked cake possessed a considerably enlarged bioactivity that was generated from the high antioxidant complex particles. The overall results indicated that the soybean protein isolates could be developed to be a green, sustainable and functional

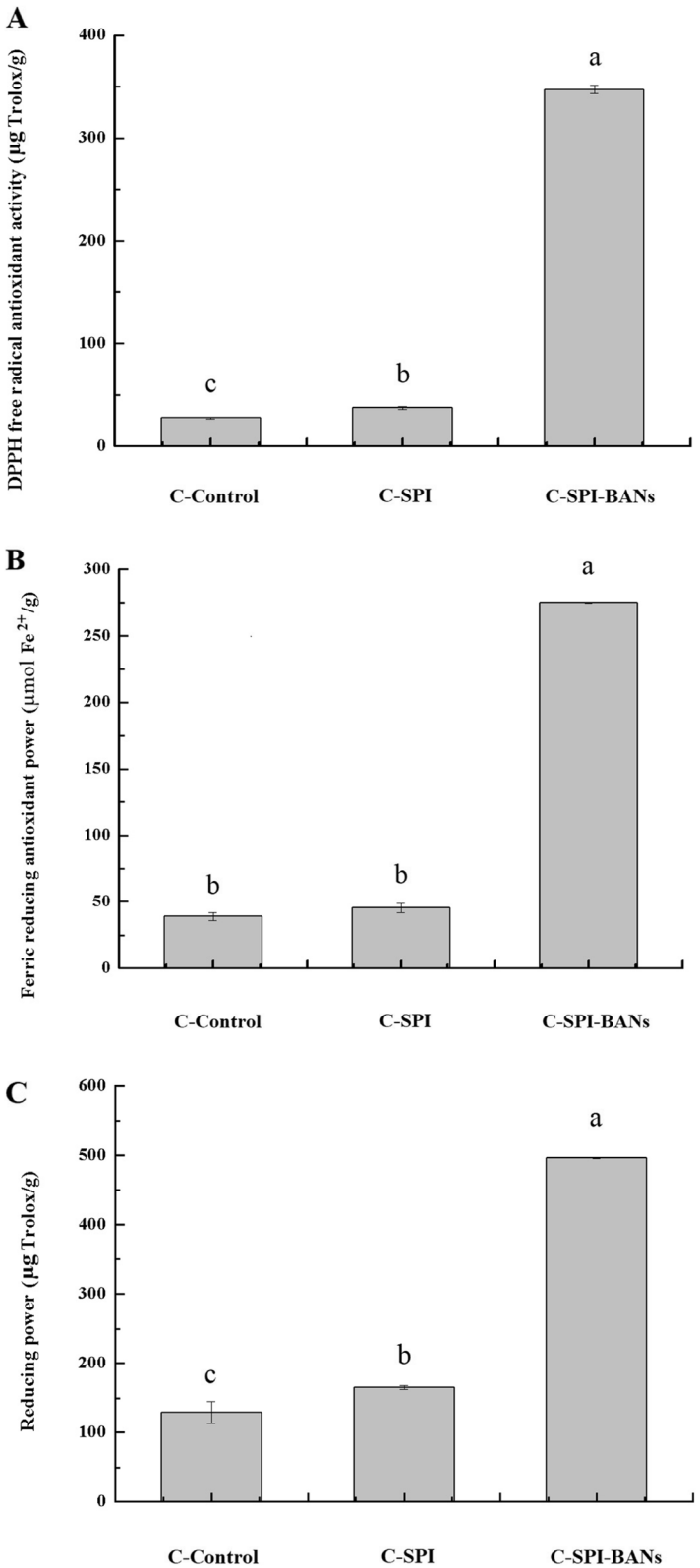


Fig. 6 The DPPH free radical scavenging activity (**A**), ferric reducing antioxidant power (FRAP) (**B**) and reducing power (**C**) of extracts of different chiffon cakes. Different letters indicated the significant differences among different samples ($P < 0.05$). C-Control represented the chiffon cake without soybean protein isolates nor blueberry anthocyanins; C-SPI and C-SPI-BANs represented the chiffon cakes with soybean protein isolates and those complexed with blueberry anthocyanins as partial replacements for egg white, respectively

protein-based ingredient by interacting with blueberry anthocyanins. This novel ingredient was multifunctional and thermal stable for the application in the baking industry, not only replacing animal proteins to some extent but also increasing the added value of cake products to better meet the requirements of customers in modern society.

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Authors' contributions
Conceptualization: H. Wu, J. Z. Zhou; Data curation: X. L. Liu, L. Zhong; Formal analysis: H. Wu, M. Corradini; Funding acquisition: H. Wu, J. Z. Zhou, X. L. Liu; Investigation: H. Wu, B. Li; Methodology: L. Zhong; Project administration: J. Z. Zhou; Resources: B. Li, X. L. Liu; Software: M. Corradini; Supervision: H. Wu, X. L. Liu; Validation: H. Wu; Visualization: L. Zhong; Writing—original draft: H. Wu; Writing—review & editing: H. Wu, X. L. Liu.

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Data availability
The data and materials are available if necessary.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

Abeyrathne, E. D. N. S., Lee, H. Y., & Ahn, D. U. (2013). Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents—A review. *Poultry Science,96*, 3292–3299. https://doi.org/10.3382/ps.2013-03391

Antolovich, M., Prenzler, P. D., Patsalides, E., McDonald, S., & Robards, K. (2002). Methods for testing antioxidant activity. *The Analyst,127*, 183–198. https://doi.org/10.1039/b009171p

Aslan, M., & Bilgiçli, N. (2021). Improvement of functional cake formulation with fermented soyu (*Glycine max*) and lupin (*Lupinus albus* L) powders. *International Journal of Gastronomy and Food Science,26*, 100429. https://doi.org/10.1016/j.ijgfs.2021.100429

Attaribo, T., Jiang, X., Huang, G., Zhang, B., & Gui, Z. (2020). Studies on the interactional characterization of preheated silkworm pupae protein (SPP) with anthocyanins (C3G) and their effect on anthocyanin stability. *Food Chemistry,326*, 126904. https://doi.org/10.1016/j.foodchem.2020.126904

Cao, H., Jia, X., Shi, J., Xiao, J., & Chen, X. (2016). Non-covalent interaction between dietary stilbenoids and human serum albumin: Structure–affinity relationship, and its influence on the stability, free radical scavenging activity and cell uptake of stilbenoids. *Food Chemistry,202*, 383–388. https://doi.org/10.1016/j.foodchem.2016.02.003

Cao, Y., & Xiong, Y. L. (2015). Chlorogenic acid-mediated gel formation of oxidatively stressed myofibrillar protein. *Food Chemistry,180*, 235–243. https://doi.org/10.1016/j.foodchem.2015.02.036

Cao, Y., & Xiong, Y. L. (2017). Interaction of whey proteins with phenolic derivatives under neutral and acidic pH conditions. *Journal of Food Science,82*, 409–419. https://doi.org/10.1111/1750-3841.13607

Chen, S., Zhang, N., & Tang, C. H. (2016). Influence of nanocomplexation with curcumin on emulsifying properties and emulsion oxidative stability of soy protein isolate at pH 3.0 and 7.0. *Food Hydrocolloids,61*, 102–112. https://doi.org/10.1016/j.foodhyd.2016.04.048

Cheng, J. R., Zhu, M. J., & Liu, X. M. (2020). Insight into the conformational and functional properties of myofibrillar protein modified by mulberry polyphenols. *Food Chemistry,308*, 125592. https://doi.org/10.1016/j.foodchem.2019.125592

Dragsted, L. O., Pedersen, A., Hermetter, A., Basu, S., Hansen, M., Haren, G. R., Kall, M., Breinholt, V., Castenmiller, J. J. M., Stagsted, J., Jakobsen, J., Skibsted, L., Rasmussen, S. E., Loft, S., & Sandström, B. (2004). The 6-a-day study: Effects of fruit and vegetables on markers of oxidative stress and antioxidative defense in healthy nonsmokers. *The American Journal of Clinical Nutrition,79*, 1060–1072. https://doi.org/10.1093/ajcn/79.6.1060

Fei, Q. Q., Gao, Y., Zhang, X., Sun, Y., Hu, B., Zhou, L., Jabbar, S., & Zeng, X. X. (2014). Effects of Oolong tea polyphenols, EGCG, and EGCG3″Me on pancreatic α-amylase activity *in vitro*. *Journal of Agriculture and Food Chemistry,62*, 9507–9514. https://doi.org/10.1021/jf5032907

Ge, G., Guo, W. X., Zheng, J. B., Zhao, M. M., & Sun, W. Z. (2021). Effect of interaction between tea polyphenols with soymilk protein on inactivation of soybean trypsin inhibitor. *Food Hydrocolloids,111*, 106117. https://doi.org/10.1016/j.foodhyd.2020.106177

Guo, Y., Bao, Y. H., Sun, K. F., Chang, C., & Liu, W. F. (2020). Effects of covalent interactions and gel characteristics on soy protein-tannic acid conjugates prepared under alkaline conditions. *Food Hydrocolloids,112*, 106293. https://doi.org/10.1016/j.foodhyd.2020.106293

Huang, W. Y., Wu, H., Li, D. J., Song, J. F., Xiao, Y. D., Liu, C. Q., Zhou, J. Z., & Sui, Z. Q. (2018). Protective effects of blueberry anthocyanins against H_2O_2-induced oxidative injuries in human retinal pigment epithelial cells. *Journal of Agricultural and Food Chemistry,66*, 1638–1648. https://doi.org/10.1021/acs.jafc.7b06135

Huang, Y., Li, A., Qiu, C., Teng, Y., & Wang, Y. (2017). Self-assembled colloidal complexes of polyphenol-gelatin and their stabilizing effects on emulsions. *Food and Function,8*, 3145–3154. https://doi.org/10.1039/C7FO00705A

Jauregi, P., Guo, Y., & Adeloye, J. (2021). Whey proteins-polyphenols interactions can be exploited to reduce astringency or increase solubility and stability of bioactives in foods. *Food Research International,141*, 110019. https://doi.org/10.1016/j.foodres.2020.110019

Jiang, J., Zhang, Z., Zhao, J., & Liu, Y. (2018). The effect of non-covalent interaction of chlorogenic acid with whey protein and casein on physicochemical and radical scavenging activity of in vitro protein digests. *Food Chemistry,268*, 334–341. https://doi.org/10.1016/j.foodchem.2018.06.015

Jiang, T., Shuai, X., Li, J., Yang, N., Deng, L., Li, S., He, Y., Guo, H., Li, Y., & He, J. (2020). Protein-bound anthocyanin compounds of purple sweet potato ameliorate hyperglycemia by regulating hepatic glucose metabolism in high-fat diet/streptozotocin-induced diabetic mice. *Journal of Agricultural and Food Chemistry,68*, 1596–1608. https://doi.org/10.1021/acs.jafc.9b06916

Jongberg, S., Gislason, N. E., Lund, M. N., Skibsted, L. H., & Waterhouse, A. L. (2011). Thiol-quinone adduct formation in myofibrillar proteins detected by LC-MS. *Journal of Agricultural and Food Chemistry,59*, 6900–6905. https://doi.org/10.1021/jf200965s

Ju, M. N., Zhu, G., Huang, G., Shen, X. C., Zhang, Y., Jiang, L. Z., & Sui, X. N. (2020). A novel pickering emulsion produced using soy protein-anthocyanin

complex nanoparticles. *Food Hydrocolloids,99*, 105329. https://doi.org/10.1016/j.foodhyd.2019.105329

Kulmyrzaev, A., Levieux, D., & Dufour, É. (2005). Front-face fluorescence spectroscopy allows the characterization of mild heat treatments applied to milk. Relations with the denaturation of milk proteins. *Journal of Agricultural and Food Chemistry,53*, 502–507. https://doi.org/10.1021/jf049224h

Li, C., Dai, T., Chen, J., Li, X., & Mcclements, D. J. (2021). Protein-polyphenol functional ingredients: The foaming properties of lactoferrin are enhanced by forming complexes with procyanidin. *Food Chemistry,339*, 128145. https://doi.org/10.1016/j.foodchem.2020.128145

Liang, G. J., Chen, W. P., Zhang, X. M., Zeng, M. M., Qin, F., He, Z. Y., Goff, H. D., Chen, J., & Wang, Z. J. (2024). Incorporating soy protein hydrolysate and temperature-induced gelling polysaccharide as partial egg replacements for enhanced texture in sponge cake. *Food Bioscience,57*, 103574. https://doi.org/10.1016/j.fbio.2024.103574

Lin, C. H., Wei, Y. T., & Chou, C. C. (2006). Enhanced antioxidative activity of soybean koji prepared with various filamentous fungi. *Food Microbiology,23*, 628–633. https://doi.org/10.1016/j.fm.2005.12.004

Lin, M. Y., Tay, S. H., Yang, H. S., Yang, B., & Li, H. L. (2017). Replacement of eggs with soybean protein isolates and polysaccharides to prepare yellow cakes suitable for vegetarians. *Food Chemistry,229*, 663–673. https://doi.org/10.1016/j.foodchem.2017.02.132

Liu, F. G., Wang, D., Sun, C., Mcclements, D. J., & Gao, Y. (2016). Utilization of interfacial engineering to improve physicochemical stability of β-carotene emulsions: Multilayer coatings formed using protein and protein–polyphenol conjugates. *Food Chemistry,205*, 129–139. https://doi.org/10.1016/j.foodchem.2016.02.155

Liu, H. N., Wu, H., Wang, Y., Wang, F., Liu, X. L., & Zhou, J. Z. (2021). Enhancement on antioxidant and antibacterial activities of Brightwell blueberry by extraction and purification. *Applied Biological Chemistry,64*, 78. https://doi.org/10.1186/s13765-021-00649-8

Liu, Y. X., Zhang, D., Hu, J. M., Liu, G. M., Chen, J., Sun, L. C., Jiang, Z. D., Zhang, X. C., Chen, Q. C., & Ji, B. P. (2015). Visible light-induced lipid peroxidation of unsaturated fatty acids in the retina and the inhibitory effects of blueberry polyphenols. *Journal of Agricultural and Food Chemistry,63*, 9295–9305. https://doi.org/10.1021/acs.jafc.5b04341

Makori, S. I., Mu, T. H., & Sun, H. N. (2021). Physicochemical properties, antioxidant activities, and binding behavior of 3,5-di-O-caffeoylquinic acid with beta-lactoglobulin colloidal particles. *Food Chemistry,347*, 129084. https://doi.org/10.1016/j.foodchem.2021.129084

Maqsood, S., Benjakul, S., & Shahidi, F. (2013). Emerging role of phenolic compounds as natural food additives in fish and fish products. *Critical Reviews in Food Science and Nutrition,53*, 162–179. https://doi.org/10.1080/10408398.2010.518775

Mau, J. L., Lee, C. C., Chen, Y. P., & Lin, S. D. (2017). Physicochemical, antioxidant and sensory characteristics of chiffon cake prepared with black rice as replacement for wheat flour. *LWT-Food Science and Technology,75*, 434–439. https://doi.org/10.1016/j.lwt.2016.09.019

Meng, Y., & Li, C. (2021). Conformational changes and functional properties of whey protein isolate-polyphenol complexes formed by non-covalent interaction. *Food Chemistry,364*, 129622. https://doi.org/10.1016/j.foodchem.2021.129622

Pasukamonset, P., Pumalee, T., Sanguansuk, N., Chumyen, C., Wongvasu, P., & Adisakwattana, S. (2018). Physicochemical, antioxidant and sensory characteristics of sponge cakes fortified with clitoria ternatea extract. *Journal of Food Science and Technology,55*, 2881–2889. https://doi.org/10.1007/s13197-018-3204-0

Rosales, T. K. O., da Silva, M. P., Lourenço, F. R., Hassimotto, N. M. A., & Fabi, H. P. (2021). Nanoencapsulation of anthocyanins from blackberry (*Rubus* spp.) through pectin and lysozyme self-assembling. *Food Hydrocolloids,114*, 106563. https://doi.org/10.1016/j.foodhyd.2020.106563

Rose, P. M., Cantrill, V., Benohoud, M., Tidder, A., Rayner, C. M., & Blackburn, R. S. (2018). Application of anthocyanins from blackcurrant (*Ribes nigrum* L.) fruit waste as renewable hair dyes. *Journal of Agricultural and Food Chemistry,66*, 6790–6798. https://doi.org/10.1021/acs.jafc.8b01044

Sahi, S. S., & Alava, J. M. (2003). Functionality of emulsifiers in sponge cake production. *Journal of the Science of Food and Agriculture,83*, 1419–1429. https://doi.org/10.1002/jsfa.1557

Sahu, A., Kasoju, N., & Bora, U. (2008). Fluorescence study of the curcumin-casein micelle complexation and its application as a drug nanocarrier to cancer cells. *Biomacromolecules,9*, 2905–2912. https://doi.org/10.1021/bm800683f

Salah, M., & Xu, X. Y. (2020). Anthocyanin-β-lactoglobulin nanoparticles in acidic media: Synthesis, characterization and interaction study. *Journal of Molecular Structure,1232*, 129995. https://doi.org/10.1016/j.molstruc.2021.129995

Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of Functional Foods,18*, 757–781. https://doi.org/10.1016/j.jff.2015.01.047

Stubenrauch, C., Hamann, M., Preisig, N., Chauhan, V., & Bordes, R. (2017). On how hydrogen bonds affect foam stability. *Advances in Colloid and Interface Science,247*, 435–443. https://doi.org/10.1016/j.cis.2017.02.002

Sui, X., Sun, H., Qi, B., Zhang, M., Li, Y., & Jiang, L. (2018). Functional and conformational changes to soy proteins accompanying anthocyanins: Focus on covalent and non-covalent interactions. *Food Chemistry,245*, 871–878. https://doi.org/10.1016/j.foodchem.2017.11.090

Sun, H. N., Mu, B., Song, Z., Ma, Z., & Mu, T. H. (2018). The in vitro antioxidant activity and inhibition of intracellular reactive oxygen species of sweet potato leaf polyphenols. *Oxidative Medicine and Cellular Longevity,2018*, 9017828. https://doi.org/10.1155/2018/9017828

Wan, Z. L., Wang, J. M., Wang, L. Y., Yuan, Y., & Yang, X. Q. (2014). Complexation of resveratrol with soy protein and its improvement on oxidative stability of corn oil/water emulsions. *Food Chemistry,161*, 324–331. https://doi.org/10.1016/j.foodchem.2014.04.028

Wang, N., Zhou, X., Wang, W., Wang, L., Jiang, L., & Liu, T. (2021). Effect of high intensity ultrasound on the structure and solubility of soy protein isolate-pectin complex. *Ultrasonics Sonochemistry,80*, 105808. https://doi.org/10.1016/j.ultsonch.2021.105808

Wang, Y. F., & Wang, X. Y. (2015). Binding, stability, and antioxidant activity of quercetin with soy protein isolate particles. *Food Chemistry,188*, 24–29. https://doi.org/10.1016/j.foodchem.2015.04.127

Wei, Z., Yang, W., Fan, R., Yuan, F., & Gao, Y. (2015). Evaluation of structural and functional properties of protein-EGCG complexes and their ability of stabilizing a model beta-carotene emulsion. *Food Hydrocolloids,45*, 337–350. https://doi.org/10.1016/j.foodhyd.2014.12.008

Wilderjans, E., Luyts, A., Brijs, K., & Delcour, J. A. (2013). Ingredient functionality in batter type cake making. *Trends in Food Science and Technology,30*, 6–15. https://doi.org/10.1016/j.tifs.2013.01.001

Wu, H., Chai, Z., Hutabarat, R. P., Zeng, Q., Niu, L., Li, D. J., Yu, H., & Huang, W. Y. (2019). Blueberry leaves from 73 different cultivars in southeastern China as nutraceutical supplements rich in antioxidants. *Food Research International,122*, 548–560. https://doi.org/10.1016/j.foodres.2019.05.015

Wu, H., Di, Q. R., Zhong, L., Zhou, J. Z., Shan, C. J., Liu, X. L., & Ma, A. M. (2022a). Enhancement on antioxidant, anti-hyperglycemic and antibacterial activities of blackberry anthocyanins by processes optimization involving extraction and purification. *Frontiers in Nutrition,9*, 1007691. https://doi.org/10.3389/fnut.2022.1007691

Wu, H., Liu, H. N., Ma, A. M., Zhou, J. Z., & Xia, X. D. (2022b). Synergetic effects of Lactobacillus plantarum and Rhizopus oryzae on physicochemical, nutritional and antioxidant properties of whole-grain oats (Avena sativa L.) during solid-state fermentation. *LWT-Food Science and Technology,154*, 112687. https://doi.org/10.1016/j.lwt.2021.112687

Wu, H., Rui, X., Li, W., Xiao, Y., Zhou, J. Z., & Dong, M. S. (2018). Whole-grain oats (*Avena sativa* L.) as a carrier of lactic acid bacteria and a supplement rich in angiotensin I-converting enzyme inhibitory peptides through solid-state fermentation. *Food and Function,9*, 227. https://doi.org/10.1039/c7fo01578j

Wu, W., Clifford, M., & Howell, N. K. (2010). The effect of instant green tea on the foaming and rheological properties of egg albumen proteins. *Journal the Science of Food and Agriculture,87*, 1810–1819. https://doi.org/10.1002/jsfa.2809

Yan, S. H., Xu, J. W., Zhang, X. Y., Xie, F. Y., Zhang, S., Jiang, L. Z., Qi, B. K., & Li, Y. (2020). Effect of pH-shifting treatment on the structural and functional properties of soybean protein isolate and its interactions with (-)-epigallocatechin-3-gallate. *Process Biochemistry,101*, 190–198. https://doi.org/10.1016/j.procbio.2020.10.016

Yang, J. H., Mau, J. L., Ko, P. T., & Huang, L. C. (2000). Antioxidant properties of fermented soybean broth. *Food Chemistry,71*, 249–254. https://doi.org/10.1016/S0308-8146(00)00165-5

Yang, Y. X., Wang, Q. M., Lei, L., Li, F. H., Zhao, J. C., Zhang, Y. H., Li, L., Wang, Q., & Ming, J. (2020). Molecular interaction of soybean glycinin and β-conglycinin with (-)-epigallocatechin gallate induced by pH changes.

Food Hydrocolloids,108, 106010. https://doi.org/10.1016/j.foodhyd.2020.106010

Yao, L., Xu, J., Zhang, L., Liu, L., & Zhang, L. (2021). Nanoencapsulation of anthocyanin by an amphiphilic peptide for stability enhancement. *Food Hydrocolloids,118*, 106741. https://doi.org/10.1016/j.foodhyd.2021.106741

Ye, J., Deng, L., Wang, Y., Mcclements, D. J., & Liu, C. (2021). Impact of rutin on the foaming properties of soybean protein: Formation and characterization of flavonoid-protein complexes. *Food Chemistry,362*, 130238. https://doi.org/10.1016/j.foodchem.2021.130238

Yin, Z., Wu, Y., Chen, Y., Qie, X., & He, Z. (2020). Analysis of the interaction between cyanidin-3-o-glucoside and casein hydrolysates and its effect on the antioxidant ability of the complexes. *Food Chemistry,340*, 127915. https://doi.org/10.1016/j.foodchem.2020.127915

Yong, Y. H., Yamaguchi, S., & Matsumura, Y. (2006). Effects of enzymatic deamidation by protein-glutaminase on structure and functional properties of wheat gluten. *Journal of Agricultural and Food Chemistry,54*, 6034–6040. https://doi.org/10.1021/jf060344u

Zhang, L., Cheng, L., Jiang, L., Wang, Y., Yang, G., & He, G. (2010). Effects of tannic acid on gluten protein structure, dough properties and bread quality of Chinese wheat. *Journal the Science of Food and Agriculture,90*, 2462–2468. https://doi.org/10.1002/jsfa.4107

Zhong, Z., & Xiong, Y. L. (2020). Thermosonication-induced structural changes and solution properties of mung bean protein. *Ultrasoncs-Sonochemistry,62*, 104908. https://doi.org/10.1016/j.ultsonch.2019.104908

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