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ORIGINAL PAPER

Effects of high hydrostatic pressure combined with heat treatment on the antigenicity and conformation of β-conglycinin

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Abstract

Soybean protein is considered as a main food allergen. This study illustrates the effects of a combination of high hydrostatic pressure (HHP) with heat treatment on soybean protein allergen. The main allergic protein β-conglycinin was isolated from low-temperature defatted soybean meal by the alkali-soluble acid precipitation method. The antigenicity and structure of β-conglycinin treated by HHP combined with heat treatment were analyzed by enzyme-linked immunosorbent assay (ELISA), western blot, non-reduced SDS-PAGE, Fourier transform infrared spectroscopy (FTIR), and fluorescence spectra. The antigenicity and immunogenicity of β-conglycinin was reduced significantly after HHP combined with heat treatment. Compared with the untreated protein, the antigenicity of β-conglycinin was lower, and it was reduced by 87.04% at 400 MPa and 100 °C for 20 min. The secondary structure of β-conglycinin changed after the combined treatment. The contents of α-helix and β-sheet decreased, but the contents of β-turn and random coils increased. The structure of the protein changed from order to disorder. Fluorescence spectroscopy showed that β-conglycinin exposed more hydrophobic groups after the combination treatment. HHP combined with heat treatment reduced the antigenicity of β-conglycinin by changing its conformation.

Keywords β-conglycinin · Antigenicity · High hydrostatic pressure · Heat treatment

Introduction

Food allergy is a common disease that affects 220–250 million people around the world [1]. Food allergy is the body's allergic reaction to food allergens. At present, the issue of food allergy has drawn much attention. Soybean cultivation originated in China and has been providing protein and oil to Asian people for thousands of years. Soybean and its derivatives are used widely in the production of human food and animal feed because of their nutritional value [2]. However, soybean is one of the eight allergic food components, which accounts for 0.1–3.2% of adult food allergies and 0.1–5.7% of children food allergies [3]. Recent studies have shown that the prevalence of soy allergy is 13% in children [4]. Of 34 IgE-mediated sensitizing proteins, β-conglycinin is a storage protein that has been identified as a major allergenic protein [5–7]. All three subunits of β-conglycinin are sensitized [8]. Soy protein can trigger various allergic reactions, and its sensitization threshold is very low, so consumers with soy allergies must avoid the intake of soy and its derivatives [9]. However, due to the wide range of uses, avoiding the ingestion of soybeans and their soy products can be challenging [10, 11]. Therefore, it is important to reduce or eliminate the allergenicity of soybean protein through processing.

At present, some processing technologies have been used to reduce the antigenicity of soybean protein allergens, such as hydrolysis, glycation, heat, and high hydrostatic pressure. As a convenient and quick method, heat treatment has been used widely in food processing. Different heat treatment methods reduce the potential allergenicity of soy protein allergens [12]. The spatial structure of the protein is changed by heat treatment, so that the antibody cannot recognize the original epitopes, and thereby, the person avoids the occurrence of hypersensitivity [13]. Nevertheless, higher temperature treatment may damage the original nutritional value of the food. HHP is a non-thermal processing method that can maintain the nutritional value and flavor of food. It was reported that the sensitization of soy protein isolate in infant formula was minimized after HHP (300 MPa for 15 min) [14]. In addition, the antigenicity of germinated bean sprouts decreased significantly with increasing

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pressure, although the antigenicity of soybean seeds after HHP showed the opposite trend [15]. Studies have showed that the combination of HHP and heat treatment can affect allergens more than any single method [16]. Compared with boiling treatment, the IgE binding ability of peanut allergen Ara h 2 decreased significantly when the sample was treated at 55 °C and 600 MPa for > 10 min [17]. In another study, compared with high temperature short-time pasteurization, milk protein showed the lowest antigenicity when treated at 30 °C with 600 MPa for 15 min [18]. However, there is little research on the effects of the combination treatment on soybean allergens.

β-conglycinin is the major allergen in soybean protein. This study was designed to reveal the effects of the combination of HHP and heat treatment on the antigenicity and structures of *β*-conglycinin. *β*-conglycinin was extracted by alkali-soluble acid precipitation, and then it was treated with a combination of pressure and temperature. The immunoactivity of *β*-conglycinin was analyzed with an enzyme-linked immunological method and western blot. The structural characteristics of the protein were determined by non-reduced SDS-PAGE, Fourier transform infrared spectroscopy (FTIR), and fluorescence spectra. This study provides an important theoretical basis for selecting suitable treatment conditions to reduce the sensitivity of soy protein during food processing.

Materials and methods

Materials

Defatted soybean flour was obtained from Henan Kunhua Biological Technology Group Co. Ltd (Anyang, China). HRP-labeled goat anti-rabbit IgG (A6154) and *β*-conglycinin (C5868) were obtained from Sigma Chemical Co. Ltd (St. Louis, MO, USA). TMB chromogenic single-component liquid was obtained from Beijing Solarbio Science and Technology Co. Ltd. (Beijing, China). The anti-*β*-conglycinin serum from rabbits was generated by our own laboratory. All chemicals were of analytical grade.

The extraction of *β*-conglycinin

β-conglycinin was prepared from defatted soy flour according to the method of Thanh et al. [19] and Liu et al. [20]. The low-temperature defatted soy flour was mixed with 0.03 M Tris–HCl (pH 8.5) solution at the ratio of flour to water of 1:15 and stirred at 45 °C for 1 h. The protein solution was then centrifuged using a high speed freezing centrifuge at 4 °C (10,000 r/min, 20 min). $NaHSO_3$ (0.01 M) and $CaCl_2$ (5 mM) were added to the supernatant, adjusted to pH 6.4, and centrifuged. The supernatant was adjusted to pH 5.5 with HCl (2 M) and centrifuged. Then the supernatant was diluted with twice the volume of ice water and stirred for 1 h, adjusted to pH 4.8, and centrifuged. The precipitate was collected and dissolved in 0.03 M Tris–HCl buffer and adjusted the pH to 7.0. The protein solution was on dialysis for 2 days, and we changed water every 3 h. The extracted *β*-conglycinin was freeze-dried and stored at − 20 °C.

HHP combined with heat treatment

The extracted *β*-conglycinin was diluted with phosphate buffer (pH 7.0) to the protein concentration (5 mg/mL) and packed into a sterile homogeneous bag (8 cm × 10 cm). The bag was sealed tightly by a vacuum packaging machine. The pressure was set to 100–600 MPa, and the pressing time was 20 min. After the high hydrostatic pressure (HHP), the samples were divided equally into three portions and treated at 60 °C, 80 °C, and 100 °C for 20 min respectively. After the treatment was completed, the solution was cooled in an ice-water bath immediately. Then the solution of samples was freeze-dried and stored at − 20 °C. The samples of HHP treatment (100–600 MPa) were used as a control. The protein content of the samples was measured using a micro-spectrophotometer (Nano Drop 2000, Thermo Scientific, Massachusetts, USA). The effects of different pressure combined with temperature on *β*-conglycinin was studied, and the immunogenicity and structure of the proteins were analyzed.

Antigenicity assay by indirect competitive ELISA

The antigenicity of *β*-conglycinin samples was analyzed by an indirect competitive ELISA method [21]. Microtiter plates with 96 wells (Costar 3590 High Binding, Corning, NY, USA) were coated with 100 µL per well of *β*-conglycinin antigen (0.4 µg/mL) in 50 mM carbonate buffer and incubated at 4 °C overnight. *β*-conglycinin samples (1 µg/mL) were mixed with an equivalent volume of rabbit anti-*β*-conglycinin serum (1:6400 dilution) diluted in 0.01 M phosphate-buffered saline (PBS, pH 7.4) that contained 1% BSA and 0.1% Tween-20 at 4 °C overnight. The following process followed the approach we reported earlier [22]. An uninhabited serum sample (no sample added) was used as a control. The absorbance was determined at dual wavelengths of 450 nm and 630 nm by a microplate reader (Thermo Fisher Scientific Instrument Co. Ltd., New York, USA).

The antigenicity of samples was expressed by the inhibition percentages (%), which was calculated as follow:

$$\text{Inhibition rate } (\%) = \left(B_0 - B\right)/B_0 \times 100$$

where B is the OD of samples, and B_0 is the absorbance obtained with the uninhibited serum sample control. A low percentage of inhibition reflects a low allergenicity of samples.

Western blot

The immunogenicity of β-conglycinin was analyzed by western blot [23]. The treated samples were subjected to SDS-PAGE, then the protein bands were transferred with a PVDF membrane. After washing three times (10 min each time) with PBST (PBS of pH 7.4 and 0.1% Tween-20), the membranes were blocked with blocking buffer (1% BSA and 0.1% Tween-20 in PBS of pH 7.4) for 1 h at room temperature. Subsequently, membranes were washed three times. Then the membrane was incubated with polyclonal rabbit antibodies (1:6400 dilution) at 4 °C overnight. After washing three times with PBST, the membranes were incubated with HRP-labeled goat anti-rabbit IgG antibody (1:20,000 dilution) for 2 h at room temperature. After washing again as described above, the membranes were incubated with an enhanced chemiluminescence (ECL, Millipore, MA, USA) for 2 min. The following operations were carried out in a dark room. When fluorescent strips appeared on the PVDF membranes, the membranes were exposed to X-ray film. The color depth indicated the strength of β-conglycinin immunogenicity.

Fourier transform infrared spectroscopy

The β-conglycinin samples were analyzed by Fourier transform infrared spectroscopy (FTIR) using a WQF-510 Fourier transform infrared spectrum equipped with a computer (Beijing Ruili Analytical Instruments Co. Ltd. Beijing, China). The lyophilized samples were mixed with potassium bromide (KBr) at a ratio of 1:100 (w/w). The mixture was then pressed into a transparent sheet. For each sample, an average of 32 scans was recorded at 4 cm^{-1} resolution in the range of 4000–400 cm^{-1} [24]. Finally, the secondary structure of samples was analyzed using Peak Fit Version 4.12 software.

SDS-PAGE

Reduced SDS-PAGE and non-reduced SDS-PAGE were performed using a 12% separating gel and a 5% stacking gel [25]. A 3 mg amount of each sample was stirred in 1 mL sample buffer. There was β-mercaptoethanol in the sample buffer of reduced SDS-PAGE, but there was no β-mercaptoethanol in non-reduced SDS-PAGE. A 10 µL of sample solution was loaded on the gels. The process was carried out for 40 min at 20 mA in the stacking gels and for 1.5 h at 40 mA in the separating gels. The gels were stained for protein with Coomassie brilliant blue and then were put into a destaining solution so that the bands became clear. Finally, the bands were analyzed by Gel Analyzer Software (Version 4.0).

Surface hydrophobicity

The surface hydrophobicity (H_0) of proteins was determined by the method of Lakshmanan [26], with slight modification. β–conglycinin samples were diluted to 5 mg/mL with PBS (10 mM, pH 7.0). Twenty µL ANS (8.0 mM) was added to the diluted solution of β-conglycinin (4 mL), then the solution was shaken with a vortex mixer for 20 s. The fluorescence intensity of each sample was measured with a fluorescence spectrophotometer at excitation and emission wavelengths of 370 nm and 490 nm, respectively [27]. The initial slope of the curve obtained from the fluorescence intensity compared with a protein concentration plot was used as an index of H_0. Measurement of each sample was performed in triplicate.

Statistical analysis

Data were analyzed using SPSS 16.0 (SPSS Statistical Software, Inc., Chicago, IL, USA), and data were expressed as the mean values ± standard deviation. Statistical significance was determined by one-way ANOVA followed by Duncan's multiple range test. Three determinations for each treatment were performed, and the significance level was set at $P < 0.05$.

Results and discussion

Extraction of β-conglycinin

The electrophoresis bands of protein maker, soybean protein isolate (SPI) and β-conglycinin extracted by alkali-soluble acid precipitation were shown in Fig. 1. The protein content of β-conglycinin was 92.7% using the kjeldahl method. SDS-PAGE showed that the characteristic bands of the extracted β-conglycinin coincided with that of β-conglycinin in SPI, and the bands of α', α, and β subunits were clear. The purity of the protein was 72.68% based on Gel Analyzer Software.

The antigenicity of β-conglycinin

The effects of HHP combined with heat treatment on the antigenicity of β-conglycinin were measured by the indirect competitive ELISA method (Fig. 2). In this experiment, the antigenicity of untreated β-conglycinin was 76.70%. Antigenicity of β-conglycinin after the combined treatment decreased significantly compared with the

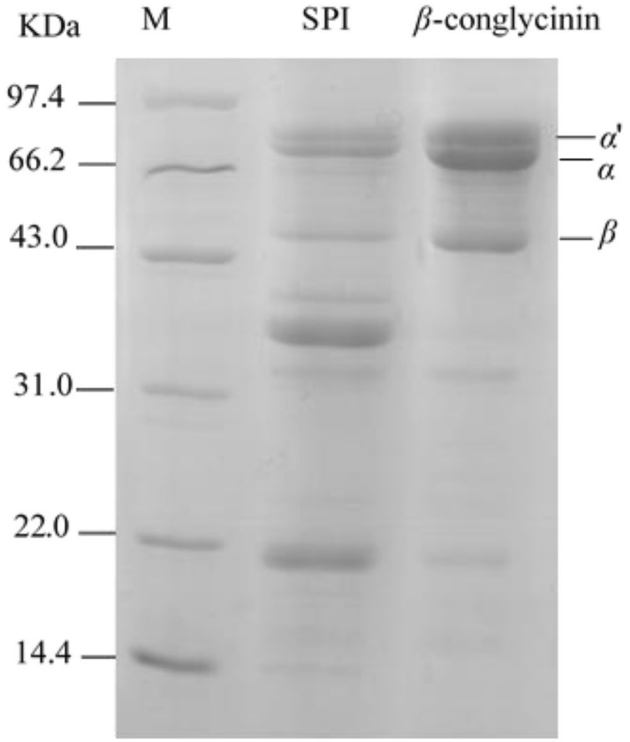

Fig. 1 SDS-PAGE profile of β-conglycinin. M: standard protein; SPI: soy protein isolate; β-conglycinin: extracted β-conglycinin

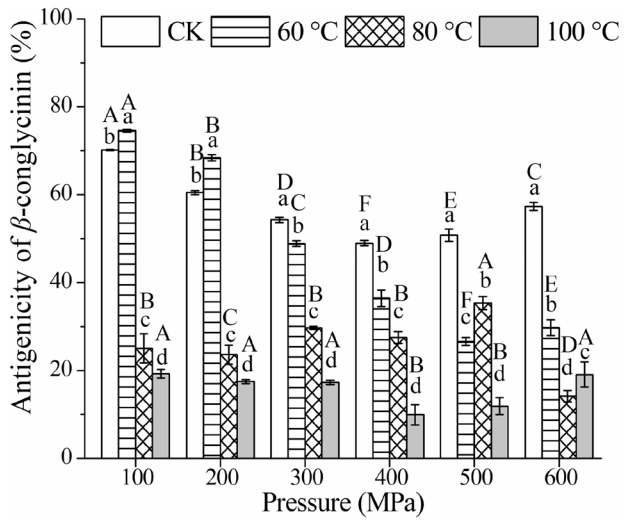

Fig. 2 Effects of HHP combined with heat treatment on β-conglycinin antigenicity. CK: only HHP treatment. The different capital letters indicate significant difference in values under the different pressure groups at the same temperature ($P < 0.05$). The different lowercase letters indicate significant difference in values between the different temperature groups at the same pressure ($P < 0.05$)

untreated β-conglycinin. When β-conglycinin was treated by HHP treatment only, the antigenicity decreased with increasing pressure (< 400 MPa) and reduced by 36.22% at 400 MPa. As the pressure increased further, antigenicity of β-conglycinin increased. The effect of HHP on antigenicity was similar to Xi et al. [28]. When β-conglycinin was treated by HHP combined with temperature, antigenicity of β-conglycinin decreased by 65.30% at 500 MPa and 60 °C for 20 min, this indicated that the combined treatment was more effective in reducing antigenicity than HHP treatment [28]. It is very obvious in Fig. 2 that antigenicity of β-conglycinin can be reduced greatly by applying only 100 MPa at 80 °C and 100 °C. Compared with untreated β-conglycinin, antigenicity of β-conglycinin was lowest and decreased by 87.04% at 400 MPa and 100 °C for 20 min. In general, HHP prior to heat treatment obviously reduced the binding of β-conglycinin and antibodies compared with HHP alone. Moreover, the combination treatment of higher pressure (300–500 MPa) and higher temperature could be an effective method to reduce antigenicity of β-conglycinin.

HHP combined with heat treatment can change the antigenicity of food allergens. Kleber et al. [29] reported that β-lactoglobulin was treated with different pressures (200, 400, and 600 MPa) and holding times (0, 10, and 30 min) from 25 °C to 68 °C. When the temperature was 40 °C and 50 °C, the antigenicity of β-lactoglobulin increased, but higher temperatures (60 and 68 °C) reduced the antigenicity at 600 MPa for 10 min, which indicated that higher temperatures and pressures were beneficial to reduce antigenicity [30]. In another study, when the prion tropomyosin was treated at 500 MPa for 10 min at 55 °C, the serum IgE binding capacity of the patient decreased by 73.59%. This combined method was better than the boiling water treatment or HHP alone [31]. In addition, the IgE binding capacity of walnut allergen decreased by up to 86.37% when the HHP was 650 MPa at 100 °C for 15 min, which was better than the single treatment [32]. Although the materials and treatment methods in the above studies were different from those in our experiment, these results showed that HHP combined with heat treatment reduced the antigenicity of allergens [29, 31]. Compared with a single treatment, HHP and heat treatment might have a synergism that promotes the reduction of sensitization.

In this study, we found that the combination of HHP and heat treatment was an effective method to reduce the antigenicity in β-conglycinin. The reason for the decrease in antigenicity may be that the combined treatment changed the protein structure, which masked or destroyed the original antigenic epitopes [29, 31, 32]. However, the results indicated that antigenicity was not removed completely; there may be optimal processing conditions between 300 and 500 MPa at 100 °C that would produce the lower antigenicity. It is important to choose the appropriate mode of combined processing, and for that reason, effects of simultaneous treatment of HHP during heat treatment on the antigenicity of β-conglycinin will be researched in our future work [33].

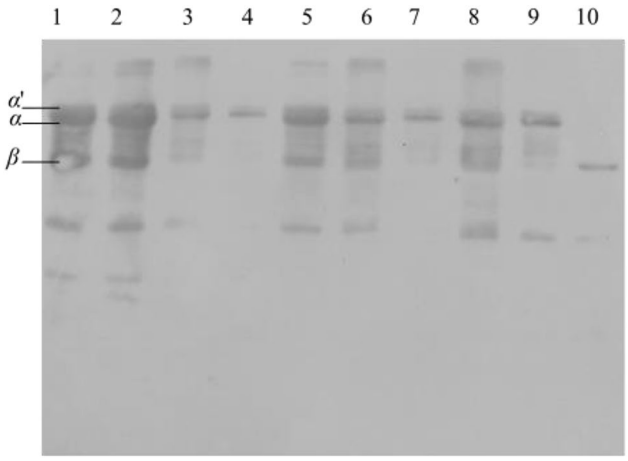

Fig. 3 Western blot of β-conglycinin after HHP combined with heat treatment. 1: blank; 2: 200 MPa, 60 °C; 3: 200 MPa, 80 °C; 4: 200 MPa, 100 °C; 5: 400 MPa, 60 °C; 6: 400 MPa, 80 °C; 7: 400 MPa, 100 °C; 8: 600 MPa, 60 °C; 9: 600 MPa, 80 °C; 10: 600 MPa, 100 °C

The immunogenicity of β-conglycinin

Figure 3 showed the western blot of β-conglycinin. All subunits of the untreated β-conglycinin (lane 1) had a deeper color, which indicated higher immunoreactivity (Fig. 3). As the pressure increased, the color of the bands (lanes 2, 5, 8; lanes 3, 6, 9; lanes 4, 7, 10) became lighter, which indicated a decrease in immunoreactivity. Protein bands did not disappear completely at 600 MPa pressure. When a combination of HPP combined with temperature was used, the intensity of protein bands (lanes 2, 3, 4; lanes 5, 6, 7; lanes 8, 9, 10) faded significantly with increasing temperature, or even disappeared. The method of HHP combined with heat treatment reduced the immunogenicity of the proteins to a great extent, but it did not eliminate them completely. This result was consistent with the above results on reducing antigenicity using an indirect competitive ELISA.

FTIR analysis

FTIR is a common technique for characterizing the secondary conformation of proteins [34]. Figure 4 showed the infrared scanning pattern of β-conglycinin after HHP combined with heat treatment. Amide I band (1700–1600 cm^{-1}), II band (1575–1480 cm^{-1}) and III band (1260–1330 cm^{-1}) are the most prominent vibrational bands in the protein backbone [35, 36]. Compared with the untreated sample, the transmittance of amide I, II and III bands were enhanced (Fig. 4), this indicated that the structure of β-conglycinin changed after combined treatment. To study the secondary structure of β-conglycinin, amide I band in a specific band of deconvolution was further analyzed [37].

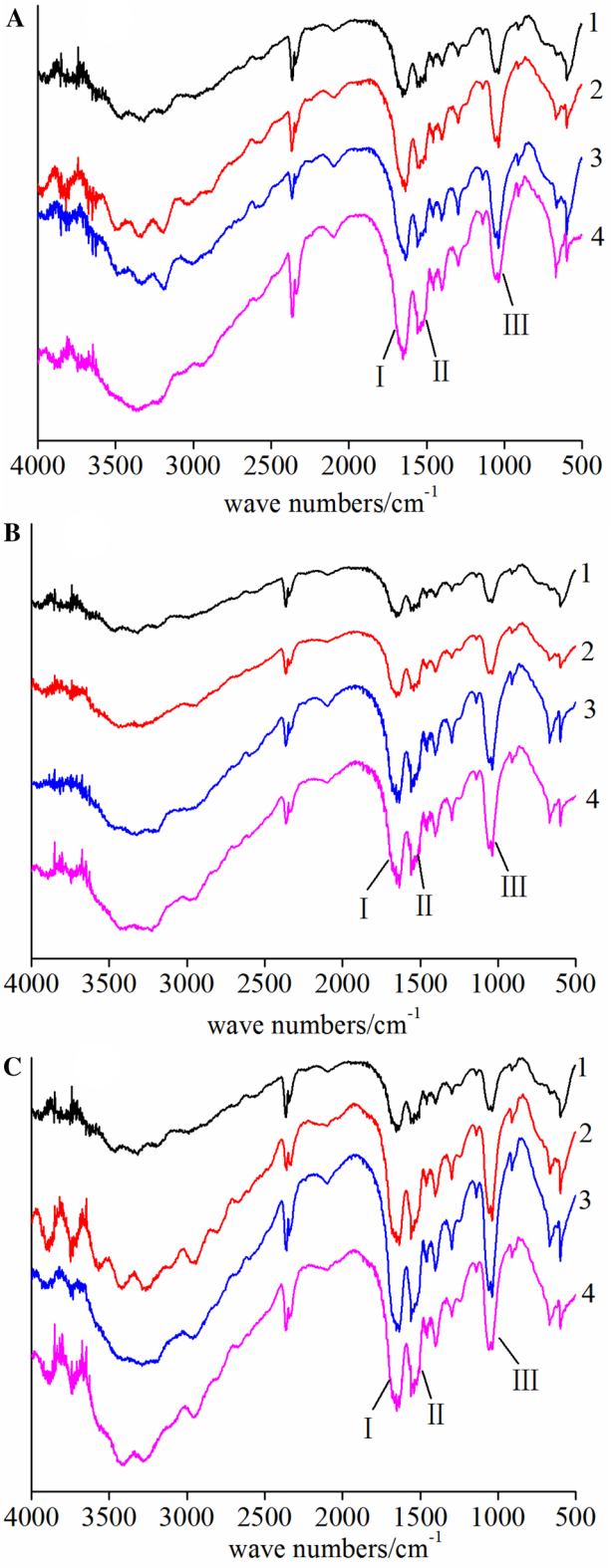

Fig. 4 Infrared scanning pattern of β-conglycinin after HHP combined with heat treatment. **a** 200 MPa; **b** 400 MPa; **c** 600 MPa; 1: Blank; 2: 60 °C; 3: 80 °C; 4: 100 °C

The primary secondary structures of native β-conglycinin were β-sheet and β-turn, followed by α-helix and random coils (Table 1). After HHP combined with high temperature, the contents of α-helix and β-sheet decreased, but the contents of β-turn and random coils increased. In general, protein structures tended to shift from order to disorder [38]. Moreover, the antigenicity of β-conglycinin decreased after the combination treatment (Fig. 2). This suggested that HHP combined with heat treatment reduced the sensitization of β-conglycinin by changing the secondary structure. Based on the published research, the reason for the decrease of the antigenicity of β-conglycinin might relate to the content of β-turn and random coils [28]. Changes in protein structure can destroy or mask the original antigenic epitopes of the allergen, thereby changing its antigenicity [39–41].

SDS-PAGE analysis

SDS-PAGE patterns of β-conglycinin were showed in Fig. 5. Compared with reduced SDS-PAGE patterns (Fig. 5a), the non-reduced electrophoresis patterns (Fig. 5b) of β-conglycinin increased a new band with a high molecular weight, which indicated that the protein was cross-linked by the disulfide bond. It can be seen from Fig. 5a, the lanes of β-conglycinin with HHP treatment at the same temperature showed small changes in color intensity (lanes 2, 5, 8; lanes 3, 6, 9; lanes 4, 7, 10). In Fig. 5b, the corresponding lanes for heat treatment at the same pressure changed significantly, and some bands even disappeared (lanes 2, 3, 4; lanes 5, 6, 7; lanes 8, 9, 10). The changes in color intensity of lanes indicated that the effect of heat treatment on the structure of β-conglycinin was greater than that of HHP. The disappearance of bands may be attributed to the polymerization between β-conglycinin subunits [25].

Non-reducing electrophoresis showed that in the absence of a reducing agent, there were a number of high molecular weight proteins that were cross-linked by disulfide bonds. Epitopes on the surface of proteins could be masked due to the accumulation of proteins, which may be responsible for the decrease in antigenicity [42]. The combination of HHP and heat treatment affected disulfide bonds, which affected the antigenicity of β-conglycinin.

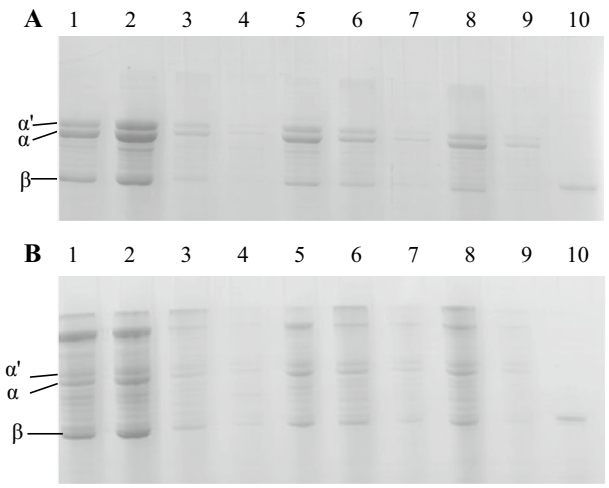

Fig. 5 SDS-PAGE of β-conglycinin after HHP combined with heat treatment (**a**). Non-reducing SDS-PAGE of β-conglycinin after HHP combined with heat treatment (**b**). 1: blank; 2: 200 MPa, 60 °C; 3: 200 MPa, 80 °C; 4: 200 MPa, 100 °C; 5: 400 MPa, 60 °C; 6: 400 MPa, 80 °C; 7: 400 MPa, 100 °C; 8: 600 MPa, 60 °C; 9: 600 MPa, 80 °C; 10: 600 MPa, 100 °C

Surface hydrophobicity

Surface hydrophobicity of β-conglycinin was measured by fluorescence spectrophotometer. The surface hydrophobicity is the main force that maintains the tertiary structure and the quaternary structure of the protein. Changes in surface hydrophobicity can reflect changes in the tertiary and

Table 1 Secondary structure contents of β-conglycinin after HHP combined with heat treatment

Samples	Content of secondary structure (%)			
	α-Helix	β-Sheet	β-Turn	Random coil
β-conglycinin	12.71 ± 0.03^{a}	37.29 ± 0.01^{a}	37.55 ± 0.16^{f}	12.54 ± 0.01^{f}
60 °C 200 MPa	12.59 ± 0.03^{b}	36.98 ± 0.21^{a}	37.73 ± 0.16^{f}	12.71 ± 0.03^{e}
80 °C 200 MPa	12.11 ± 0.03^{e}	36.45 ± 0.07^{b}	38.63 ± 0.02^{e}	12.82 ± 0.02^{d}
100 °C 200 MPa	12.07 ± 0.06^{e}	35.57 ± 0.23^{c}	39.45 ± 0.28^{d}	12.96 ± 0.04^{c}
60 °C 400 MPa	12.40 ± 0.02^{c}	35.86 ± 0.10^{c}	39.13 ± 0.08^{d}	12.62 ± 0.04^{f}
80 °C 400 MPa	12.30 ± 0.01^{d}	35.62 ± 0.34^{c}	39.34 ± 0.33^{d}	12.75 ± 0.05^{de}
100 °C 400 MPa	11.83 ± 0.09^{f}	34.29 ± 0.33^{e}	40.72 ± 0.21^{b}	13.18 ± 0.04^{b}
60 °C 600 MPa	12.09 ± 0.01^{e}	37.16 ± 0.12^{a}	37.80 ± 0.09^{f}	12.97 ± 0.02^{c}
80 °C 600 MPa	11.90 ± 0.01^{f}	34.96 ± 0.09^{d}	39.95 ± 0.03^{c}	13.20 ± 0.07^{b}
100 °C 600 MPa	11.25 ± 0.01^{g}	33.51 ± 0.36^{f}	41.49 ± 0.35^{a}	13.72 ± 0.05^{a}

The values shown are the mean of three replications ± SD. Values of the same column with different superscript letters are significantly different ($P < 0.05$)

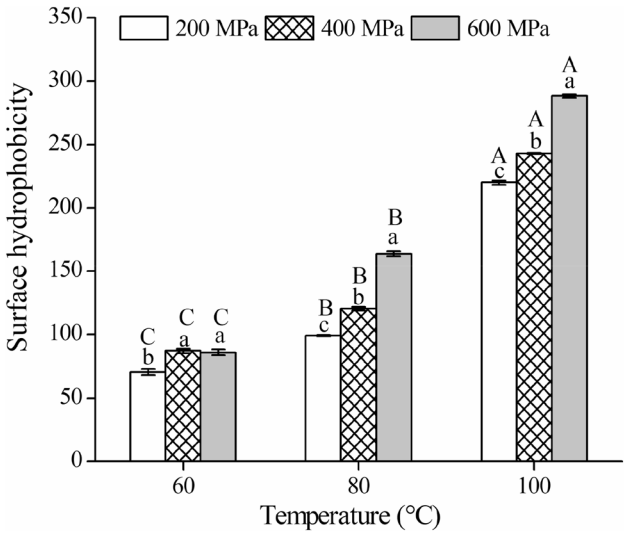

Fig. 6 Effects of HHP combined with heat treatment on surface hydrophobicity of β-conglycinin. The different capital letters indicate significant difference in values under the different temperature groups at the same pressure ($P < 0.05$). The different lowercase letters indicate significant difference in values between the different pressure groups at the same temperature ($P < 0.05$)

quaternary structure of proteins. Wang et al. [43] reported that the denaturation of globulin included changes in the spatial structure and the exposure of hydrophobic sites. Surface hydrophobicity increased significantly as temperature and pressure increased (Fig. 6). The changes in surface hydrophobicity indicated that after HHP combined with heat treatment, the structure stretched and the inside hydrophobic regions in β-conglycinin were more exposed [29, 44]. Because epitopes are mainly located in the hydrophilic region of the protein, increased hydrophobicity of the treated β-conglycinin may be responsible for the decrease in antigenicity [45].

Conclusions

The antigenicity and structural characteristics of β-conglycinin after HHP combined with heat treatment were studied in this paper. Antigenicity and immunogenicity of β-conglycinin reduced significantly after the combined treatment. Compared with the untreated sample, the antigenicity of β-conglycinin was lowest and reduced by 87.04% at 400 MPa and 100 °C for 20 min. After the combined treatment, the contents of α-helix and β-sheet in the secondary structure of β-conglycinin decreased, and the contents of β-turn and random coils increased. The secondary structure of proteins changed from order to disorder. The results of electrophoresis showed that the combined treatment changed the polymerization of the protein significantly. The surface hydrophobicity of β-conglycinin revealed that its structures unfolded and exposed more hydrophobic regions after the combined treatment. These structural changes may have caused the reduction in antigenicity of β-conglycinin. However, the effect of HHP combined with heat treatment on antigenic epitopes of proteins is not yet clear. Changes in the antigenic epitopes using the combined treatment will be studied in our future research.

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Compliance with ethical standards

Conflict of interest All the authors declare that there are no conflicts of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

References

1. Martorell-Aragonés A, Echeverra-Zudaire L, Alonso-Lebrero E, Boné-Calvo J, Martín-Muñoz MF, Nevot-Falcó S, Piquer-Gibert M, Valdesoiro-Navarrete L (2015) Position document: IgE-mediated cow's milk allergy. Allergol Immunopathol 43(5):507–526
2. Friedman M, Brandon DL (2001) Nutritional and health benefits of soy proteins. J Agr Food Chem 49(3):1069–1086
3. Katz Y, Gutierrez-Castrellon P, Gonz Lez MG, Rivas R, Lee BW, Alarcon P (2014) A comprehensive review of sensitization and allergy to soy-based products. Clin Rev Allerg Immunol 46(3):272–281
4. 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. Mol Immunol 70:125–133
5. Yang H, Gao JY, Yang AS, Lu J, Chen HB (2015) Allergenicity characteristics of germinated soybean proteins in a BALB/c mouse model. Regul Toxicol Pharm 72(2):249–255
6. Plaimein A, Elviragonzalez DM (2010) Purification, characterisation, and quantification of the soy allergen profilin (Gly m 3) in soy products. Food Chem 119(4):1671–1680
7. Holzhauser T, Wackermann OW, Bk B-W, Bindslev-Jensen C, Scibilia J, Perono-Garoffo L, Utsumi S, Poulsen LK, Vieths S (2009) Soybean (Glycine max) allergy in Europe: Gly m 5 (beta-conglycinin) and Gly m 6 (glycinin) are potential diagnostic markers for severe allergic reactions to soy. J Allergy Clin Immun 123(2):452–458
8. Krishnan HB, Kim WS, Jang SC, Kerley MS (2009) All three subunits of soybean β-conglycinin are potential food allergens. J Agric Food Chem 57(3):938–943
9. Song YS, Frias J, Martinez-Villaluenga C, Vidal-Valdeverde C, Gonzalezde Mejia E (2008) Immunoreactivity reduction of soybean

meal by fermentation, effect on amino acid composition and antigenicity of commercial soy products. Food Chem 108(2):571–581
10. You JM, Sun P, Df Li, Qiao SY, Qu MR, Wang ZR, Li GH, Pan K (2009) A novel method using immuno-affinity chromatography for isolating β-conglycinin from soybean proteins. Food Chem 117(2):371–374
11. Van Boxtel EL, Van Den Broek LA, Koppelman SJ, Gruppen H (2008) Legumin allergens from peanuts and soybeans: effects of denaturation and aggregation on allergenicity. Mol Nutr Food Res 52(6):674–682
12. Verma AK, Kumar S, Das M, Dwivedi PD (2012) Impact of thermal processing on legume allergens. Plant Food Hum Nutr 67(4):430–441
13. Huang L, Clare Mills EN, Carter JM, Morgan MRA (1998) Analysis of thermal stability of soya globulins using monoclonal antibodies. Biochem Biophys Acta 1388(1):215–226
14. Li HJ, Zhu KX, Zhou HM, Peng W (2012) Effects of high hydrostatic pressure treatment on allergenicity and structural properties of soybean protein isolate for infant formula. Food Chem 132(2):808–814
15. Peñas E, Gomez R, Frias J, Baeza ML, Vidal-Valverde C (2010) High hydrostatic pressure effects on immunoreactivity and nutritional quality of soybean products. Food Chem 125(2):423–429
16. Peñas E, Préstamo G, Polo F, Gomez R (2006) Enzymatic proteolysis, under high pressure of soybean whey: analysis of peptides and the allergen Gly m 1 in the hydrolysates. Food Chem 99(3):569–573
17. Long FY, Yang X, Sun J, Zhong QY, Wei JP, Qu P, Yue TL (2016) Effects of combined high pressure and thermal treatment on the allergenic potential of peanut in a mouse model of allergy. Innov Food Sci Emerg 35:133–138
18. Bogahawaththa D, Buckow R, Chandrapala J, Vasiljevic T (2018) Comparison between thermal pasteurization and high pressure processing of bovine skim milk in relation to denaturation and immunogenicity of native milk proteins. Innov Food Sci Emerg 47:301–308
19. Thanh VH, Shibasaki K (1976) Major proteins of soybean seeds. A straightforward fractionation and their characterization. J Agric Food Chem 24(6):1117–1121
20. Liu C, Wang HL, Cui ZM, He XL, Wang XS, Zeng XX, Ma H (2007) Optimization of extraction and isolation for 11S and 7S globulins of soybean seed storage protein. Food Chem 102(4):1310–1316
21. Bu GH, Zhu TW, Chen FS, Zhang N, Liu KL, Zhang LF, Yang HS (2015) Effects of saccharide on the structure and antigenicity of β-conglycinin in soybean protein isolate by glycation. Eur Food Res Technol 240(2):285–293
22. Bu GH, Zhang N, Chen FS (2015) The influence of glycosylation on the antigenicity, allergenicity, and structural properties of 11S-lactose conjugates. Food Res Int 76:511–517
23. Dhakal S, Liu C, Zhang Y, Roux KH, Sathe SK, Sathe VM (2014) Effect of high pressure processing on the immunoreactivity of almond milk. Food Res Int 62(1):215–222
24. Savadkoohi S, Bannikova A, Mantri N, Kasapis S (2016) Structural modification in condensed soy glycinin systems following application of high pressure. Food Hydrocolloid 53:115–124
25. Luo Y, Li M, Zhu KX, Guo XN, Guo XN, Zhou HM (2016) Heat-induced interaction between egg white protein and wheat gluten. Food Chem 197:699–708
26. Lakshmanan R, Lamballerie MD, Jung S (2006) Effect of soybean-to-water ratio and pH on pressurized soymilk properties. J Food Sci 71(9):E384–E391
27. Puppo MC, Beaumal V, Speroni F, de Lamballerie M, Añón MC, Añón M (2011) β-Conglycinin and glycinin soybean protein emulsions treated by combined temperature–high-pressure treatment. Food Hydrocolloid 25(3):389–397
28. Xi J, He MX (2017) High hydrostatic pressure (HHP) effects on antigenicity and structural properties of soybean β-conglycinin. J Food Sci Tech 55(2):630–637
29. Kleber N, Maier S, Hinrichs J (2007) Antigenic response of bovine β-lactoglobulin influenced by ultra-high pressure treatment and temperature. Innov Food Sci Emerg 8(1):39–45
30. Zhang T, Lv CY, Yun SJ, Liao XY, Zhao GH, Leng XJ (2012) Effect of high hydrostatic pressure (HHP) on structure and activity of phytoferritin. Food Chem 130(2):273–278
31. Long FY, Yang X, Wang RR, Hu XS, Chen F (2015) Effects of combined high pressure and thermal treatments on the allergenic potential of shrimp (*Litopenaeus vannamei*) tropomyosin in a mouse model of allergy. Innov Food Sci Emerg 29:119–124
32. Yang X, Sun J, Tao JM, Ma YS, Wei JP, Long FY (2017) The allergenic potential of walnuts treated with high pressure and heat in a mouse model of allergy. Innov Food Sci Emerg 39:165–170
33. Meinlschmidt P, Brode V, Sevenich R, Ueberham E, Schweiggert-Weisz U, Lehmann J, Lehmann C, Knorr D, Eisner P (2016) High pressure processing assisted enzymatic hydrolysis. An innovative approach for the reduction of soy immunoreactivity. Innov Food Sci Emerg 40:58–67
34. Wang JM, Yang XQ, Yin SW, Zhang Y, Tang CH, Li BS, Yuan DB, Guo J (2011) Structural rearrangement of ethanol-denatured soy proteins by high hydrostatic pressure treatment. J Agric Food Chem 59(13):7324–7332
35. Huson MG, Strounina EV, Kealley CS, Rout MK, Rout JS, Appelqvist IAM, Gidley MJ, Gilbert EP (2011) Effects of thermal denaturation on the solid-state structure and molecular mobility of glycinin. Biomacromol 12(6):2092–2102
36. Dzwolak W, Kato M, Taniguchi Y (2002) Fourier transform infrared spectroscopy in high-pressure studies on proteins. BBA-Biomembranes 1595(1):131–144
37. Dong AC, Huang P, Caughey WS (1992) Redox-dependent changes in beta-extended chain and turn structures of cytochrome c in water solution determined by second derivative amide I infrared spectra. Biochemistry 31(1):182–189
38. Bu GH, Zhu TW, Chen FS (2017) The structural properties and antigenicity of soybean glycinin by glycation with xylose. J Sci Food Agr 97(7):2256–2262
39. Azdad O, Mejrhit N, El-Kabbaoui M, Chda A, Ouahidl I, Tazi A, Bencheikh R, Aarab L (2018) Effect of heating and enzymatic hydrolysis on casein cow milk sensitivity in Moroccan population. Food Agr Immunol 29(1):424–433
40. Tong P, Gao JY, Chen HB, Li X, Zhang Y, Jian S, Wichers H, Wu ZH, Yang AS, Liu FH (2012) Effect of heat treatment on the potential allergenicity and conformational structure of egg allergen ovotransferrin. Food Chem 131(2):603–610
41. Yang AS, Deng H, Zu QQ, Lu J, Wu ZH, Li X, Tong P, Chen HB (2018) Structure characterization and IgE-binding of soybean 7S globulin after enzymatic deglycosylation. Int J Food Prop 21(1):171–182
42. Abu JO, Müller K, Duodu KG, Minnaar A (2006) Gamma irradiation of cowpea (*Vigna unguiculata* L. Walp) flours and pastes: effects on functional, thermal and molecular properties of isolated proteins. Food Chem 95(1):138–147
43. Wang ZJ, Li Y, Jiang LZ, Qi BK, Zhou LY (2014) Relationship between secondary structure and surface hydrophobicity of soybean protein isolate subjected to heat treatment. J Chem 2014:1–10
44. Arzeni C, Martínez K, Zema P, Arias A, Pérez OE, Pérez AMR (2012) Comparative study of high intensity ultrasound effects on food proteins functionality. J Food Eng 108(3):463–472
45. Sun XL, Shan XH, Yan ZH, Zhang YZ, Guan L (2013) Prediction and characterization of the linear IgE epitopes for the major soybean allergen β-conglycinin using immunoinformatics tools. Food Chem Toxicol 56:254–260