The Effect of Glycosylated Soy Protein Isolate on the Stability of Lutein and Their Interaction Characteristics

Xia Wang 1, Shaojia Wang *1, Duoxia Xu 1, Jingwei Peng 2, Wei Gao *2 and Yanping Cao *1

1 Beijing Advanced Innovation Center for Food Nutrition and Human Health (BTBU), School of Food and Health, Beijing Higher Institution Engineering Research Center of Food Additives and Ingredients, Beijing Technology and Business University (BTBU), Beijing, China, 2 Chenguang Biotech Group Co., Ltd., Handan, China

Lutein is a natural fat-soluble carotenoid with various physiological functions. However, its poor water solubility and stability restrict its application in functional foods. The present study sought to analyze the stability and interaction mechanism of the complex glycosylated soy protein isolate (SPI) prepared using SPI and inulin-type fructans and lutein. The results showed that glycosylation reduced the fluorescence intensity and surface hydrophobicity of SPI but improved the emulsification process and solubility. Fluorescence intensity and ultraviolet–visible (UV–Vis) absorption spectroscopy results showed that the fluorescence quenching of the glycosylated soybean protein isolate by lutein was static. Through thermodynamic parameter analysis, it was found that lutein and glycosylated SPI were bound spontaneously through hydrophobic interaction, and the binding stoichiometry was 1:1. The X-ray diffraction analysis results showed that lutein existed in the glycosylated soybean protein isolate in an amorphous form. The Fourier transform infrared spectroscopy analysis results revealed that lutein had no effect on the secondary structure of glycosylated soy protein isolate. Meanwhile, the combination of lutein and glycosylated SPI improved the water solubility of lutein and the stability of light and heat.

Keywords: soy protein isolate, inulin-type fructans, lutein, stability, interaction

INTRODUCTION

Lutein, an oxygen-containing fat-soluble carotenoid, is widely distributed in flowers, vegetables (1), fruits, egg yolks (2), algae, grains (3), etc. and is mainly derived from marigolds (4). Lutein is also present in the macular pigment of human eyes and plays an indispensable role in preventing eye diseases, such as age-related macular degeneration (AMD) (5). However, it cannot be synthesized within the human body and should be supplied from external sources, such as food (6). Lutein possesses a carbon skeleton and a polyolefin chain (7), a major chromophore group (8). The carbon chain is supported by hydroxyl-containing ionone rings on each side with three stereocenters, β-ionone ring containing a stereo center (3R), and ε-ionone ring containing two stereocenters (3 R and 6 R) (9). The eight conjugated olefins in the carbon chain of the lutein molecule make lutein susceptible to degradation by the external environment (10).
The encapsulation systems, including liposomes, emulsions, gels, and molecular complexes, are often used to inhibit the degradation and improve the stability of biologically active substances, such as lutein (11). Of them, the macromolecules’ cavity (such as protein) has attracted increasing interest in the encapsulation of biologically active substances. It is reported that egg white albumin improves the stability of marigold lutein ester extract during storage, and lutein dipalmitate binds spontaneously to egg protein through van der Waals forces and hydrogen bonding (12). Yi et al. (13) found that the stability of lutein increased with the increase of milk protein content, the protective effect of sodium caseinate (SC) on lutein was stronger than the whey protein isolate (WPI), and the milk protein interacted with lutein through hydrophobic bond.

Soy protein isolate (SPI) is a plant protein (about 90% protein) with high nutritional value and is widely used in the food industry (14, 15). It is mainly composed of β-conglycinin (7S, 180–210 kDa) and glycamin (11S, approximately 360 kDa) and accounts for about 70% of the total protein. The 11S component consists of 6 acidic and 6 basic polypeptide chains linked together by disulfide bonds, whereas the 7S is stabilized by hydrophobic interactions (16). In recent years, SPI has been used as a carrier in food-grade delivery systems to improve the stability of biologically active substances. Cao et al. (17) found that SPI could improve the thermal stability of chlorophyll. Tapal and Tiku (18) improved water solubility and bioavailability of curcumin using SPI. Wan et al. (19) also showed that SPI could be used as a carrier of resveratrol (RES) in functional foods, which could improve the water solubility and stability of RES. However, the functional properties of SPI, such as solubility in neutral and mildly acidic environments, severely limit its use as an encapsulation wall material. As such, the physical, chemical, and enzymatic properties of SPI are often modified (16). Physical modification mainly relies on high temperature and high pressure or shearing, making the equipment requirements relatively high and large-scale production difficult. Enzymatic modification is expensive, and protein hydrolysis can make the taste worse. Chemical modification has a high effect on the functional properties of the protein, mainly by introducing food-grade ingredients (20). Glycosylation is a typical chemical modification, which modifies the structure of the protein and improves the functional properties of the protein through a covalent bond between the amino groups of protein and the carbonyl group of the reducing sugar (21). The glycosylation of glucan and protein improves the freeze–thaw stability of SPI hydrolysate (SPIH) emulsion (22), the binding and transporting ability of casein phosphopeptide to calcium (23), the solubility, emulsifying, and foaming ability of peanut protein isolate (24). The emulsifying properties and emulsifying stability of the conjugates of inulin and WPI were significantly higher than the WPI at a pH of 3–7 (25). Inulin-type fructans (ITFs) are linear fructose polymers with predominantly or only β-(2→1) fructosyl–fructose linkages (26). Stabilizing the intestinal mucosal barrier, reducing the risk of colon cancer, promoting calcium absorption, and improving constipation are the physiological functions of ITFs (27–33). ITFs are widely used as a prebiotic food ingredient (34). However, the modification effects of ITFs on SPI and ITF-modified SPI on the stability of lutein are still unknown.

Therefore, the present study aimed to study the modification effect of ITFs on SPI and ITF-modified SPI (glycosylated soybean protein isolate, GSPI) on the stability of lutein and the interaction between GSPI and lutein. The study results will provide insights into the preparation of protein-encapsulating systems for embedding lutein and other similar biologically active compounds.

MATERIALS AND METHODS

Materials

Soy protein isolate was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). ITFs and lutein extract were provided by Chenguang Biotech Group Co., Ltd. (Hebei, China). Phosphates were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Lutein (97%) was purchased from ChromaDex, USA. Bovine serum albumin (BSA, purity 97%) was obtained from Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China). Sodium 8-naphthalenesulfonate-1-anilino (ANS) ≥97% was purchased from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). G250 Coomassie Brilliant Blue Ultra pure grade was purchased from Beijing Boao Tuoda Science and Technology Co., Ltd.

Absolute ethanol (chromatographic grade), methyl tert-butyl ether (chromatographic grade), methanol (chromatographic grade), cyclohexane (chromatographic grade), and N-hexane (chromatographic grade) were purchased from Beijing Merida Technology Co., Ltd. (Beijing, China). Ethyl acetate (analytical grade) and potassium hydroxide (KOH) (analytical grade) were purchased from Tianjin Fuchen Chemical Reagent Company (Tianjin, China). 2, 6-Di-tert-butyl-p-cresol (BHT) (analytical grade) was purchased from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China).

Sample Preparation

Soy protein isolate and ITFs (mass ratio 1:1) were stirred overnight with 10 mM Na2HPO4 to make them fully hydrated and adjusted to pH 11 with 0.1 M NaOH to make the final protein concentration of 2% (w/v). The SPI/ITF mixtures were incubated in a water bath at 100°C for 4.7 h (previous research work has confirmed that the GSPI has the highest emulsification ability under this experimental condition). HSPI was the only heated SPI (100°C for 4.7 h), and G-SPI was the physical mixture of SPI and ITFs.

Lutein extract was dissolved in absolute ethanol and stored at 4°C protecting from light. The lutein content in the lutein ethanol solution was expressed as 10 mg/ml with 97% lutein standard product.

For stable analysis of protein and lutein complexes preparation, 15 ml of GSPI and SPI were adjusted to pH 7.0, and then 0.5 ml of lutein extract in absolute ethanol was added dropwise to GSPI and SPI, respectively. Finally, the volume was adjusted to 100 ml with 10 mM pH 7.0 phosphate-buffered saline (PBS) and incubated for 1 h at room temperature.
The complexes of different concentrations of 97% lutein (10, 20, 30, 40, 50, 60, and 70 μM) and GSPI (0.2 mg/ml) were also prepared to explore the interaction between lutein and protein.

**Fourier Transform Infrared Spectroscopy**

The freeze-dried sample was mixed with KBr at a ratio of 1:100 with KBr as the background. The wave number was 400–4,000 cm\(^{-1}\), and the resolution was 4 cm\(^{-1}\) for 32 scans with IS5 infrared spectrometer (Thermo Fisher, USA).

The spectral range of 1,700–1,600 cm\(^{-1}\) (amide I) of GSPI and GSPI–lutein were subjected to Fourier deconvolution and second derivative analysis by PeakFit 4.12.

**Fluorescence Spectra**

Later, 200 μl of all the samples were transferred to 96-well plates for fluorescence spectroscopy, and the intrinsic fluorescence spectrum was measured by M200 Pro TECAN Infinite multifunctional microplate reader (Tecan Inc., Switzerland).

**Excitation Spectrum**

The fluorescence excitation spectra of SPI, HSPI, G-SPI, and GSPI were measured at 293 K. The excitation wavelength was set from 310 to 460 nm, and the excitation wavelength was 420 nm (25).

**Emission Spectrum**

The fluorescence emission spectra of SPI, HSPI, G-SPI, and GSPI were measured at 293, 303, and 313 K. The emission wavelength was set from 325 to 380 nm, and the emission wavelength was 310 nm (excitation wavelength) and 470 nm (emission wavelength) using M200 Pro TECAN Infinite multifunctional microplate reader. The slope of the protein concentration and its corresponding fluorescence intensity were \(H_0\).

**Solubility and Emulsification**

**Solubility**

The samples were centrifuged at 4,500 r/min for 15 min using an Avanti JXN-30 floor-standing centrifuge (Beckman, USA). The supernatant was diluted by 200 times, then 1 ml of the diluted solution was added with 5 ml of G250 Coomassie Brilliant Blue, mixed uniformly, and the absorbance was measured at 595 nm using an UVmini-1240 UV Spectrophotometer (Shimadzu, Japan). The samples were measured within 2–30 min. BSA was used as the standard curve to calculate the protein content.

**Emulsification**

Approximately 40 ml of GSPI and 10 ml of soybean oil were put into a 100-ml beaker, homogenized at a speed of 12,000 r/min for 2 min using T25 digital homogenizing and dispersing instrument (IKA, Germany) (36). Then, 50 μl was taken from the bottom and mixed with 5 ml of 0.1% SDS, and the absorbance values were measured at 500 nm. Afterward, 50 μl of deionized water and 5 ml of 0.1% SDS were adjusted to zero. The emulsifying activity index (EAI) was calculated by Equation (5).

\[
\text{EAI (m}^2/\text{g)} = \frac{2 \cdot 2.303 \cdot A \cdot n}{C \cdot (1 - \phi') \cdot 10^4}
\]

where, \(A\) is the absorbance value at 500 nm, \(n\) is the dilution factor, \(C\) is the protein concentration (g/ml), and \(\phi\) is the oil phase volume.

**UV–Vis Absorption Spectroscopy**

The mixtures of GSPI and lutein in 2.2 were diluted four times, namely GSPI (0.025 mg/ml) with lutein standards (2.5, 5.0, 7.5, 10, and 12.5 μM). The ultraviolet–visible (UV–Vis) spectra were measured in the wavelength range of 190–350 nm using a 10-mm quartz cell with TU-1900 Dual-Beam UV–Visible Spectrophotometer (Beijing Puxi General Instrument Co., Ltd., China.).
**X-Ray Diffraction**
The crystal of lutein, GSPI, and GSPI–lutein was measured by a D8 Advance X-ray Diffractometer (Bruker Technology Co., Ltd., Germany) at a speed of approximately 2°/min and scanning at an angle (2θ) from 5° to 40°.

**Stability of Lutein**
Thermal stability: Around 8 ml of lutein, SPI–lutein, and GSPI–lutein were added into a pressure test tube, tightened the tube mouth, and then heated in an oil bath at 120°C for 50 min. The samples were taken every 10 min.

Light stability: Lutein, SPI–lutein, and GSPI–lutein were placed in a 100-ml sample bottle, irradiated under a 302-nm UV lamp for 12 h, and the samples were taken every 2 h for analysis. (The light was perpendicular to the sample, and the distance was about 2 cm.)

**Color Measurements**
The color characteristics were measured by a CR-800 Spectrophotometer under a reflection mode (Beijing Colorimeter Instrument Equipment Co., Ltd., China). The total color difference (ΔE) was calculated by Equation (6):

\[
ΔE = \sqrt{(L^* - L_{0}^*)^2 + (a^* - a_{0}^*)^2 + (b^* - b_{0}^*)^2}
\]

where, ΔE means total color difference. \(L_{0}^*, a_{0}^*, \) and \(b_{0}^*\) mean the initial color values of the sample. \(L^*, a^*, \) and \(b^*\) mean the color values of the sample at time \(t\). \(L^*\) means light and dark; \(a^*\) means red and green, and \(b^*\) means yellow and blue.

**Content and Degradation Kinetics Analysis**
Around 5 ml of the above sample was used for stability analysis. Then, 10 ml of absolute ethanol was mixed with 10 ml of 60% KOH, then shaken at room temperature for 3h, and extracted with cyclohexane:hexane:tert-butyl ether = 1:2:2. The extraction was repeated 3 times. The extracts were combined and rotary evaporated to near dryness in a 30°C water bath, then dissolved to 10 ml using a 0.1% BHT absolute ethanol for HPLC analysis. A standard curve was plotted using 97% lutein to characterize the content changes of lutein during the degradation process.

The change in lutein content was determined by Waters 2695 HPLC (Waters Technology Co., Ltd., USA). The chromatographic column model was Venusil XBP C30 (5 μm, 250 mm × 4.6 mm). Phase A (methanol:water = 88:12), phase B (methyl tert-butyl ether). At 0–18 min, phase A changed from 100% to 10%; at 18.1 min, phase A changed from 10% to 100%, and kept it for 10 min. The flow rate was 1.0 ml/min, and the injection volume was 50 μl. The degradation rate was calculated by Equation (7), and the degradation kinetics was determined by Equations (8–10).

\[
\text{Degradation rate} = \frac{C_0 - C_t}{C_0} \times 100\%
\]

**Kinetic equations (8–10) (37)**

\[
C_0C_t = k_0t
\]

\[
\ln \left(\frac{C_t}{C_0}\right) = k_1t
\]

\[
\frac{1}{C_t} - \frac{1}{C_0} = k_2t
\]

where, \(C_0\) represents the initial content of lutein before stability analysis, and \(C_t\) represents lutein content at time \(t\) during stability analysis. \(k_0, k_1, \) and \(k_2\) represent the kinetic constants.

**Size Distribution**
The particle size of lutein, GSPI, and GSPI–lutein was measured by an S3500 laser particle size analyzer (Microtrac, Germany). The sample was added dropwise to the sample cell until the screen showed ready, and then proceeded to the particle size distribution measurement.

**Statistical Analysis**
The images were generated by Origin 2021 software. The data were analyzed by one-way analysis of variance using IBM SPSS statistics 26. The significance analysis was done by LSD and Duncan’s test (\(p < 0.05\)).

**RESULTS AND DISCUSSION**

**Effect of Glycosylation on Protein Properties**

**Fourier Transform Infrared Spectroscopy**
Fourier transform infrared (FTIR) could determine the occurrence of glycosylation reaction. The amide I bands (1,700–1,600 cm\(^{-1}\)) and amide II bands (1,600–1,500 cm\(^{-1}\)) of protein are the most sensitive regions related to protein conformation (38). The C=O stretching vibration of the peptide bond, the C–N stretching vibration of the amino group, and the N–H bending of the amino group are the characteristics of this amide zone (39). **Figure 1A** depicts the FTIR results of SPI, HSPI, ITFs, and GSPI under the range of 4,000–400 cm\(^{-1}\). The amide I region of the GSPI absorption peak shifted from 1659.09 to 1658.38 cm\(^{-1}\), and the absorption intensity was slightly reduced, which might be due to the decrease in the carbonyl content. The Schiff base and pyrazine formed during glycosylation showed an absorption at 1658.38 cm\(^{-1}\) (23). The amide II band shifted from 1536.99 to 1548.99 cm\(^{-1}\), and the absorption peak of GSPI was stronger than SPI, indicating that the glycosylation product was formed by covalent bonds. This result was consistent with the results of glycosylated modified egg white protein pretreated by ball milling (40). The absorption intensity of GSPI was higher than SPI at the wavenumber of 1057.58 cm\(^{-1}\), indicating that GSPI produces a new C–N covalent bond, which was consistent with the findings of WPI and inulin (25). Additionally, there was a
Wang et al. Glycosylated Soy Protein Isolate

**FIGURE 1** | (A) The Fourier transform infrared spectroscopy of SPI, HSPI, ITFs, and GSPI. (B) Fluorescence excitation spectroscopy of 0.1 mg/ml SPI, HSPI, G-SPI, GSPI, T = 293 K, pH = 7.0, λ\textsubscript{exc} = 280 nm, and λ\textsubscript{em} = 310–460 nm. (C) Fluorescence emission spectroscopy of 0.1 mg/ml SPI, HSPI, G-SPI, GSPI, T = 293 K, pH = 7.0, λ\textsubscript{em} = 420 nm, and λ\textsubscript{ex} = 250–380 nm. (D) Surface hydrophobicity of 0.01–0.09 mg/ml SPI, HSPI, G-SPI, GSPI, T = 293 K, pH = 7.0, λ\textsubscript{em} = 470 nm, and λ\textsubscript{ex} = 390 nm. Different letters indicate significant differences.

**Fluorescence Intensity**

Glycosylation is a chemical method for modifying proteins by covalent bonding between the proteins and sugars (40). The fluorescent substances with characteristic peaks between 340 and 370 nm are produced during a reaction (42). As depicted in **Figure 1B**, SPI, HSPI, G-SPI, and GSPI had a characteristic protein excitation wavelength at 280 nm, whereas GSPI had another maximum excitation wavelength at 351 nm. These results confirmed the occurrence of the Maillard reaction and the production of fluorescent substances. This was consistent with the previous research result showing that WPI and inulin had a maximum fluorescence excitation wavelength of 344 nm (25). **Figure 1C** depicts the fluorescence emission spectrum, indicating that the fluorescence intensity of SPI and G-SPI had not much difference. The fluorescence intensity of GSPI was less than SPI, suggesting that ITFs had a shielding effect on the fluorescence of SPI. Some previous studies had also manifested that the fluorescence intensity of glycosylated protein was less than the original protein (43), and this effect increased with the decrease of dextran molecular weight. Notably, heating increased the fluorescence intensity of the protein, and the fluorescence intensity of WPI increased by dry heat treatment (44). However, in this study, the fluorescence intensity of HSPI was less than
SPI. Therefore, it was speculated that this phenomenon was caused by extreme pH. Zhao et al. (45) revealed that extreme alkaline pH treatment of PSE-like chicken proteins had the same result.

Surface Hydrophobicity
Surface hydrophobicity is one of the essential functional properties of the protein, reflecting protein conformation and structure (46). ANS is commonly used as a probe to determine $H_0$ of proteins, which binds to the hydrophobicity of proteins efficiently (47). Figure 1D depicts the change in $H_0$ of SPI, G-SPI, HSPI, and GSPI, indicating that the physical mixture of SPI and ITFs was not significantly different from the $H_0$ of SPI. Compared with SPI, the $H_0$ of HSPI was significantly lower, which might be due to the fact that the alkaline conditions could make the protein fold and reduce the exposure of the SPI hydrophobic group. The $H_0$ decreased significantly after glycosylation and the exposed hydrophobic groups of SPI bound to ITFs, resulting in a decrease in $H_0$. In previous studies, the same results were obtained after glycosylation of SPI with Pleurotus eryngii polysaccharide (PEP) or dextran (DX) (20, 43). $H_0$ was consistent with the fluorescence emission spectroscopy results, and similar results were obtained under extremely acidic pH excursions and mild heating conditions for SPI (48).

Emulsification and Solubility
The EAI of protein is involved in several parameters, such as solubility, hydrophobicity, and structural flexibility (49). EAI is often used to indicate the emulsification of protein. As shown in Figure 2A, the EAI of SPI and GSPI were measured at different pH conditions, and the results showed that the emulsifying ability of GSPI was greater than SPI under the pH range of 5.5–7.5. Compared with GSPI, the EAI values of SPI were much more affected by pH. The poor solubility of SPI limits its application. The solubility of glycosylation significantly increased, and the protein content of GSPI diluted by centrifugation was 56.29 ± 0.96–59.30 ± 1.85 µg/ml, with no significant change in pH 5.5–7.5, whereas SPI had poor solubility in neutral and weakly acidic conditions (Figure 2B).

Interaction Between GSPI and Lutein
Fluorescence Quenching
Fluorescence quenching methods could reveal the protein conformation and/or dynamic changes in the macromolecular systems. The Stern–Volmer equation is the easiest method to determine the quenching pattern (50). Quenching occurs when the quencher is located near or in contact with the fluorophore (51). Protein fluorescence arises from the presence of multiple fluorophores, mainly including three aromatic amino acids—Phe, Tyr, and Trp (52). The changes in the amino acid environment change the fluorescence intensity. As depicted in Figure 3A, with the increase of lutein (10, 20, 30, 40, and 50 µM), the fluorescence intensity of GSPI continued to decrease, accompanied by a red shift, indicating an interaction between lutein and GSPI, which changed the microenvironment of the fluorophore (53).

Fluorescence quenching includes static quenching, dynamic quenching, and combined static and dynamic quenching. Static quenching is caused by the formation of complexes, whereas dynamic quenching is caused by intermolecular collisions. The dynamic and static combination quenching is caused by the formation of complexes and intermolecular collisions. As shown in Figure 3B and Table 1, the slope ($K_{SV}$) of 303 and 313 K was >293 K, indicating that the fluorescence quenching of GSPI was caused by the collision of lutein and GSPI, which was a sign of dynamic quenching. However, the value of $K_q$
Wang et al. Glycosylated Soy Protein Isolate

FIGURE 3 | (A) Fluorescence spectra of GSPI (0.2 mg/ml) with different concentrations of lutein (0–50 µM), T = 293 K, pH = 7.0, and λ<sub>ex</sub> = 280 nm. (B) The linear Stern–Volmer plot for lutein binding to GSPI at 293, 303, and 313 K. (C) The Stern–Volmer plot for lutein binding to GSPI with lutein (0–70 µM) at 293 K. (D) UV–Vis spectra of GSPI (0.2 mg/L) with different concentrations of lutein (0–50 µM), T = 293 K, and pH = 7.0.

far exceeded the maximum value of the dynamic quenching rate constant [10<sup>10</sup>/(mol s)], indicating that the quenching was static. Thus, it could not be concluded if it is a static or dynamic quenching.

The quenching effects of high lutein concentration on GSPI fluorescence and UV–Vis were studied to evaluate the type of quenching between GSPI and lutein. In the case of dynamic quenching, the relationship between <i>F<sub>0</sub></i>/<i>F</i> and high-lutein concentration might concave toward the Y-axis (54). Figure 3C depicts a good linear relationship between increasing lutein concentration and <i>F<sub>0</sub>/F</i> (<i>R</i><sup>2</sup> = 0.9901), with no concave to the Y-axis (55). It also confirmed that there was no dynamic quenching between lutein and GSPI.

| T (K) | <i>K</i><sub>SV</sub> (10<sup>4</sup>/mol) | <i>K</i><sub>q</sub> (10<sup>12</sup>/(mol s)) | <i>R</i><sup>2</sup> |
|-------|----------------|-----------------|---------|
| 293   | 1.3689         | 1.3689          | 0.9899  |
| 303   | 1.7122         | 1.7122          | 0.9739  |
| 313   | 1.5495         | 2.7171          | 0.9715  |

TABLE 1 | Lutein induces the Stern–Volmer quenching constant (<i>K</i><sub>SV</sub>) and bimolecular quenching rate (<i>K</i><sub>q</sub>) of GSPI at different temperatures (293, 303, and 313 K).

Ultraviolet–visible is a simple and effective method for the interaction between small molecules and proteins (56). Dynamic quenching does not change the absorption spectrum of the
fluorophore, whereas complex formation (static quenching) changes the absorption spectrum of the fluorophore (57, 58). As depicted in Figure 3D, with the addition of lutein, the absorption intensity of the ultraviolet spectrum increased, which was accompanied by a significant red shift (Δλ = 10 nm). This indicated that lutein formed a complex with GSPI (59), leading to a red shift in the spectrum (60, 61). Therefore, lutein quenched the fluorescence of GSPI by static quenching.

Binding Forces and Thermodynamic Parameters
The binding constant (K_b value) reflects the strength of the binding force (62). As depicted in Figure 4, the log ([F_0 - F]/F) and log [Q] presented a good linear relationship (R^2 > 0.98). The n-values of the three different temperatures at 293, 303, and 313 K were 0.8551, 0.9237, and 0.9861, respectively, indicating that there was only one binding site. The K_b values of lutein and GSPI interaction at 293, 303, and 313 K showed an increasing trend with the increase in temperature (Table 2), suggesting that the GSPI–lutein complex was relatively stable at higher temperatures (63).

The thermodynamic parameters, such as ΔG, ΔH, and ΔS, could reveal the GSPI–lutein interaction (64). The value and sign of ΔH and ΔS could reflect the interaction force between small molecules and proteins (65). The forces between the biologically active substances and proteins mainly include hydrophobic interaction, hydrogen bonding, van der Waals force, electrostatic attraction, etc. (66). The interaction force could be divided into four types: for ΔH > 0 and ΔS > 0, the main force is hydrophobic interaction; for ΔH > 0 and ΔS < 0, the main force is electrostatic and hydrophobic interaction; for ΔH < 0 and ΔS < 0, hydrogen bonding and van der Waals force play a major role; and for ΔH < 0 and ΔS > 0, electrostatic interaction plays a principal role (67). As summarized in Table 2, ΔH > 0 and ΔS > 0 demonstrated that the interaction force between lutein and GSPI was mainly hydrophobic interaction. Yi et al. (13) reported similar results for WPI and SC (13). Furthermore, the values of ΔG (−20.09, −22.85, and −24.91 kJ mol⁻¹) were negative at 293, 303, and 313 K, indicating spontaneous binding of lutein with GSPI.

X-Ray Diffraction
The crystal analysis of the sample was performed by XRD. Figure 5 depicts the diffraction patterns of lutein, GSPI, and GSPI–lutein. Lutein showed a strong diffraction peak within 10°–25° (20), suggesting that lutein had a highly crystalline structure. After the combination of lutein and GSPI, the frontal characteristic diffraction peak of lutein did not appear, showing the presence of lutein in GSPI in an amorphous form (68).

Secondary Structure Analysis
The combined effect of lutein on the structural changes of GSPI was studied by an FTIR. GSPI was used as a control. As described in Section 3.1.2, the amide I region was the most sensitive region related to protein conformation. The amide I region in the FTIR spectra of GSPI and GSPI–lutein was analyzed, and the secondary structural data were obtained by calculating the integrated area of each sub-peak in the 1,600–1,700 cm⁻¹ spectrum. The ratio of each secondary structure of GSPI and GSPI–lutein is depicted in Figure 6. The FTIR spectra indicated that GSPI–lutein was bound through hydrophobic interaction and had little effect on its secondary structure. These results were consistent with the previous study results reporting that SPIHs interacting with cyanin-3-O-glucoside (Cy3G) (60) and SPI (unheated and heated) combined with curcumin (69) had no significant effect on the secondary structure of the protein.

Effect of GSPI on the Stability of Lutein

Color Analysis
In addition to physiological functions, lutein is also popular as a coloring agent. Therefore, studying its color changes has high significance. The smaller the total color difference (ΔE), the more stable the lutein (70). ΔE > 3 indicated a very significant

![Figure 4](image-url)
difference, $1.5 < \Delta E < 3$ indicated a significant difference, $\Delta E < 1.5$ indicated a small difference (71). As depicted in Figure 7A, $\Delta E$ of lutein reached $11.83 \pm 0.18$ at 5 h of light exposure. At this time, the $\Delta E$ of SPI–lutein and GSPI–lutein was lower, accounting for $1.04 \pm 0.06$ and $0.41 \pm 0.74$, respectively. This result indicated that protein had a positive effect on the photostability of lutein. The difference between SPI–lutein and GSPI–lutein appeared with the prolongation of light time. When the light time was 12 h, the $\Delta E$ of SPI–lutein and GSPI–lutein was significantly different, accounting for $7.65 \pm 1.34$ and $1.91 \pm 0.18$, respectively. GSPI–lutein exhibited better light stability than lutein and SPI–lutein. However, the advantage of thermal stability was not good as light stability (Figure 7B). At 120°C oil bath for 50 min, the $\Delta E$ of lutein was $9.42 \pm 0.52$, the $\Delta E$ of SPI–lutein was $6.20 \pm 0.08$, and the $\Delta E$ of GSPI–lutein was $5.58 \pm 0.08$. Overall, GSPI had a better effect on the color stability of lutein than SPI.

Degradation Rate
Lutein is sensitive to environmental factors, such as light and heat, due to its high degree of unsaturation. Figure 7C depicts the degradation rates of lutein, SPI–lutein, and GSPI–lutein under the influence of light and heat, respectively. The degradation rate and total color difference showed the same trend. When lutein was exposed to light for 4 h, the degradation rate reached as high as $95.41\% \pm 0.34$. At this time, the degradation rates of SPI–lutein and GSPI–lutein were $15.78\% \pm 5.56$ and $9.40\% \pm 2.13$, respectively. Compared with lutein, the degradation rate of GSPI–lutein was reduced by about $86.01\%$. Lutein was completely degraded at 5 h (not shown). With the increase in light time, the degradation rate of GSPI–lutein was always lower than SPI–lutein. Thermal degradation for 10 min showed a significant protective effect of the protein, but there was no significant difference in the protective effect of SPI and GSPI. After 50 min, the degradation rate of lutein was $89.46\% \pm 0.21$, the degradation rate of SPI–lutein was $77.48\% \pm 0.94$, and the degradation rate of GSPI–lutein was $65.96\% \pm 0.82$ (Figure 7D). Overall, GSPI–lutein has excellent light stability and fine stability at high temperatures and short time. Additionally, lutein (with or without protein) followed the first-order degradation kinetics at $120^\circ$C and zero-order degradation kinetics under UV light at $25^\circ$C (Table 3).

Particle Size Analysis
The particle size affects the stability of the lutein system (32, 72). The smaller the particle size, the greater the stability in the aqueous solution. As depicted in Figure 8, the average particle
size of lutein in the aqueous solution was about 2 \( \mu \)m. The average particle sizes of SPI and SPI–lutein were about 83.34 and 84.15 \( \mu \)m, respectively. The average particle sizes of GSPI and GSPI–lutein were about 212 and 219 nm, respectively. GSPI and GSPI–lutein had lower particle sizes, indicating that they were more stable in the aqueous solutions.

**CONCLUSION**

In this study, the effects of glycosylation on the structure and stability of SPI were investigated by fluorescence spectroscopy, FTIR, emulsification, and solubility. The results showed that the glycosylation reaction occurred, and GSPI had better emulsification and solubility than SPI. Then, the interaction between GSPI and lutein was investigated by fluorescence quenching, thermodynamic binding parameters, UV–Vis, and
XRD. The results indicated that lutein was spontaneously bound to GSPI at a stoichiometric of 1:1. The fluorescence of GSPI was quenched by static quenching during the binding process, which slightly affected the secondary structure of GSPI. Finally, the total color difference and degradation rate results showed that GSPI had a better stabilization effect on lutein.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

AUTHOR CONTRIBUTIONS

XW: data management, investigation, validation, and writing—original draft. SW: methodology, project management, and writing—review and editing. DX: fund acquisition and supervision. JP: resources, concepts, and supervision. WG: conceptualization, project management, and supervision. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by the National Natural Science Foundation of China (31871808), the School Level Cultivation Fund of Beijing Technology and Business University for Distinguished and Excellent Young Scholars (BTBUYP2020), the Construction of Service Capability of Scientific and Technological Innovation (PXM2019_014213_000010, PXM2018_014213_000033, PXM2018_014213_000014, PXM2018_0142_13_000041, and 19005857058), and the Cultivation and Development of Innovation Base (Z171100002217019).

ACKNOWLEDGMENTS

We would like to thank MogoEdit (https://www.mogoedit.com) for its English editing during the preparation of this manuscript.

REFERENCES

1. Chung RWS, Leanderson P, Gustafsson N, Jonasson L. Liberation of lutein from spinach: Effects of heating time, microwave-heating and liquefaction. Food Chem. (2019) 277:573–8. doi: 10.1016/j.foodchem.2018.11.023
2. Youjia F, Jingde Y, Longwei J, Lili R, Jiang Z. Encapsulation of lutein into starch nanoparticles to improve its dispersity in water and enhance stability of chemical oxidation. Starch/Stärke. (2019) 71:1800248. doi: 10.1002/star.201800248
3. Ziegler Ju, Wahl S, Wurschum T, Longin CF, Carle R, Schweiggert RM. Lutein and lutein esters in whole grain flours made from 75 genotypes of 5 triticum species grown at multiple sites. J Agric Food Chem. (2015) 63:5061–71. doi: 10.1021/acs.jafc.5b01477
4. Cheng X, Zhao XZ, Huang CL, Zhan J, Su G. Lutein content in petals and leaves of marigold and analysis of lutein synthesis gene expression. Acta Physiologiae Plantarum. (2019) 41:128. doi: 10.1007/s11738-019-2913-y
5. Hentschel V, Kranl K, Hollmann J, Lindhauer MG, Bohm V, Bitsch R. Spectrophotometric determination of yellow pigment content and evaluation of carotenoids by high-performance liquid chromatography in durum wheat grain. J Agric Food Chem. (2002) 50:6663–8. doi: 10.1021/jf025701p
6. Yoshizako H, Hara K, Takai Y, Kaidzu S, Obana A, Ohira A. Comparison of macular pigment and serum lutein concentration changes between free lutein and lutein esters supplements in Japanese subjects. Acta Ophthalmol. (2016) 94:e11–e6. doi: 10.1111/aos.13106
7. Jiaqi Z, Wang F, Liu P, Zhang K, Sun Q, Bai X, et al. One-pot preparation of lutein block methoxy polyethylene glycol copolymer-coated lutein nanoemulsion. Colloid Polym Sci. (2021) 299:1055–62. doi: 10.1007/s00396-021-04824-7
8. Rodriguez-Conception M, Avalos J, Luisa Bonet M, Boronat A, Gomez-Gomez L, Hornero-Mendez D, et al. A global perspective on carotenoids: Metabolism, biotechnology, and benefits for nutrition and health. Prog Lipid Res. (2018) 70:62–93. doi: 10.1016/j.plipres.2018.04.004
9. Horvath MP, George EW, Tran QT, Baumgardner K, Zharov G, Lee S, et al. Structure of the lutein-binding domain of human STARD3 at 174 angstrom resolution and model of a complex with lutein. Acta Crystallogr F Struct Biol Commun. (2016) 72:609–18. doi: 10.1107/S2053230X16010694
10. Huang J, Bai F, Wu Y, Ye Q, Liang D, Shi C, et al. Development and evaluation of lutein-loaded alginate microspheres with improved stability and antioxidant. J Sci Food Agric. (2019) 99:5195–201. doi: 10.1002/jsfa.9766
11. Steiner BM, McClements DJ, Davidov-Pardo G. Encapsulation systems for lutein: a review. Trends Food Sci Technol. (2018) 82:71–81. doi: 10.1016/j.tifs.2018.10.003
12. Qi X, Xu DX, Zhu JH, Wang SJ, Peng JW, Gao W, et al. Interaction of ovalbumin with lutein dipalmitate and their effects on the color of...
stability of marigold lutein esters extracts. *Food Chem.* (2022) 372:131211. doi: 10.1016/j.foodchem.2021.131211

13. Yi J, Fan Y, Yokoyama W, Zhang Y, Zhao L. Characterization of milk proteins-lutein complexes and the impact on lutein chemical stability. *Food Chem.* (2016) 200:91–7. doi: 10.1016/j.foodchem.2016.01.035

14. Chee-Yuen G, Wen-Hwei O, Lee-Min W, Azhar Mat E. Effects of ribose, microbial transglutaminase and soy protein isolate on physical properties and in-vitro starch digestibility of yellow noodles. *LWT – Food Sci Technol.* (2009) 42:174–9. doi: 10.1016/j.lwt.2008.05.004

15. Zhang Y, Chen S, Qi B, Sui X, Jiang L. Complexation of thermally-denatured soybean protein isolate with anthocyanins and its effect on the protein structure and in vitro digestibility. *Food Res Int.* (2018) 106:619–25. doi: 10.1016/j.foodres.2018.01.040

16. Sharif HR, Williams PA, Sharif MK, Abbas S, Majeed H, Masamba KG, et al. Current progress in the utilization of native and modified legume proteins as emulsifiers and encapsulants - a review. *Food Hydrocolloids.* (2018) 76:2–16. doi: 10.1016/j.foodhyd.2017.01.002

17. Cao JR Li FW, Li YY, Chen HP, Liao XI, Zhang Y. Hydrophobic interaction driving the binding of soybean protein isolate and chlorophyll: Improvements to the thermal stability of chlorophyll. *Food Hydrocolloids.* (2021) 113:106465. doi: 10.1016/j.foodhydrocol.2020.106465

18. Tapal A, Tiku PK. Complexation of curcumin with soy protein isolate and its implications on solubility and stability of curcumin. *Food Chem.* (2012) 130:960–5. doi: 10.1016/j.foodchem.2011.08.025

19. Wang ZL, Wang JM, Wang LQ, Yuan Y, Yang XQ. Complexation of resveratrol with soy protein and its improvement on oxidative stability of corn oil/water emulsions. *Food Chem.* (2014) 161:324–31. doi: 10.1016/j.foodchem.2014.04.028

20. He M. Y., Li L, Wu C, Zheng L, Jiang L, Huang Y, et al. Effects of glycation and acylation on the structural characteristics and physicochemical properties of soy protein isolate. *J Food Sci.* (2021) 86:1737–50. doi: 10.1111/1750-3841.15688

21. Zhang H, Chi Y. Modified soy protein isolate with improved gelling stability. *J Food Sci Technol.* (2011) 46:14–22. doi: 10.1011/j.1365-2621.2010.03355.x

22. Zhang Z, Wang X, Yu J, Chen S, Ge H, Jiang L. Freeze-thaw stability of oil-in-water emulsions stabilized by soy protein isolate-dextran conjugates. *Lwt-Food Sci Technol.* (2017) 78:241–9. doi: 10.1016/j.lwt.2016.12.051

23. Jiang L, Li S, Wang N, Zhao S, Chen Y, Chen Y. Preparation of dextran-casein phosphopeptide conjugates, evaluation of its calcium binding capacity and digestion in vitro. *Food Chem.* (2021) 352:129332. doi: 10.1016/j.foodchem.2021.129332

24. Liu Y, Zhao GL, Zhao MM, Ren JY, Yang B. Improvement of functional properties of peanut protein isolate by conjugation with dextran through Maillard reaction. *Food Chem.* (2012) 131:901–6. doi: 10.1016/j.foodchem.2011.09.074

25. Wang W, Li C, Bin Z, Huang Q, You L, Chen C, et al. Physicochemical properties and bioactivity of whey protein isolate-inulin conjugates obtained by Maillard reaction. *Int J Biol Macromol.* (2020) 150:326–35. doi: 10.1016/j.jbiomac.2020.02.086

26. Apolinario AC, Goulart de. Lima Damasceno BP, de Macedo Beltrao NE, Pessoa A, Converti A, da Silva JA. Inulin-type fructans: a review on different aspects of biochemical and pharmaceutical technology. *Carbohydrate Polymers.* (2014) 101:368–78. doi: 10.1016/j.carbpol.2013.09.081

27. Closa-Manestrello R, Ferre N, Castillo-DeVillasante G, Luque V, Gispert-Llaurado M, Zaragoza-Jordana M, et al. The use of inulin-type fructans improves stool consistency in constipated children. a randomised clinical trial: pilot study. *Int J Food Sci Technol.* (2017) 68:587–94. doi: 10.1002/1096-8374.2016.126305

28. Coxam V. Current data with inulin-type fructans and calcium, targeting bone health in adults. *J Nutr.* (2007) 137:2527–35S. doi: 10.1093/jn/137.11.2527S

29. Kleessen B, Blaut M. Modulation of gut mucosal biofilms. *Br J Nutr.* (2005) 93:535–40. doi: 10.1079/BJN20041346

30. Pool-Zobel BL. Inulin-type fructans and reduction in colon cancer risk: review of experimental and human data. *Br J Nutr.* (2005) 93:573–90. doi: 10.1079/BJN20041349

31. Roberfroid MB. Introducing inulin-type fructans. *Br J Nutr.* (2005) 93:513–25. doi: 10.1079/BJN20041350

32. Roberfroid MB. Inulin-type fructans: Functional food ingredients. *J Nutr.* (2007) 137:2493S–502. doi: 10.1093/jn/137.11.2493S

33. Scholz-Ahrens KE, Schrezenmeir J. Inulin and oligofructose and mineral metabolism: The evidence from animal trials. *J Nutr.* (2007) 137:2513S–23. doi: 10.1093/jn/137.11.2513S

34. Song LF, Li DJ, Pang HL, Liu CQ. Effect of ultrasonic waves on the stability of all-trans lutein and its degradation kinetics. *Ultrasan Sonoch.* (2015) 27:602–8. doi: 10.1021/uts604200

35. Qi X, Xiu DX, Zhu JJ, Wang SJ, Peng JW, Gao W, et al. Studying the interaction mechanism between bovine serum albumin and lutein dimers: multi-spectroscopic and molecular docking techniques. *Food Hydrocolloids.* (2021) 113:106513. doi: 10.1016/j.foodhydrocol.2020.106513

36. He M, Wu C, Li L, Zheng L, Tian J, Jiang L, et al. Effects of cavitation jet treatment on the structure and emulsification properties of oxidized Soy Protein Isolate. *Foods.* (2020) 10:2. doi: 10.3390/foods10100002

37. Sharafodin H, Haysun A, Robinson D, Haysun A, et al. Ultrasound-induced gelation and foam stability of gelatine and fish gelatine gels containing soy protein isolate. *J Agric Food Chem.* (2017) 65:8684–91. doi: 10.1021/acs.jafc.7b00825

38. Apolinario AC, Goulart de. Lima Damasceno BP, de Macedo Beltrao NE, Pessoa A, Converti A, da Silva JA. Inulin-type fructans: a review on different aspects of biochemical and pharmaceutical technology. *Carbohydrate Polymers.* (2014) 101:368–78. doi: 10.1016/j.carbpol.2013.09.081

39. Closa-Manestrello R, Ferre N, Castillo-DeVillasante G, Luque V, Gispert-Llaurado M, Zaragoza-Jordana M, et al. The use of inulin-type fructans improves stool consistency in constipated children. a randomised clinical trial: pilot study. *Int J Food Sci Technol.* (2017) 68:587–94. doi: 10.1002/1096-8374.2016.126305

40. Coxam V. Current data with inulin-type fructans and calcium, targeting bone health in adults. *J Nutr.* (2007) 137:2527–35S. doi: 10.1093/jn/137.11.2527S

41. Kleessen B, Blaut M. Modulation of gut mucosal biofilms. *Br J Nutr.* (2005) 93:535–40. doi: 10.1079/BJN20041346

42. Pool-Zobel BL. Inulin-type fructans and reduction in colon cancer risk: review of experimental and human data. *Br J Nutr.* (2005) 93:573–90. doi: 10.1079/BJN20041349

43. Roberfroid MB. Introducing inulin-type fructans. *Br J Nutr.* (2005) 93:513–25. doi: 10.1079/BJN20041350
nanoparticles. *Colloids Surfaces a-Physicochem Engineer Aspects*. (2021) 628. doi: 10.1016/j.colsurfa.2021.127233. [Epub ahead of print].

52. Hinderink EBA, Berton-Carabin CC, Schroen K, Raublanc A, Houinsou-Houssou B, Boire A, et al. Conformational changes of whey and pea proteins upon emulsification approached by front-surface fluorescence. *J Agric Food Chem*. (2021) 69:6601–12. doi: 10.1021/acs.jafc.1c01005

53. Bayraktutan T, Organger Y. Biophysical influence of coumarin 35 on bovine serum albumin: Spectroscopic study. *Spectrochim Acta A Mol Biomol Spectrosc*. (2017) 171:90–6. doi: 10.1016/j.saa.2016.07.043

54. Wang BL, Pan DQ, Zhou KL, Lou YY, Shi JH. Multi-spectroscopic approaches and molecular simulation research of the intermolecular interaction between the angiotensin-converting enzyme inhibitor (ACE inhibitor) benazepril and bovine serum albumin (BSA). *Spectrochim Acta A Mol Biomol Spectrosc*. (2019) 212:15–24. doi: 10.1016/j.saa.2018.12.040

55. Zhang YF, Zhou KL, Lou YY, Pan DQ, Shi JH. Investigation of the binding interaction between estazolam and bovine serum albumin: multi-spectroscopic methods and molecular docking technique. *J Biomol Struct Dyn*. (2017) 35:3605–14. doi: 10.1080/07391102.2016.1264889

56. You Y, Yang L, Chen H, Xiong L, Yang F. Effects of (−)-Epigallocatechin-3-gallate on the functional and structural properties of Soybean Protein Isolate. *J Agric Food Chem*. (2021) 69:2306–15. doi: 10.1021/acs.jafc.0c07337

57. Zhang D, Zhang X, Liu Y-C, Huang S-C, Ouyang Y, Hu Y-J. Investigations of the molecular interactions between nisoldipine and human serum albumin in vitro using multi-spectroscopy, electrochemistry and docking studies. *J Mol Liq*. (2018) 258:155–62. doi: 10.1016/j.molliq.2018.03.010

58. Tantimongcolwat T, Prachayasittikul S, Prachayasittikul V. Unravelling the interaction mechanism between clioquinol and bovine serum albumin by spectroscopic and molecular docking approaches. *Spectrochim Acta A Mol Biomol Spectrosc*. (2019) 216:25–34. doi: 10.1016/j.saa.2019.03.004

59. Yue Y, Yin C, Huo F, Chao J, Zhang Y. The application of natural drug-curcumin in the detection hypochlorous acid of real sample and its bioimaging. *Sensors Actuators B-Chem*. (2014) 202:531–6. doi: 10.1016/j.snb.2014.05.119

60. Chen Z, Wang C, Gao X, Chen Y, Kumar Santhanam R, Wang C, et al. 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. *Food Chem*. (2019) 271:266–73. doi: 10.1016/j.foodchem.2018.07.170

61. Wang YX, Zhang L, Wang P, Xu XL, Zhou GH. pH-shifting encapsulation of curcumin in egg white protein isolate for improved dispersity, antioxidant capacity and thermal stability. *Food Res Int*. (2020) 137:109366. doi: 10.1016/j.foodres.2020.109366

62. Chen YS, Zhou YF, Chen M, Xie BJ, Yang JF, Chen JG, et al. Isoenriemann interaction with human serum albumin: Multi-spectroscopic analyses and docking simulation. *Food Chem*. (2018) 258:393–9. doi: 10.1016/j.foodchem.2018.02.105

63. Yang L, Baohua L, Lianzhou J, Regenstein JM, Nan J, Poivas V, et al. Interaction of soybean protein isolate and phosphatidylcholine in nanoemulsions: a fluorescence analysis. *Food Hydrocoll*. (2019) 87:814–29. doi: 10.1016/j.foodhyd.2018.09.006

64. Lelis CA, Nunes NM, de Paula HMC, Coelho YL, da Silva LHM, Pires ACD. Insights into protein-curcumin interactions: kinetics and thermodynamics of curcumin and lactoferrin binding. *Food Hydrocoll*. (2020) 105:105825. doi: 10.1016/j.foodhyd.2020.105825

65. Mohammadi F, Moeeni M. Study on the interactions of trans-resveratrol and curcumin with bovine alpha-lactalbumin by spectroscopic analysis and molecular docking. *Matter Sci Eng C Mater Biol Appl*. (2015) 50:358–66. doi: 10.1016/j.msec.2015.02.007

66. Song Z, Yuan W, Zhu R, Wang S, Zhang C, Yang B. Study on the interaction between curcumin and CopC by spectroscopic and docking methods. *Int J Macromolecules*. (2017) 96:192–9. doi: 10.1016/j.ijbiomac.2016.11.099

67. Wang C, Xie Y. Interaction of protein isolate with anthocyanin extracted from black Soybean and its effect on the anthocyanin stability. *J Food Sci*. (2019) 84:3140–6. doi: 10.1111/1750-3841.14816

68. Zhao C, Cheng H, Jiang P, Yao Y, Han J. Preparation of lutein-loaded particles for improving solubility and stability by Polyvinylpyrrolidone (PVP) as an emulsion-stabilizer. *Food Chem*. (2014) 156:123–8. doi: 10.1016/j.foodchem.2014.01.086

69. Chen FP Li BS, Tang CH. Nanoencapsulation of curcumin and soy protein isolate: influence on curcumin stability/bioaccessibility and in vitro protein digestibility. *J Agric Food Chem*. (2015) 63:3559–69. doi: 10.1021/jf506329d

70. Jimenez-Aguilar DM, Ortega-Regules AE, Lozada-Ramirez JD, Perez-Perez MCI, Vernon-Carter EJ, Welti-Chanes J. Color and chemical stability of spray-dried blueberry extract using mesquite gum as wall material. *J Food Composition Analysis*. (2011) 24:889–94. doi: 10.1016/j.jfca.2011.04.012

71. Bellary AN, Indiramma AR, Prakash M, Baskaran R, Rastogi NK. Anthocyanin infused watermelon rind and its stability during storage. *Innovative Food Sci Emerg Technol*. (2016) 33:554–62. doi: 10.1016/j.ifset.2015.10.010

72. Mengyao L, Fuli W, Chuanfen P, Wenting T, Qingjie S. Nanoencapsulation of lutein within lipid-based delivery systems: characterization and comparison of zein peptide stabilized nano-emulsion, solid lipid nanoparticle, and nano-structured lipid carrier. *Food Chem*. (2021) 358:129840. doi: 10.1016/j.foodchem.2021.129840

**Conflict of Interest:** JP and WG were employed by Chenguang Biotech Group Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Wang, Wang, Xu, Peng, Gao and Cao. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.