

The effects of thermal treatments on the antigenicity and structural properties of soybean glycinin

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Abstract

The effects of thermal treatments on the antigenicity and structural properties of soybean glycinin were investigated. An indirect enzyme-linked immunosorbent assay (iELISA) result indicated that the antigenicity of the soybean glycinin decreased to the lowest level when the heat treatment time was 50 min and the temperature was set at 110°C. The reducing sodium dodecyl sulfate-Gel electrophoresis results showed that the heat treatment had promoted the formation of small molecular weight protein subunits and concentrated protein. The free sulfhydryl (-SH) group of glycinin had increased significantly ($p < .05$) under the conditions of temperature between 90 and 120°C and 40 to 50 min heat treatments. The maximum fluorescence wavelength of the intrinsic fluorescence also changed significantly when compared with the control. The circular dichroism showed that the number of disordered structures had also increased significantly. These results provided evidence that the heat-induced structural modifications of glycinin will alter the antigenicity of soybean glycinin.

Practical applications

The relationship between antigenicity and structural properties of heat-treated soybean globulin was studied by different protein structural research methods. The results showed that the antigenicity of the protein was minimized at 110°C 50 min, which could be used in food safety production. This study chose to analyze the changes in the antigenicity and structural characteristics of soybean glycinin, both prior to and after heat treatments, for the purpose of promoting the development of low allergenic food products.

KEYWORDS

antigenicity, heat treatments, soybean glycinin, structural properties

1 | INTRODUCTION

Soybean is a main source of plant protein because of its high protein content and amino acid balance (Lambrecht et al., 2018). However, soybean proteins contain a variety of antitrophic factors which can potentially affect digestion and nutrient absorption, as well as causing allergic reactions (He et al., 2015). Allergen proteins in soybean include Gly m 5, Gly m Bd 30K, Gly m Bd 28K, Gly m 6 (soybean glycinin), and Gly m Ti (trypsin inhibitor) (Huang et al., 2019; Rouquié et al., 2010).

Soybean glycinin is a hexamer consisting of six subunits, which are composed of acid and basic polypeptides connected by disulfide bonds. The molecular weights of the acid polypeptides A1a, A1b, A2, and A4 are approximately 33.57 kDa. Meanwhile, the molecular weight of the A3 is approximately 42 kDa. The total molecular weight of the five basic polypeptides is approximately 20.65 kDa (Moreira et al., 1979). The following five different subunits are formed by different acid-base peptide pairings: A1aB1b (G1, 53.6 kDa); A2B1a (G2, 52.4 kDa); A1bB2 (G3, 52.2 kDa); A5A4B3 (G4, 61.2 kDa); and A3B4

(G5, 55.4 kDa) (Maruyama et al., 2018; Prak et al., 2005) (Maruyama et al., 2018; Prak et al., 2005). The subunits form hollow oblate cylinders, some or all which are non-spherical, causing partial obstruction of the central pore (Badley et al., 1975). There have been many reports presented regarding the sensitization characteristics of glycinin. For example, it has been confirmed that glycinin is the most important soybean allergen (Beardslee et al., 2000; Ping et al., 2002; Saeed et al., 2016). In Japan, soybean crops have traditionally been an important part of daily food intake. Previous studies have shown that the majority of people (whether allergic or not) produce IgE against Gly m 6 (Ito et al., 2011). For a long period of time, a variety of acidic polypeptides of soybean glycinin have been proven to be human allergens. Some previous studies have shown that the IgE antibodies of patients with soybean allergies have displayed strong binding power with the acid polypeptides (Beardslee et al., 2000).

Therefore, it is extremely important at the present time to identify appropriate processing methods for reducing or even eliminating the allergenic of soybean glycinin. How processing affects antigenicity is usually examined through using purified food proteins rather than allergens in a natural food matrix (Kroghsbo et al., 2014). Heat treatments are the conventional and most commonly used processing technology for many foods in order to reduce pathogen loads, prolong shelf life, and improve quality. It is generally believed that the conformation and molecular sizes of proteins can be changed when they are processed at different temperatures. These heat-induced structural changes of food proteins have been widely studied (Rahaman et al., 2017; Stanciu et al., 2017; Verma et al., 2012). The durations of the heat treatments, along with the concentrations of the samples, tend to affect the detections of soybean allergens in food. It has been found that the recovery rates of the soybean allergens will decrease with increase in the baking time (Gomaa & Boye, 2013). In addition, the water dispersion (5 to 15% w/v) studies of the soybean protein isolates, it was found that they had become completely denatured at 80 and 100°C for 30 min, and at 100°C, the soybean β -conglycinin and glycinin were completely denatured. Furthermore, at 80°C, the 7S proteins were completely denatured, while the glycinin was not completely denatured (Sorgentini et al., 1995). In addition, the effect of heat treatment (water boiling) on glycinin antigenicity and structure of soybean was more significant than that of baking (Kroghsbo et al., 2014; Meeyong et al., 2013). This study chose to analyze the changes in the antigenicity and structural characteristics of soybean glycinin both prior to and after heat treatments for the purpose of promoting the development of low allergenic food products.

2 | MATERIALS AND METHODS

2.1 | Material

The defatted soybean powder (The production process is to remove the impurities, peel the soybean, leach the soybean by petroleum liquefaction agent, and finally crush the soybean) utilized in this study was purchased from Kunhua Biotechnology Co., Ltd. (Henan Province,

China). The 2-nitrobenzoic acid (DTNB) was obtained from Sigma (St. Louis, MO, USA). The rainbow 245 broad spectrum protein marker (11–245 kDa) and 1,8-anilinonaphthale-nesulfonate (ANS) were purchased from Beijing Solarbio Science and Technology Co., Ltd. (China). The thick wall pressure bottles used in this study's experiments were obtained from Shanghai Rego Instrument Co., Ltd. (China).

2.2 | Purification of the soybean glycinin

Glycinin was first extracted by following the method presented by Liu et al. (2007). Mix the defatted soybean flour and the distilled water according to 1:15 (m/V), adjust pH to 8, static 2 hr, 9,000 r/min centrifugation for 30 min, add NaHSO_3 (0.98 g/L) in the supernatant, adjust pH to 6.4, and then incubated at 4°C overnight, the final centrifugal precipitation is glycinin. The glycinin was then purified using a Sepharose CL-6B gel filtration column (Beijing Solarbio Science and Technology Co., Ltd.). The antiserum was prepared in Henan Academy of Agricultural Sciences. New Zealand rabbits were immunized with the extracted and purified glycinin (native immunogen). The specificity of the rabbit antiserum was good. The results of this study's by Western Blot analysis indicated that the purified glycinin could specifically bind to the glycinin antiserum, which confirmed the immunological reactivity of the purified glycinin.

2.3 | Thermal treatments

Thermal treatments were performed using an electric blast drying oven supplied by Shanghai Qixin Scientific Instrument Co., Ltd. (China). The glycinin powder was diluted to 10 mg/ml using a phosphate buffer (PBS 0.01 M, pH 7.4), and 1 ml protein samples were placed into each thick wall pressure bottle. The reason for which we chose 10 mg/ml is that the antigenicity of the protein was lowest at this concentration when the temperature was 110°C and heating time was 50 min. Then, thermal treatments at 80, 90, 100, 110, and 120°C were selected and conducted for 10, 20, 30, 40, 50, and 60 min, respectively. The samples were immediately cooled following the heating processes in order to avoid thermal denaturation. The treatments of each sample were conducted in triplicate, and the heated samples were stored at -20°C for further analysis.

2.4 | Evaluations of the glycine antigenicity using an indirect enzyme-linked immunosorbent assay method

The antigenicity of the thermally treated samples was analyzed using an indirect enzyme-linked immunosorbent assay (iELISA) method (Ji et al., 2018). The concentrations of the component reagents for the iELISA, as well as the different assay parameters, were optimized. The most effective concentrations of antigen coating, and the best diluted multiples of the primary antibody, were then determined.

In the present study, 96-well ELISA plates were coated with 100 μ l of the purified glycinin prior to and following the thermal treatments (2.5 μ g/ml), which had been diluted in a carbonate bicarbonate buffer (CBS, 0.05 M, pH 9.6) and incubated at 4°C overnight. The plates were washed with PBS containing 0.05% Tween-20 (PBST), and then closed with 1% BSA in PBS for 2 hr at 37°C. At that point in the experimental process, the serum samples (rabbit polyclonal antibody) were diluted 6,400 times in PBS and incubated at 37°C for 60 min. Then, the plates were washed five times with PBST. After the wells had been washed, the goat anti-rabbit IgG-HRP was diluted 6,400 times and incubated at 37°C for 0.5 hr. After which, 3,3',5,5'-tetramethyl benzidine (TMB, 100 μ l/well) was added and incubated for 15 min. The plates were read at 450 nm using an Multisan FC enzyme reader (Thermo, USA) after the reaction had been terminated with 2 M of sulfuric acid. It was determined in this study that the lower the absorbance value was, the lower the antigenicity of the glycinin would be.

2.5 | SDS-gel electrophoresis

The non-reducing (minus 2-mercapto ethanol) and reducing (plus 2-mercapto ethanol) SDS-PAGE were measured following the method presented by Yukiko et al. (Mizutani et al., 2019). The SDS-PAGE was carried out using a 12% separation gel and a 5% stacking gel. The concentrations of glycinin prior to and following the thermal processing were diluted to 2 mg/ml and mixed with a loading buffer. The gel was stained with Coomassie Brilliant Blue and decolorized with a decolorizer.

2.6 | Determination of the free sulfhydryl group content

This study referenced the research results presented by Beveridge et al. for the determination of the free sulfhydryl group content (Beveridge et al., 2006). Ellman Reagent analysis method was also adopted. The samples were dissolved in a Tris-Glycine buffer (0.086 mol/L, pH 8.0) containing 0.09 M of glycine, 0.04 M of EDTA, and 8 M of urea (in which the free SH was measured). Then, following a vortex shock treatment with 40 μ l of Ellman reagent, the suspension was maintained at 20°C for 1 hr. An absorbance value of 412 nm was determined from the mixture without adding protein as a blank (A_{412}). The sulfhydryl content (μ mol/g) = $73.53 \times A_{412}/C$ was obtained using the formula $73.53 = 10^6/1.36 \times 10^4$, in which 1.36×10^4 was determined to be the molar extinction coefficient, and C represents the concentration level of the sample (mg/ml).

2.7 | Intrinsic emission fluorescence spectroscopy

In the present study, the protein samples were configured to 0.1 mg/ml with PBS (0.01 M, pH 7.4) before and after the heat treatments.

The results were obtained using a type of immolation spectrophotometer (Varian company, Cary Eclipse) at a 295 nm excitation wavelength, and then scanned from 310 to 420 nm, with the PBS as a blank.

2.8 | Circular dichroism spectra

The circular dichroism (CD) spectra were measured based on the method described by Xi and He (2018). The CD spectrophotometry results were recorded at 25°C using a MOS-450 spectrophotometer (Biologic science instrument, France). The parameter settings were as follows: 1. A step resolution of 1 nm and a bandwidth of 0.5 nm were established; 2. The protein sample was dissolved with PBS (0.01 M, pH 7.4) to a final concentration of 0.1 mg/ml, and the scanning wavelength was set at between 190 and 250 nm (far ultraviolet region); 3. Each CD spectrum was scanned with 30 nm/min, and the average value of five scans was taken as the final spectrum, which was expressed using the average molar roundness rate $[\theta]$ (deg cm²/dmol).

2.9 | Statistical analysis results

Three independent experiments were carried out for each parameter in this study. SPSS 16.0 (IBM, New York, USA) was used to analyze the experimental data and establish a significance level of $p < .05$. Duncan's multi-range tests were used to detect the differences between the average values.

3 | RESULTS AND DISCUSSION

3.1 | Effects of the thermal treatments on the antigenicity of soybean glycinin

As can be seen from Figure 1, different temperatures and heating times have different effects on the antigenicity of soybean glycinin, while 80°C has little influence on the antigenicity of soybean glycinin. It can be seen from Figure 1 that when the temperature was 90°C and the test duration ranged between 10 and 20 min, the antigenicity trend decreased. The obtained results indicated soybean glycinin had denaturation, which was consistent with the previous related experimental results (Sorgentini et al., 1995). Soybean glycinin is less antigenic when heated at temperatures above 90°C than when heated at 80°C. The antigenicity was observed to be the lowest when the duration and temperature were 50 min and 110°C, respectively (Figure 1). There are also many studies on the antigenicity of food protein destroyed by heat treatment. For example, the results of previous studies have confirmed reductions in the allergen reactivity of peanut protein following the frying and boiling of de-shelled peanuts (Mondoulet et al., 2005). In other related studies, it was observed that the

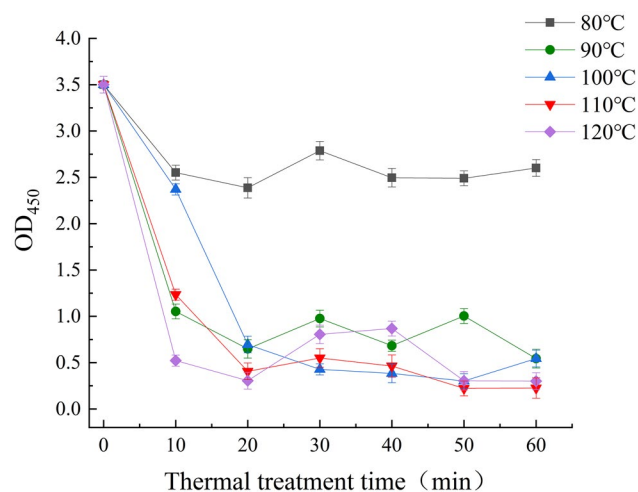


FIGURE 1 Effect of heat treatment on antigenicity of soybean glycinin (data are expressed as mean $\pm$ SD)

antigenicity of whey protein decreased significantly during heat treatments, and that the IgE binding of boiled shrimp was lower than that of raw shrimp, as demonstrated by dot blot and ELISA results (Liu et al., 2010). Furthermore, in other related research, although egg white protein had been heat treated at 100°C for 20 and 3 min, it was found that it could still be detected using immunology (Gu et al., 1983). However, this may have been related to the thermal stability of the protein.

3.2 | SDS-gel electrophoresis results

The reduced SDS-PAGE showed that temperature and durations of the heat treatments had no effects on the molecular weight of the glycinin, which indicated that the amino acid sequence of the protein had remained unchanged (Figure 2b,d). In other words, the primary structure of the protein had remained unaffected by the heat treatments. This phenomenon may have been due to the inability of the heat treatments to degrade, depolymerize, or cross-link the soy glycinin into smaller subunit units.

The non-reducing SDS-PAGE, which does not contain 2-mercaptoethanol, was used to examine the disulfide bond breakage of proteins before and after the heat treatments. It had not the ability to break the disulfide bonds between the acidic and basic chains of the soybean glycinin. In the present study, the observed phenomena were found to be similar to those described by Mizutani et al. (2019), in which one lane contained raw soybean glycinin bands. Under the non-reducing conditions (Figure 2a,c), the intermediary products of the glycinin (11S) were observed (Mizutani et al., 2019). Non-reductive SDS-PAGE showed that heat treatment breaks the disulfide bond between the linked subunits. It was found that the molecular weight distribution had changed when the heating duration exceeded 10 min. These findings indicated that glycinin had generated low molecular weight protein due to the soybean glycinin depolymerization. The formation of large molecular weight

proteins of different sizes, as well as accumulations of proteins, was observed. It was determined that brief heat treatments of 10 min at 110°C had resulted in the proteins becoming aggregated and depolymerized. However, the antigenicity of glycinin had not reached the minimum, and there were still some antigen-binding epitopes. When the protein was heated for 20 min, it was found that after the 20-min point, the bands were basically similar, and the antigenicity was low. These findings were consistent with the results obtained indirectly using iELISA.

3.3 | Effects of the thermal treatments on the free sulfhydryl (-SH) content of the glycinin

The free sulfhydryl groups (-SH) and disulfide bonds (-S-S-) are important functional groups of proteins, in which the content changes can reflect the denaturation degrees of the proteins, as well as having important effects on their functional properties.

As detailed in Figure 3a, heating duration of 50 min had significantly increased ($p < .05$) the content levels of the free sulfhydryl groups in the glycinin in the temperature range of 90 to 110°C and a timeframe of 10 to 50 min, when compared with the control (Figure 3a). The free sulfhydryl groups had increased with the extension of heating treatment time (Figure 3b). It was observed that under the conditions of a temperature of 120°C and 60 min of heating time, the content levels of the free sulfhydryl groups decreased significantly ($p < .05$). Also, under the aforementioned conditions, the sulfhydryl groups exposed to the surfaces of the soybean glycinin molecules were oxidized to varying degrees, resulting in decreases in the sulfhydryl groups. The aggregation of the proteins caused by the hydrophobic effects, along with the disulfide bond exchanges due to the extension of the heat treatment time, had resulted in decreases in the numbers of the total free sulfhydryl groups.

3.4 | Intrinsic emission fluorescence spectroscopy

In this study, a structural characterization of glycinin was performed by collecting the fluorescence spectra following the heat treatments. The changes in the protein fluorescence under the different treatment conditions were determined by the environmental-dependent emission characteristics of the Trp and Tyr residues, which had provided indications of the changes in the protein tertiary structures (Perez et al., 2014). The fluorescence of the glycinin was dependent on the thermal treatments' durations and temperature values. In the current study, the maximum fluorescence wavelengths of the glycinin before and after heat treatments were determined to be greater more 330 nm, which indicated that tryptophan in the soybean glycinin had been in a polar environment. The fluorescence peak of the untreated glycinin was 335.07 nm. The fluorescence spectrum of the hydrothermal soybean glycinin solution displayed a red shift (moving toward the long wave direction), as well as an increased

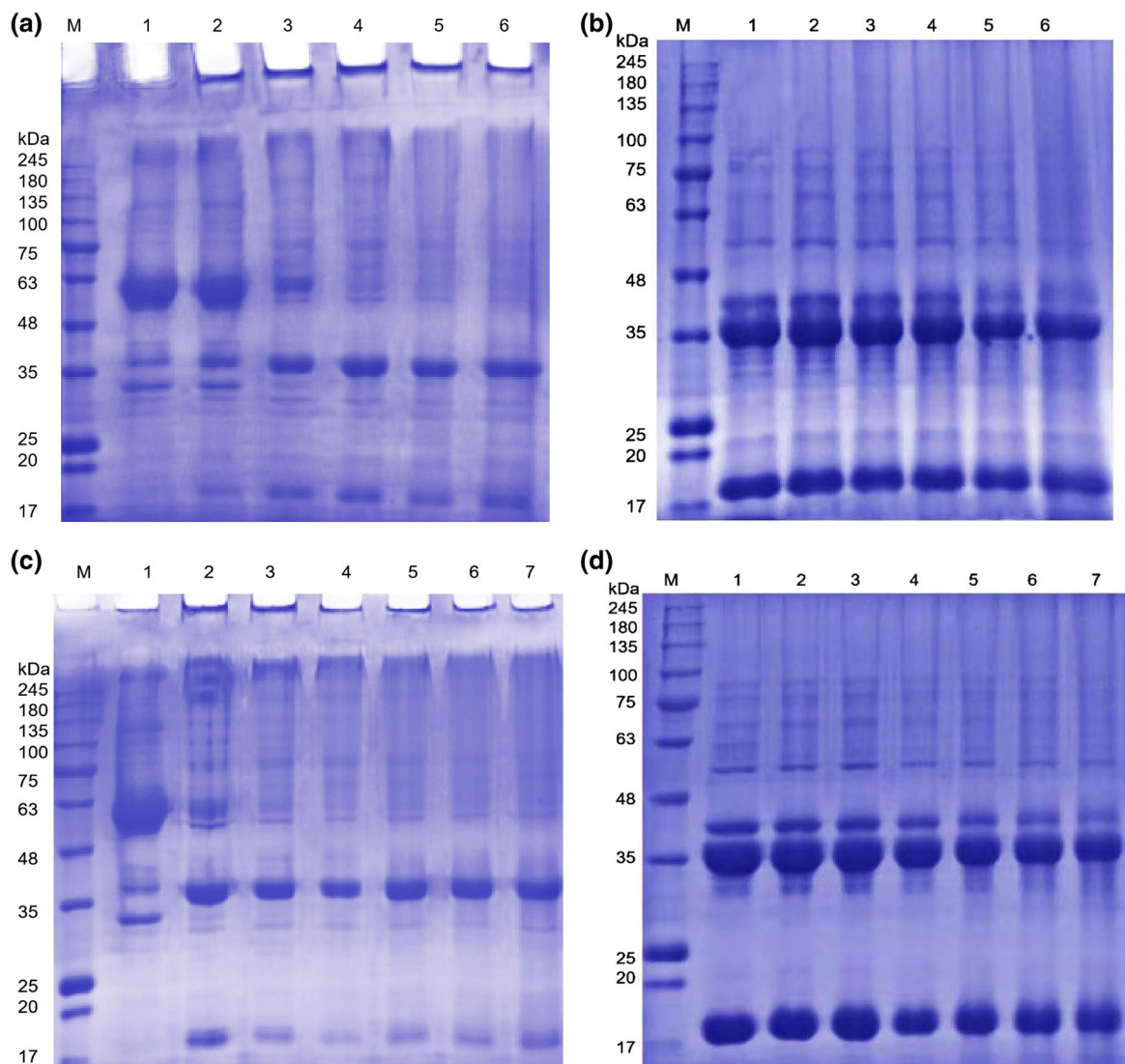


FIGURE 2 SDS-PAGE patterns of the glycinin under different of thermal treatments conditions ([a] M: Rainbow 245 broad spectrum protein marker; 1: unheated; 2 to 6: heating temperatures were 80, 90, 100, 110, and 120°C, respectively, and the duration of the heating treatment was 50 min without 2-ME; [b] Same conditions as a. with 2-ME; [c] 1: unheated; 2 to 7: heating treatment durations were 10, 20, 30, 40, 50, and 60 min, respectively, under the heating condition of 110°C without 2-mercaptoethanol; [d] Same conditions as c. with 2-mercaptoethanol)

peak intensity (Figure 4). The maximum fluorescence peak of the soybean glycinin had red shifted to 343.07 nm at 120°C, and red shifted to 348 nm when the heating duration had reached the 60 min time point. These red shifts indicated increases in the micro-environmental polarity of the tryptophan. Since the protein domains were destroyed following heat treatments, this had resulted in the tryptophan (which is a fluorescent group) becoming more exposed to the solvent (water). The changes in the fluorescence intensity and maximum absorption wavelengths ($\lambda_{\max}$) reflected the exposure degrees of the tryptophan residue, as well as the changes in the micro-environment. Previous study has shown that the maximum absorption wavelengths of endogenous fluorescence scans then to move toward the direction of the long wavelengths (redshift) when subunit dissociation occurs at 115 (Nielsen et al., 1989).

The changes in the protein tertiary structures were monitored in this study by the changes in the fluorescence emission maximum and fluorescence intensity (Shen & Chuan-HeTang, 2012). The $\lambda_{\max}$ of the samples increased by 5 and 2 nm when the temperature treatments were 90°C and above. These results indicated that under the temperature treatment conditions, a large number of subunits of glycinin had disintegrated causing the structure to expand. In addition, the tryptophan residues inside the glycinin molecules were exposed. A maximum $\lambda_{\max}$ was observed at heat treatment for 50 min time point of the heat treatments, and a longer treatment duration (60 min) had not led to a higher intrinsic fluorescence intensity. At that time, tryptophan exposure of glycinin after heating was found to be the most thorough. However, when compared with the temperature effects, the effects of the durations of the heat treatments on the maximum fluorescence intensity were not observed to be significant.

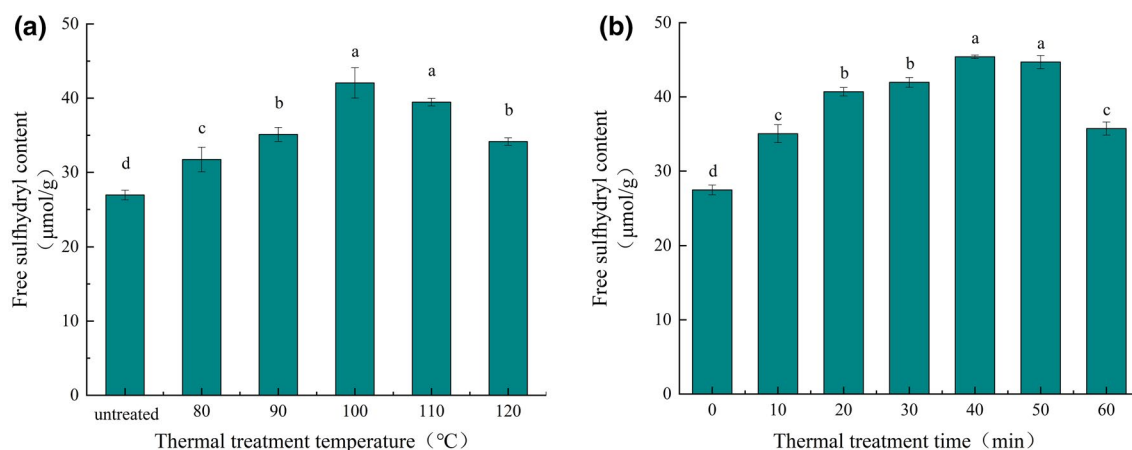


FIGURE 3 Effects of the different temperature levels and durations of the thermal treatments on the free sulfhydryl groups content levels in the glycinin ([a] Thermal treatment temperature fixed at 110°C; [b] Thermal treatment time fixed at 50 min) (data are expressed as mean $\pm$ SD; a and b indicate that the content levels of the free sulfhydryl groups were significantly different at the $p < 0.05$ level)

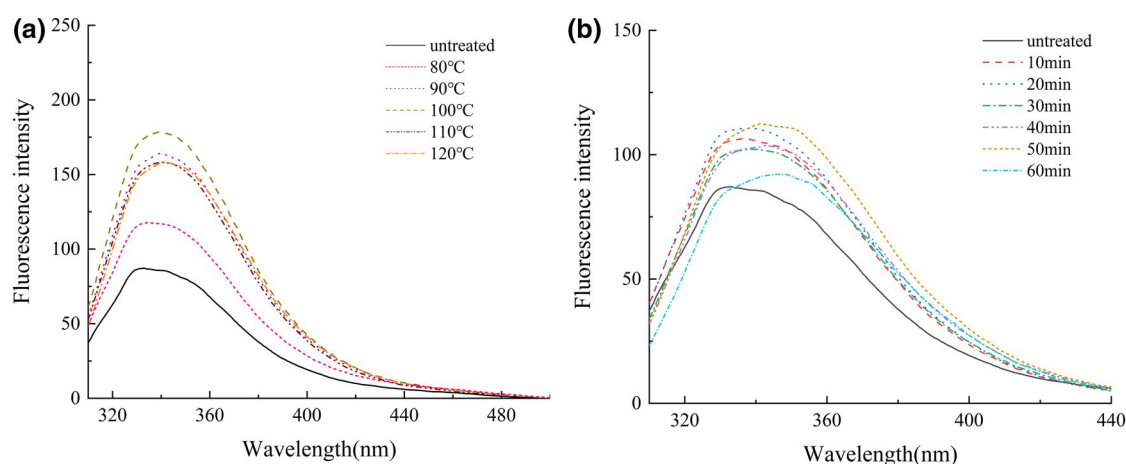


FIGURE 4 Structural changes of the glycinin monitored by emission spectrum ([a] Temperature-induced; [b] Time-induced) (the excitation wavelength was 295 nm, and the spectra were observed between 300 and 400 nm)

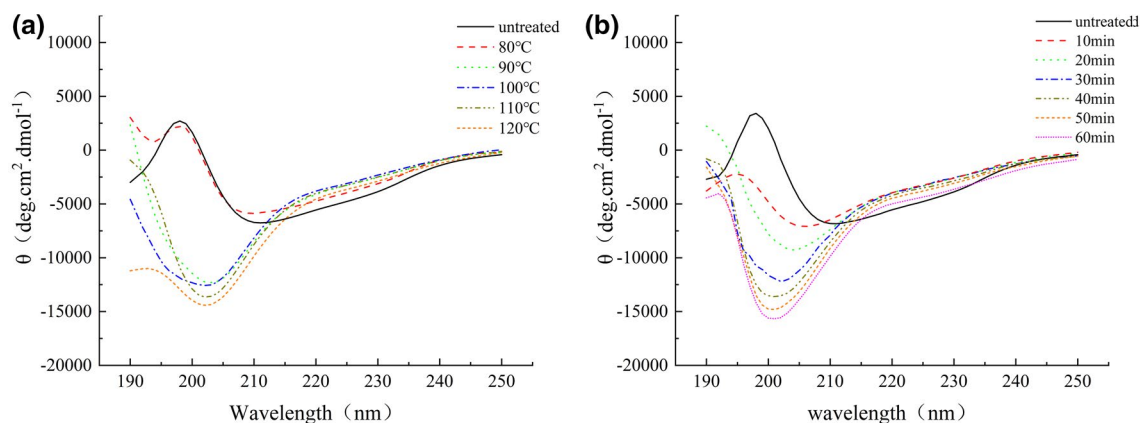


FIGURE 5 Far-UV CD spectra of diluted glycinin solution during heat treatments ([a] Temperature-induced; [b] Time-induced) (glycinin in phosphate buffer solution, and the smoothed spectra are shown)

3.5 | CD analysis

Figure 5 illustrates the changes in the CD spectra of the soybean glycinin. The spectra of the untreated samples were similar to those previously reported (Zhang et al., 2003). Therefore, the secondary structures of the natural soybean glycinin appeared to be rich in β -structure. The low peak of the untreated protein at 208 nm indicated that there was α -helix structure. It was observed that the larger the absolute value of θ value of the circular dichroism near 222 nm was, the higher the content of the α -helix structure would be. As shown in Figure 5, when compared with the control, the heat treatments with different temperatures and durations had reduced the content levels of the α -helix (Figure 5). The strong positive peak at 195 nm represented the β -sheet. The CD spectra of the untreated and heat-treated soybean glycinin at 80°C displayed this phenomenon at 195 nm (Figure 5a). The zero cross of the CD spectrum of the natural glycinin was approximately 201 nm, and had not changed at 80°C. However, under the conditions of 90°C and above, the peak shapes had obviously changed, which indicated that the heating temperatures had significant effects on the secondary structures of the soybean glycinin.

It was believed that the negative shoulder peak of 205 to 218 nm may have represented the β -sheet structure, and the more the negative shoulder peak moved to the left, the higher the content of irregular curl structures would be. As detailed in Figure 5, the different heat treatment temperatures and durations resulted in the peak moving to the left and changed the peak intensity of 205 to 218 nm (Figure 5). The moderate changes of the CD spectrum also indicated that the β -sheet content had decreased. Therefore, the instability of the α -helix and the higher content of irregular curl structures had also increased the intensity of the 200 to 220 nm peak. These results indicated that the content levels of α -helix and β -sheet decreased, while the content of irregular curl structures increased, with the increases in the heat treatment temperatures and durations. Meanwhile, the content levels of α -helix and β -sheet decreased with the decrease in antigenicity, which indicated that the antigenicity epitopes may have been located in the α -helix and β -sheet structures of the soybean glycinin.

In a previous related study conducted by Wu et al. (2010) it had been determined that the α -helix content levels of soybean protein decreased and the β -sheet content levels had increased following heat treatments at different pH values. The differences between the aforementioned study results and the results obtained in this study may have been due to the different pH and heat treatment parameters, among which the content levels of α -helix and β -sheet were observed to decrease significantly in thermally treated protein Z using circular dichroism spectra.

4 | CONCLUSIONS

It was suggested in this study that different heat treatment conditions may strongly affect the antigenicity and structure of soybean glycinin. It was observed that following heat treatments at 110°C

for a duration of 50 min, the antigenicity of the soybean glycinin had significantly decreased. The non-reduced SDS-PAGE showed that the heat treatments had changed the protein bands and promoted the formation of small molecular weight protein subunits and protein aggregate to a certain extent. It was found that under the conditions of heating temperatures ranging between 90 and 120°C, with heat treatment durations of 40 to 50 min, the free SH groups of soybean glycinin increased significantly ($p < .05$). The maximum $\lambda_{\max}$ was observed at heat treatment for 50 min. It was found that longer treatment durations had not led to higher intrinsic fluorescence intensity. In addition, following the heat treatments, the content levels of disorder of the soybean glycinin were observed to have increased significantly. Therefore, it was concluded in this study that heat treatments at a temperature of 110°C could be used to reduce the antigenicity of the soybean glycinin.

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CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Jun Xi: Formal analysis; Funding acquisition; Methodology; Project administration; Writing-original draft. **Lili Yao:** Software; Writing-original draft; Writing-review & editing. **Huibin Chen:** Software; Writing-original draft.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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REFERENCES

- Badley, R. A., Atkinson, D., Hauser, H., Oldani, D., Green, J. P., & Stubbs, J. M. (1975). The structure, physical and chemical properties of the soy bean protein glycinin. *BBA - Protein Structure*, 412(2), 214–228. [https://doi.org/10.1016/0005-2795\(75\)90036-7](https://doi.org/10.1016/0005-2795(75)90036-7)
- Beardslee, T. A., Zeece, M. G., Sarath, G., & Markwell, J. P. (2000). Soybean glycinin G1 acidic chain shares IgE epitopes with peanut allergen Ara h 3. *International Archives of Allergy & Immunology*, 123(4), 299–307. <https://doi.org/10.1159/000053642>
- Beveridge, T., Toma, S., & Nakai, S. (2006). Determination of SH-and SS-groups in some food proteins using Ellman's reagent. *Journal of Food Science*, 39, 49–51. <https://doi.org/10.1111/j.1365-2621.1974.tb00984.x>
- Gomaa, A., & Boye, J. I. (2013). Impact of thermal processing time and cookie size on the detection of casein, egg, gluten and soy allergens in food. *Food Research International*, 52(2), 483–489. <https://doi.org/10.1016/j.foodres.2013.01.019>
- Gu, J., Matsuda, T., & Nakamura, R. (1983). Immunochemical identification of the allergens in egg white. *The Journal of*

- Allergy and Clinical Immunology, 71, 481–486. [https://doi.org/10.1016/0091-6749\(83\)90465-7](https://doi.org/10.1016/0091-6749(83)90465-7)
- He, L., Han, M., Qiao, S., He, P., Li, D., Li, N., & Ma, X. (2015). Soybean antigen proteins and their intestinal sensitization activities. *Current Protein and Peptide Science*, 16(7), 613–621.
- Huang, L., Wang, C., Zhang, Y., Chen, X., Huang, Z., Xing, G., & Dong, M. (2019). Degradation of anti-nutritional factors and reduction of immunoreactivity of tempeh by co-fermentation with *Rhizopus oligosporus* RT-3 and *Actinomyces elegans* DCY-1. *International Journal of Food Science & Technology*, 54(5), 1836–1848.
- Ito, K., Sjölander, S., Sato, S., Movérare, R., Tanaka, A., Söderström, L., Borres, M., Poorafshar, M., & Ebisawa, M. (2011). IgE to Gly m 5 and Gly m 6 is associated with severe allergic reactions to soybean in Japanese children. *Journal of Allergy & Clinical Immunology*, 128(3), 673–675. <https://doi.org/10.1016/j.jaci.2011.04.025>
- Ji, S., Luo, Y., Zhang, T., Shao, L., Meng, X.-Y., Wang, Y., Gao, Y., Li, Y., Li, S. U., Sun, Y., Jin, X., & Qiu, H.-J. (2018). An improved indirect ELISA for specific detection of antibodies against classical swine fever virus based on structurally designed E2 protein expressed in suspension mammalian cells. *Archives of Virology*, 163, 1831–1839. <https://doi.org/10.1007/s00705-018-3809-7>
- Kroghsbo, S., Rigby, N. M., Johnson, P. E., Adel-Patient, K., Bøgh, K. L., Salt, L. J., Mills, E. N. C., & Madsen, C. B. (2014). Assessment of the sensitizing potential of processed peanut proteins in brown Norway rats: Roasting does not enhance allergenicity. *PLoS ONE*, 9(5), e96475. <https://doi.org/10.1371/journal.pone.0096475>
- Lambrecht, M. A., Deleu, L. J., Rombouts, I., & Delcour, J. A. (2018). Heat-induced network formation between proteins of different sources in model systems, wheat-based noodles and pound cakes. *Food Hydrocolloids*, 79, 352–370. <https://doi.org/10.1016/j.foodhyd.2017.12.032>
- Liu, C., Wang, H., Cui, Z., He, X., Wang, X., Zeng, X., & Hao, M. (2007). Optimization of extraction and isolation for 11S and 7S globulins of soybean seed storage protein. *Food Chemistry*, 102(4), 1310–1316. <https://doi.org/10.1016/j.foodchem.2006.07.017>
- Liu, G. M., Cheng, H., Nesbit, J. B., Su, W. J., Cao, M. J., & Maleki, S. J. (2010). Effects of boiling on the IgE-binding properties of tropomyosin of shrimp (*Litopenaeus vannamei*). *Journal of Food Science*, 75(1), T1–T5.
- Maruyama, N., Matsuoka, Y., Yokoyama, K., Takagi, K., Yamada, T., Hasegawa, H., Terakawa, T., & Ishimoto, M. (2018). A vacuolar sorting receptor-independent sorting mechanism for storage vacuoles in soybean seeds. *Scientific Reports*, 8(1), 1108. <https://doi.org/10.1038/s41598-017-18697-w>
- Meeyong, S., Han, Y., & Kangmo, A. (2013). The influence of the time and temperature of heat treatment on the allergenicity of egg white proteins. *Allergy Asthma & Immunology Research*, 5(2), 96–101. <https://doi.org/10.4168/aair.2013.5.2.96>
- Mizutani, Y., Shibata, M., Yamada, S., Nambu, Y., Hirotsuka, M., & Matsumura, Y. (2019). Effects of heat treatment under low moisture conditions on the protein and oil in soybean seeds. *Food Chemistry*, 275, 577–584. <https://doi.org/10.1016/j.foodchem.2018.09.139>
- Mondoulet, L., Paty, E., Drumare, M. F., Ah-Leung, S., Scheinmann, P., Willemot, R. M., Wal, J. M., & Bernard, H. (2005). Influence of thermal processing on the allergenicity of peanut proteins. *Journal of Agricultural and Food Chemistry*, 53(11), 4547–4553. <https://doi.org/10.1021/jf050091p>
- Moreira, M. A., Hermanson, M. A., Larkins, B. A., & Nielsen, N. C. (1979). Partial characterization of the acidic and basic polypeptides of glycinin. *Journal of Biological Chemistry*, 254(19), 9921–9926. [https://doi.org/10.1016/S0021-9258\(19\)83605-0](https://doi.org/10.1016/S0021-9258(19)83605-0)
- Nielsen, N. C., Dickinson, C. D., Cho, T. J., Thanh, V. H., Scallan, B. J., Fischer, R. L., & Gol Db Erg, D. (1989). Characterization of the glycinin gene family in soybean. *The Plant Cell*, 1(3), 313.
- Perez, A. A., Andermatten, R. B., Rubiolo, A. C., & Santiago, L. G. (2014). β -Lactoglobulin heat-induced aggregates as carriers of polyunsaturated fatty acids. *Food Chemistry*, 158, 66–72. <https://doi.org/10.1016/j.foodchem.2014.02.073>
- Ping, X., Beardslee, T. A., Zeece, M. G., Markwell, J. P., & Sarath, G. (2002). Identification and analysis of a conserved immunoglobulin E-binding epitope in soybean G1a and G2a and peanut Ara h3 glycinin. *Archives of Biochemistry and Biophysics*, 408(1), 51–57.
- Prak, K., Nakatani, K., Katsube-Tanaka, T., Adachi, M., Maruyama, N., & Utsumi, S. (2005). Structure-function relationships of soybean proglycinins at subunit levels. *Journal of Agricultural and Food Chemistry*, 53(9), 3650–3657. <https://doi.org/10.1021/jf047811x>
- Rahaman, T., Vasiljevic, T., & Ramchandran, L. (2017). Digestibility and antigenicity of β -lactoglobulin as affected by heat, pH and applied shear. *Food Chemistry*, 217, 517–523. <https://doi.org/10.1016/j.foodchem.2016.08.129>
- Rouquié, D., Capt, A., Eby, W. H., Sekar, V., & Hérouet-Guicheney, C. (2010). Investigation of endogenous soybean food allergens by using a 2-dimensional gel electrophoresis approach. *Regulatory Toxicology & Pharmacology*, 58(3), S47–S53. <https://doi.org/10.1016/j.yrtph.2010.09.013>
- Saeed, H., Gagnon, C., Cober, E., & Gleddie, S. (2016). Using patient serum to epitope map soybean glycinins reveals common epitopes shared with many legumes and tree nuts. *Molecular Immunology*, 70, 125–133. <https://doi.org/10.1016/j.molimm.2015.12.008>
- Shen, L., & Tang, C.-H. (2012). Microfluidization as a potential technique to modify surface properties of soy protein isolate. *Food Research International*, 48(1), 108–118. <https://doi.org/10.1016/j.foodres.2012.03.006>
- Sorgentini, D. A., Wagner, J. R., & Anon, M. C. (1995). Effects of thermal treatment of soy protein isolate on the characteristics and structure-function relationship of soluble and insoluble fractions. *Journal of Agricultural & Food Chemistry*, 43(9), 15607–15613. <https://doi.org/10.1021/jf00057a029>
- Stanciuc, N., Banu, I., Bolea, C., Patrascu, L., & Aprodu, I. (2017). Structural and antigenic properties of thermally treated gluten proteins. *Food Chemistry*, 267(30), 43–51.
- Verma, A. K., Kumar, S., Das, M., & Dwivedi, P. D. (2012). Impact of thermal processing on legume allergens. *Plant Foods for Human Nutrition*, 67(4), 430–441. <https://doi.org/10.1007/s11130-012-0328-7>
- Wu, W., Wu, X., & Hua, Y. (2010). Structural modification of soy protein by the lipid peroxidation product acrolein. *LWT - Food Science and Technology*, 43, 133–140. <https://doi.org/10.1016/j.lwt.2009.05.006>
- Xi, J., & He, M. (2018). High hydrostatic pressure (HHP) effects on antigenicity and structural properties of soybean β -conglycinin. *Journal of Food Science and Technology*, 55, 630–637. <https://doi.org/10.1007/s13197-017-2972-2>
- Zhang, H., Li, L., Tatsumi, E., & Kotwal, S. (2003). Influence of high pressure on conformational changes of soybean glycinin. *Innovative Food Science & Emerging Technologies*, 4(3), 269–275. [https://doi.org/10.1016/S1466-8564\(03\)00043-2](https://doi.org/10.1016/S1466-8564(03)00043-2)

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