

High hydrostatic pressure treatment reduces the potential antigenicity of β -conglycinin by changing the protein structure during *in vitro* digestion

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Abstract

BACKGROUND: High hydrostatic pressure (HHP) treatment has been used to alleviate the allergenicity of soybeans, but there are little data about the potential antigenicity of β -conglycinin after HHP treatment.

RESULTS: We examined the effects of HHP treatment on the antigenicity and structure of β -conglycinin. When the pressure was 300 and 400 MPa, HHP treatment reduced the immunoglobulin (Ig)G binding capacity of β -conglycinin, while its IgE binding capacity did not change significantly. After *in vitro* digestion, both the IgE and IgG binding of β -conglycinin was obviously inhibited after HHP treatment at 400 MPa and 60 °C, although its binding capacity with linear epitope antibodies increased. Moreover, HHP treatment changed the secondary structure of β -conglycinin, the content of α -helix and random coils increased, while the β -sheet and β -turn decreased. After HHP treatment, the conformational structure was unfolded so that a large number of hydrophobic regions were exposed.

CONCLUSION: HHP treatment alleviated the potential antigenicity of β -conglycinin by modifying its structure, which facilitated *in vitro* digestion and destroyed epitopes. This research provides a new insight into the mechanism of HHP treatment that affects the sensitization of soy protein allergens.

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Keywords: β -conglycinin; high hydrostatic pressure; potential antigenicity; *in vitro* digestion

INTRODUCTION

As an important economic crop, soybean (*Glycine max*) is known as a perfect functional food because of its high-quality nutritional value and excellent processing performance.^{1,2} β -Conglycinin is the main storage protein in soybeans, and it accounts for 40% of total soybean protein. The benefits of β -conglycinin in reducing obesity and metabolic diseases have attracted the attention of researchers.^{3–5} However, as a most important protein allergen, β -conglycinin induces food allergic reactions in humans.⁶ Therefore, reduction or even complete elimination of sensitization of β -conglycinin will expand its utilization in the food industry.

High hydrostatic pressure (HHP), an emerging non-thermal processing method, has been used to preserve the original nutritional value of food to the maximum extent. Numerous studies have shown that the sensitization of protein allergens after HHP treatment was reduced as a result of their conformational structure change.^{7,8} HHP treatment did not destroy the nutritional value of soybean products, but significantly eliminated the antigenicity of soybeans.⁹ This was due to the secondary and tertiary structure of allergic proteins in soybeans changing after HHP treatment, which prevented the IgE in the serum of soybean allergy patients from recognizing the epitopes.^{10,11} Xi and He¹² and Bu *et al.*¹³ revealed that HHP treatment of 400 MPa significantly inhibited the immunoglobulin (Ig)G binding capacity of β -conglycinin, and they speculated that this

was related to the disruption of original epitopes of β -conglycinin after HHP treatment. However, there are few studies on the changes in IgE binding ability and epitope of HHP-treated β -conglycinin.

In previous studies, synthetic epitope antibodies and spectroscopy were used to analyze changes in the potential antigenicity and structure of β -conglycinin after heat treatment.¹⁴ The results showed that heat treatment weakened the binding capacity of β -conglycinin with antibodies. Moreover, heated β -conglycinin exposed more enzymatic hydrolysis sites and was easier to digest by proteases, which destroyed a large number of epitopes, although some linear epitopes were retained. Thus, the use of epitope antibodies and spectroscopic methods was helpful in evaluating the effects of HHP treatment on the potential antigenicity and structure of β -conglycinin. The results of this study will provide new insights into the mechanism of how HHP treatment affects the sensitization of soy protein allergens.

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MATERIALS AND METHODS

β -Conglycinin and antibodies

The extraction of β -conglycinin was performed based on Liu *et al.*,¹⁵ with some modification. The protein content and purity of extracted β -conglycinin was 852 and 933 g kg⁻¹, respectively.¹⁶ Moreover, the anti- β -conglycinin rabbit serum was generated by our laboratory. The epitope polyclonal antibodies (PA) were prepared following the published study.¹⁶ The β -conglycinin allergic serum pool was prepared with soy allergy sera obtained from Henan Children's Hospital (Zhengzhou, China).

HHP treatment

β -Conglycinin samples were subjected to HHP treatment using a 1 L HHP vessel (Stansted Fluid Power Ltd, Stansted, UK) equipped with temperature and pressure control units (pressure range: 0–600 MPa; operating temperatures: 0–80 °C). The isolated β -conglycinin samples were diluted to 2 mg mL⁻¹ with 50 mmol L⁻¹ phosphate-buffered saline (PBS, pH 7.0) and packed into polyethylene bags. The protein solution was treated with HHP for 20 min and the pressure was set to 50, 100, 200, 300 and 400 MPa; meanwhile, each pressure group was maintained at 20, 40 and 60 °C, respectively. Untreated protein solution was used as control.

IgG and IgE binding

The IgG and IgE binding capacities of β -conglycinin were evaluated using indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) described in our previous study.¹⁶ The 100 μ L of β -conglycinin antigen (0.1 μ g mL⁻¹ for IgG, 20 μ g mL⁻¹ for IgE) was coated in a microtiter plate and incubated at 4 °C overnight. Meanwhile, the HHP-treated sample solution was mixed with an equivalent volume of serum. Next day, the plate was blocked with blocking buffer (0.01 mol L⁻¹ PBS containing 1 g L⁻¹ Tween-20 and 10 g L⁻¹ BSA) and incubated at 37 °C for 2 h. Then, 100 μ L per well of anti- β -conglycinin rabbit serum or human allergy serum was added to the plate and incubated at 37 °C for 1 h. Following that, the plates were coated with 100 μ L horseradish peroxidase (HRP)-labeled goat anti-rabbit IgG (1:5000 dilution; A6154, Sigma Chemical Co. Ltd, St Louis, MO, USA) or goat anti-human IgE (1:2 dilution; A9667, Sigma Chemical Co. Ltd) and again incubated at 37 °C for 1 h. Subsequently, 100 μ L per well

of 3,3',5,5'-tetramethylbenzidine (TMB; PR1200, Solarbio, Beijing, China) was added, and the chromogenic reaction lasted for 15 min and terminated with 50 μ L per well H₂SO₄ (2 mol L⁻¹). An ELISA plate reader was used to record the absorbance of each well at 450 and 630 nm.

Epitope polyclonal antibodies binding

Epitope polyclonal antibodies were synthesized and used to evaluate the binding of β -conglycinin with epitope polyclonal antibodies using ic-ELISA; the detailed procedure was described in our previous published work.¹⁴ The β -conglycinin (0.1 μ g mL⁻¹, 100 μ L) was coated in a microtiter plate and incubated at 4 °C overnight. After blocking, 100 μ L per well of epitope polyclonal antibodies (the concentration of addition was determined by preliminary experiment) was added to the plate and incubated at 37 °C for 1 h. Addition of only epitope polyclonal antibodies served as a positive control. The following processes refer to the earlier section on 'IgG and IgE binding'.

In vitro digestion

The digestive procedure of β -conglycinin, including gastric and intestinal simulation, was performed *in vitro* following the method from our research.^{14,17} The 10 mg of different samples was mixed in 8 mL gastric digestive juices (0.12 mol L⁻¹ NaCl) and stirred for 15 min. The mixed solution was adjusted to pH 2.0 with 1 mol L⁻¹ HCl, and its volume was brought to 10 mL. Subsequently, 0.1 mL pepsin solution (2 mg mL⁻¹) was added to each sample and gastric simulation digestion was carried out at 37 °C for 1 h. Then, the pH was raised dropwise to 6.0 with 1 mol L⁻¹ NaHCO₃, and 2.5 mL of intestinal digestive juices (containing 0.1 mg mL⁻¹ trypsin and 0.6 mg mL⁻¹ bile extract) was added. The pH was adjusted to pH 7.5 with 1 mol L⁻¹ NaOH, and the volume was brought to 15 mL. The intestinal simulation digestion was carried out at 37 °C for 2 h. Finally, the mixed solution was heated at 85 °C for 10 min to inactivate the enzyme. Digestions were performed in triplicate. All digestion samples were collected and stored at –20 °C until used.

Potential antigenicity

The potential antigenicity of β -conglycinin after HHP treatment was evaluated using an *in vitro* digestion model.

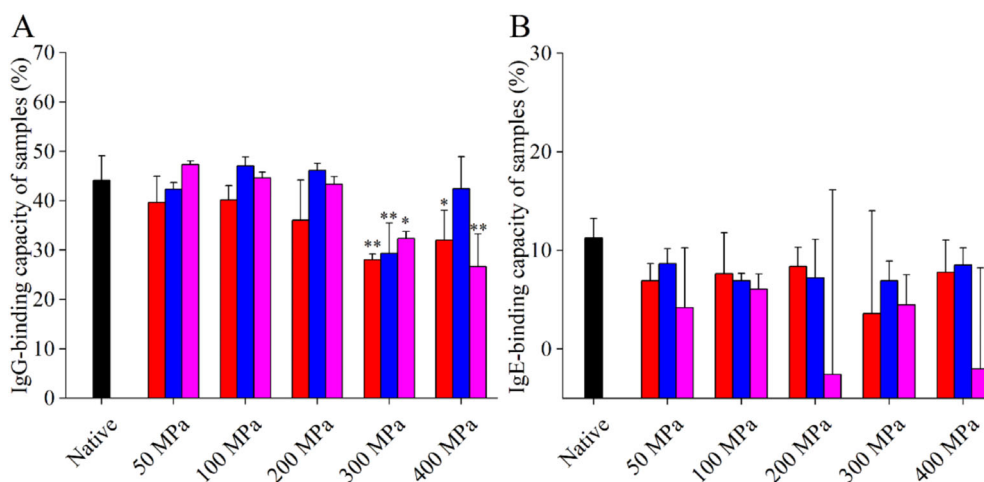


Figure 1. Binding capacity of HHP-treated β -conglycinin with IgG (A) and IgE (B). Native: native β -conglycinin; 50–400 MPa: HHP treatment of 50, 100, 200, 300 and 400 MPa. Red bars: 20 °C; blue bars: 40 °C; purple bars: 60 °C; * P < 0.05; ** P < 0.01.

ic-ELISA

The binding of β -conglycinin with IgG, IgE and epitope polyclonal antibodies after HHP treatment was analyzed by ic-ELISA. The method was carried out as described above.

Western blotting

Western blotting could visually evaluate the epitope of β -conglycinin after HHP treatment.¹⁶ First, SDS-PAGE was performed to isolate the subunits of β -conglycinin; the gels were then transferred to a polyvinylidene fluoride (PVDF) membrane. The membranes were blocked with 10 mol L⁻¹ PBS containing 50 g L⁻¹ non-fat dry milk and were incubated with antibodies. The epitope polyclonal antibodies (1 μ L PA-1 to PA-10 mixed, 1:1000 dilution) were used as primary antibodies

and HRP-labeled goat anti-rabbit IgG (1:5000 dilution) was used as secondary antibody. Subsequently, the PVDF membrane was incubated with 3-amino-9-ethylcarbazole (AEC) peroxidase substrate at room temperature for 15 min using an AEC kit (A2010, Solarbio, Beijing, China). Finally, the PVDF membrane was placed in double-distilled water to terminate the coloration reaction.

Conformational changes of β -conglycinin after HHP treatment

SDS-PAGE

The distribution of molecular weights of HHP-treated β -conglycinin was assessed with SDS-PAGE.^{10,16} Samples were stirred into 1 mL loading buffer (0.5 mol L⁻¹ Tris-HCl containing 10 g L⁻¹ SDS, 20 g L⁻¹ β -mercaptoethanol, 200 g L⁻¹ glycerol

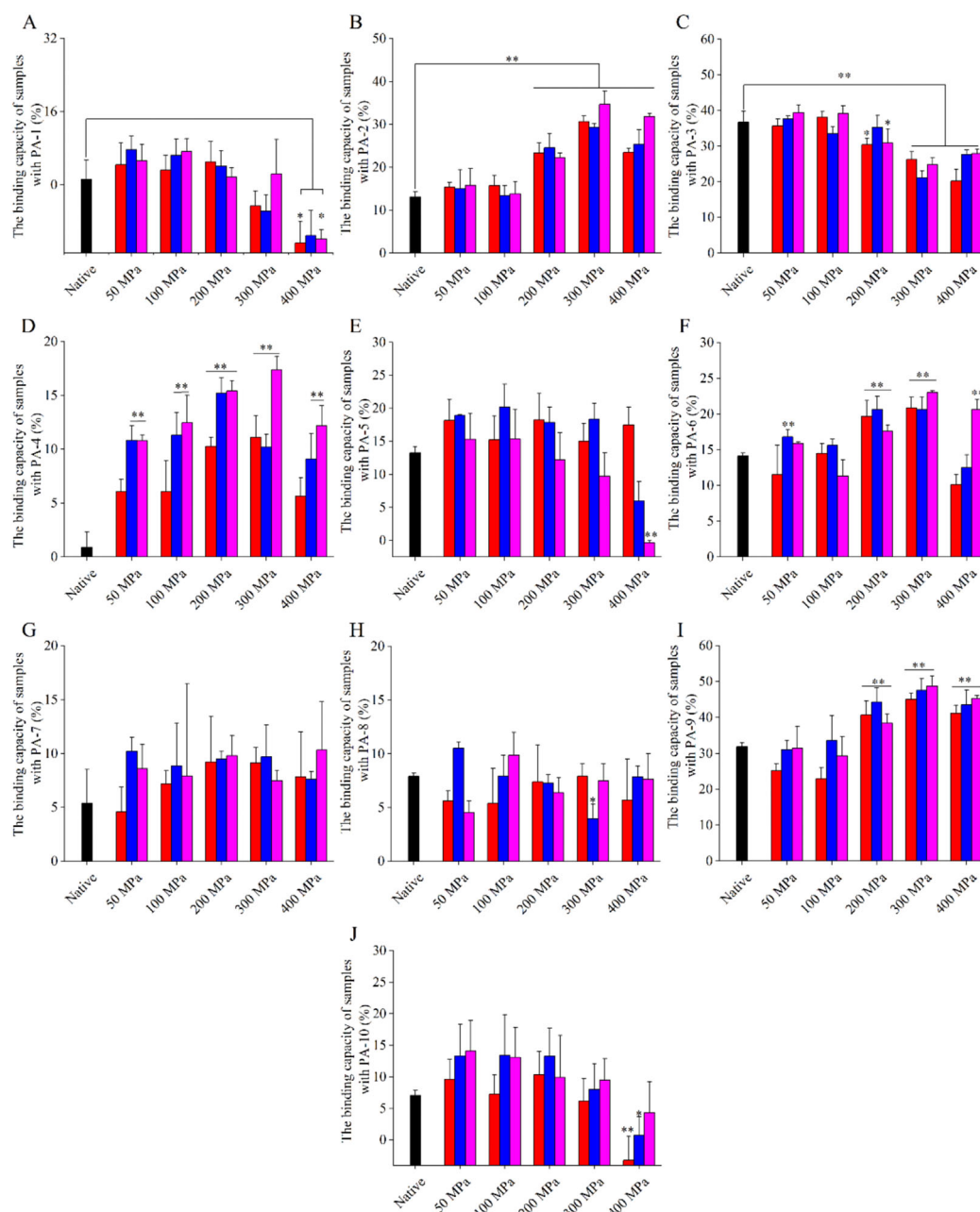


Figure 2. The binding capacity of HHP-treated β -conglycinin with epitope polyclonal antibodies. A–J: PA-1 to PA-10; Native: native β -conglycinin; 50–400 MPa: HHP treatment of 50, 100, 200, 300 and 400 MPa. Red bars: 20 °C; Blue bars: 40 °C. Purple bars: 60 °C; *, $P < 0.05$; **, $P < 0.01$.

and 0.25 g L⁻¹ bromophenol blue). In non-reduced SDS-PAGE, the loading buffer did not contain β -mercaptoethanol.

Fourier transform infrared (FTIR)

The infrared radiation of samples was recorded using a WQF-510 infrared spectrometer (Beijing Ruili Analytical Instruments Co. Ltd, Beijing, China). FTIR was carried out at ambient temperature.¹⁸ All samples were scanned 32 times with a wave number of 4000 to 400 cm⁻¹ and a resolution of 4 cm⁻¹. Peak Fit (version 4.12) was used to analyze the secondary structure of samples.

Fluorescence spectrum

The changes in hydrophobicity of HHP-treated β -conglycinin were measured with a fluorescence spectrometer (Varian Inc., Agilent Technologies, Santa Clara, CA, USA) according to the method of Yang *et al.*¹¹ The fluorescence intensity of each sample was recorded at excitation and the emission wavelengths of 390 nm and 400 to 700 nm, respectively.

UV absorption spectrum

The UV absorption spectrum of samples was measured with a UV-visible spectrophotometer (Puxi Analytical Instrument Co. Ltd, Beijing, China) and the procedure was described in our previous research.¹⁴

Statistical analysis

SPSS 16.0 was used to analyze the obtained data, which were expressed as the mean $\pm$ standard deviation. Differences between data were calculated by analysis of variance using Tukey's multiple range test.

RESULTS AND DISCUSSION

IgG and IgE binding

The serum from rabbits and soybean allergy patients were used to detect the IgG and IgE binding capacity of β -conglycinin after HHP treatment, respectively. At pressures of 300 MPa (20, 40 or 60 °C) and 400 MPa (20 or 60 °C), the IgG binding capacity of β -conglycinin decreased significantly, which was reduced by 39.61% at 400 MPa and 60 °C (Fig. 1(A)). Of note, higher temperature reduced the IgG binding capacity of HHP-treated β -conglycinin at 400 MPa, but it was enhanced at 300 MPa. In

addition, HHP treatment did not reduce the binding capacity of β -conglycinin with IgE, even though its mean values were lower at 300 and 400 MPa at 60 °C (Fig. 1(B)).

Some studies have reported that HHP effectively reduced the IgG binding capacity of β -conglycinin at 400 MPa at room temperature,^{12,13} which was similar to the results of this study (Fig. 1(A)). Moreover, HHP treatment inhibited the IgE binding capacity of soybean proteins in other studies.^{9,11} A widely accepted view was that HHP treatment changed the structure of β -conglycinin, which destroyed its epitope, so that the binding ability of the antibody with the epitope was reduced.^{19,20}

In this study, there was no change in IgE binding capacity of HHP-treated β -conglycinin due to differences in processing methods and subjects (β -conglycinin, not soybean proteins). Previous work indicated that the reduction in antigenicity of β -conglycinin at temperatures > 80 °C was mainly caused by heat treatment rather than HHP treatment.¹³ Therefore, we set a lower temperature to explain the effect of HHP on IgE/IgG binding capacity of β -conglycinin and its synergistic effect with heat treatment; meanwhile, the lower temperature ensured that both methods were conducted simultaneously. When the temperature was <60 °C, the binding of β -conglycinin with IgG became more difficult as pressure increased. Moreover, with increasing temperature, the IgG binding of β -conglycinin decreased significantly when HHP was carried out at 400 MPa, while the IgG binding capacity increased at 300 MPa. This suggested that these two methods had a synergistic effect on the IgG and IgE binding capacity of β -conglycinin.

Binding of β -conglycinin with epitope polyclonal antibodies

As shown in Fig. 2, with increasing pressure HHP treatment enhanced the binding of β -conglycinin with PA-1, PA-3, PA-5 and PA-10 compared with the native group, while binding with PA-2, PA-4, PA-6 and PA-9 decreased. Also, the binding of β -conglycinin with PA-7 and PA-8 was barely affected after HHP treatment; even the binding of β -conglycinin with PA-8 was reduced significantly at 300 MPa and 40 °C. The reason for the change may be attributed to the structural change of β -conglycinin after HHP treatment, which buried the original epitope or exposed the antigen epitope.^{11,12,21} A previous study suggested that all ten

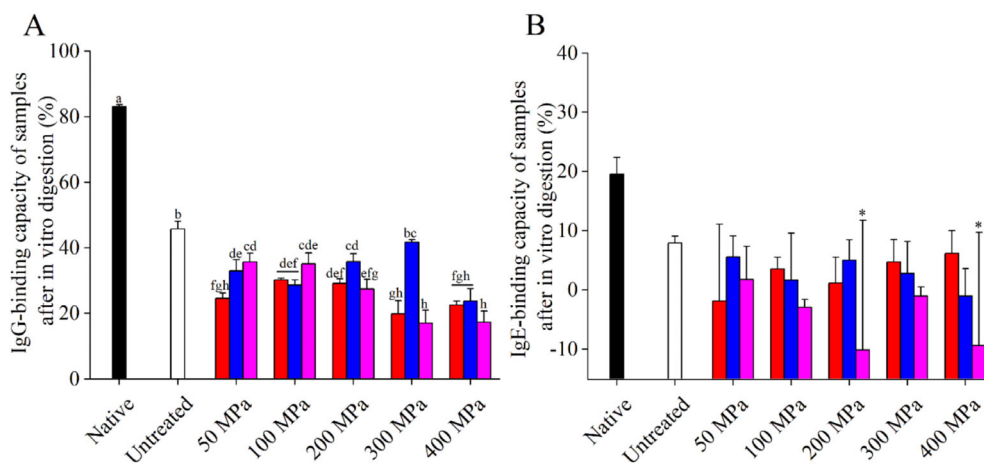


Figure 3. Binding capacity of HHP-treated β -conglycinin after *in vitro* digestion with IgG (A) and IgE (B). Native: native β -conglycinin; Untreated: untreated β -conglycinin; 50–400 MPa: HHP treatment of 50, 100, 200, 300 and 400 MPa. Red bars: 20 °C; blue bars: 40 °C; purple bars: 60 °C; * $P < 0.05$. Different lower-case letters indicate a significant difference among samples ($P < 0.05$).

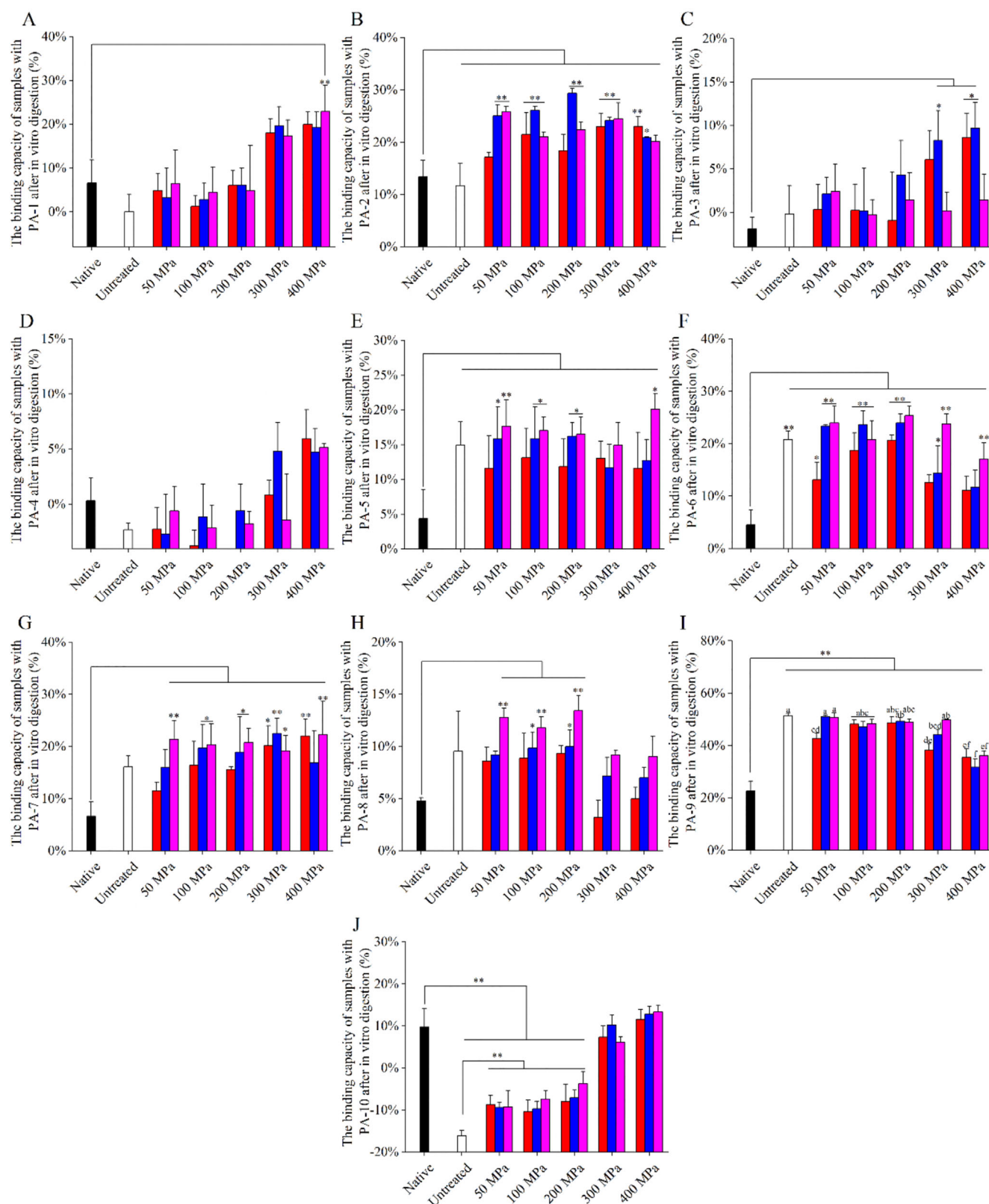


Figure 4. Binding capacity of HHP-treated β -conglycinin after *in vitro* digestion with ten epitope polyclonal antibodies. A–J: PA-1 to PA-10; Native: native β -conglycinin; Untreated: untreated β -conglycinin; 50–400 MPa: HHP treatment of 50, 100, 200, 300 and 400 MPa. Red bars: 20 °C; blue bars: 40 °C; purple bars: 60 °C; *P < 0.05; **P < 0.01. Different lower-case letters indicate a significant difference among samples (P < 0.05).

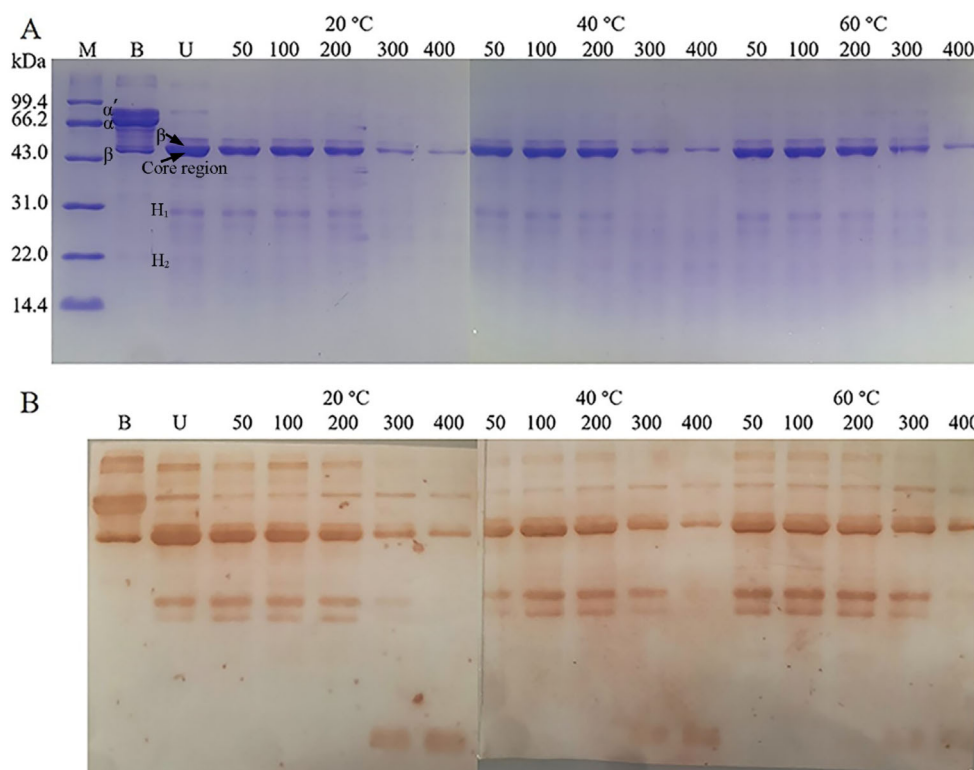


Figure 5. SDS-PAGE (A) and western blotting (B) of HHP-treated β -conglycinin after *in vitro* digestion with epitope polyclonal antibodies. M: protein standard marker; B: native β -conglycinin; U: untreated β -conglycinin; 50–400: HHP treatment of 50, 100, 200, 300 and 400 MPa.

Table 1. Molecular weight of bands of lanes B and U in Fig. 4(A), analyzed by Gel Analyzer software (Version 4.0)

Number	Name of lane B	Molecular weight (kDa)	Name of lane U	Molecular weight (kDa)
1	α'	81.053	—	—
2	α	66.200	—	—
3	β	49.246	—	—
4	—	—	β and extension	47.462
5	—	—	H ₁	29.978
6	—	—	H ₂	21.406

epitope antibodies specifically recognized IgG epitopes, and PA-2 and PA-6 also recognized IgE epitopes specifically.¹⁶ Interestingly, HHP treatment inhibited β -conglycinin binding with IgG, but not all epitope antibodies declined simultaneously (Figs 1(A) and 2). Furthermore, the PA-2 and PA-6 binding of β -conglycinin increased significantly, while its IgE binding capacity remained the same. This may be due to the destruction of conformational epitope after HHP treatment, rather than linear epitope.

The effects of HHP treatment on antigenicity and epitopes of β -conglycinin were further evaluated by *in vitro* simulated digestion experiments. On the one hand, a common characteristic of epitopes is their ability to remain intact during gastrointestinal digestion.²¹ The resistance of the protein structure to enzymes is related to the expression of epitopes after *in vitro* digestion, because epitope antibodies recognize a continuous sequence of amino acids. On the other hand, after enzymatic hydrolysis, the conformational epitopes of proteins are destroyed, and their linear epitopes are cleaved, but it is possible that some epitopes are retained or the epitopes originally inside the protein structure are exposed.^{22,23}

Evaluation of potential antigenicity

After digestion, the binding of untreated β -conglycinin with IgG and IgE decreased compared with native protein (Fig. 3(A)). Also, HHP treatment weakened the IgG binding capacity of β -conglycinin at 300 and 400 MPa at 60 °C. The binding of untreated β -conglycinin with IgE was not reduced after digestion compared with the native group (Fig. 3(B)), but the IgE binding of digested β -conglycinin decreased significantly at 60 °C under 200 and 400 MPa. Apparently, the HHP-treated β -conglycinin showed lower IgE and IgG binding capacity after *in vitro* digestion than before digestion (Figs 1 and 3). The suppression of IgE and IgG binding of β -conglycinin after HHP treatment was strong because the HHP treatment promoted the destruction of antigen epitopes during digestion. *In vitro* simulated digestion contributed to the accurate evaluation of the potential antigenicity of β -conglycinin, because the antigen epitopes that resisted enzymatic hydrolysis induced allergic reactions.²⁴

After *in vitro* digestion, the HHP-treated β -conglycinin exhibited high binding capacity with ten epitope antibodies (Fig. 4).

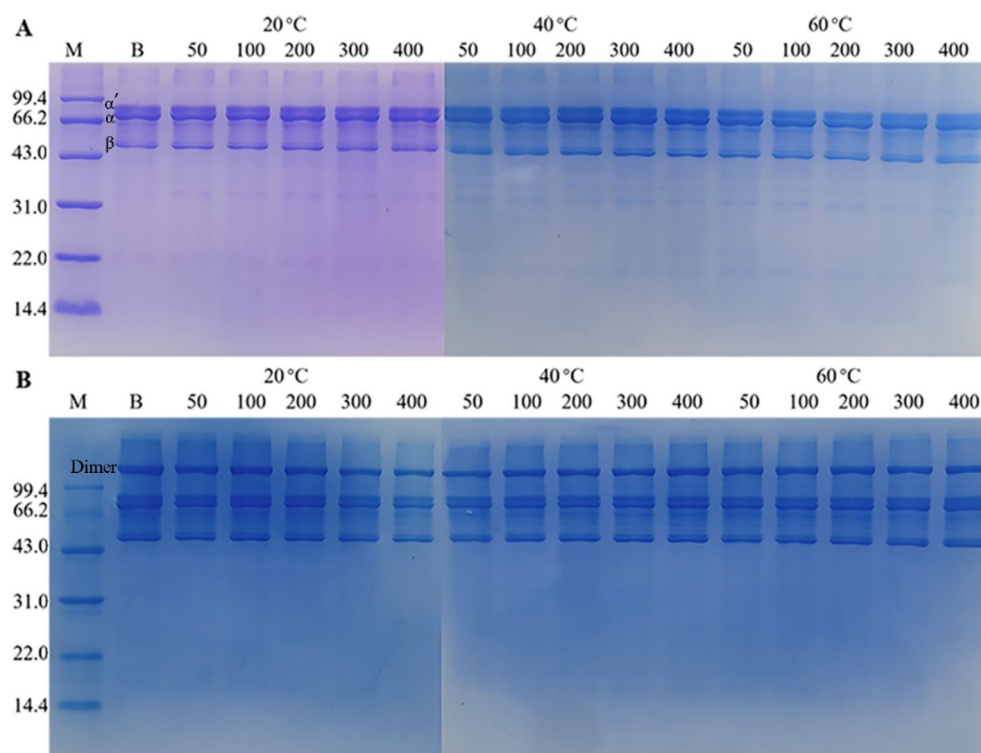


Figure 6. Reduced SDS-PAGE (A) and non-reduced SDS-PAGE (B) of HHP-treated β -conglycinin. M: protein standard marker; B: native β -conglycinin; 50–400: HHP treatment of 50, 100, 200, 300 and 400 MPa.

Although HHP treatment inhibited the binding capacity of β -conglycinin with PA-10 at low pressure (50–200 MPa), its binding capacity with PA-10 was still stronger than untreated β -conglycinin (Fig. 4(J)). In addition, HHP treatment did not affect the binding of β -conglycinin with PA-4 compared with native group, but the mean value of its binding capacity with PA-4 increased with increased pressure (Fig. 4(D)). In conclusion, the binding of HHP-treated β -conglycinin with epitope antibodies was enhanced. This may be attributed to the fact that the HHP-treated β -conglycinin was easily hydrolyzed, so more antigen epitopes that were resistant to digestion were exposed and retained.^{21–23} Furthermore, the IgG/IgE binding of HHP-treated β -conglycinin was lower after digestion than before (Fig. 3). This indicated that the conformational epitope of β -conglycinin was cleaved by the protease after HHP treatment, because the binding ability of the protein with linear epitopes was retained (Fig. 4). To confirm the above idea, the subunits of β -conglycinin were isolated using SDS-PAGE, and then the effect of HHP treatment on the linear epitopes was evaluated using epitope antibodies.

The distribution of molecular weights of HHP-treated β -conglycinin after *in vitro* digestion is shown in Fig. 5. After digestion, the band intensity of the α and the α' subunits almost disappeared, but the band intensity of the β subunit (about 47 kDa), H₁ (<31 kDa) and H₂ (<20 kDa) increased sharply (lane U in Fig. 5(A); Table 1). Possibly, the extension regions of the α' and α subunits of β -conglycinin were hydrolyzed, and their core regions remained after digestion. Compared with the core region, the extension regions were hydrophilic, rich in aspartic acid, and were disrupted easily by proteases.^{25,26}

Moreover, with the decrease in band intensity of the α and α' subunits, the band intensity of 47 kDa and H₁ increased (Fig. 5 (B)). Because both the α and α' subunits consist of a core region

and an extension region, and the amino acid sequences of the core region and the β subunit were 420–440 amino acid residues and 439 amino acid residues, respectively, the core region and the β subunit of β -conglycinin after digestion exhibited similar molecular weights. This suggested that the α and α' subunits were hydrolyzed into core regions and extension regions, which bound specifically to epitope antibodies.^{25,27,28} HHP treatment promoted the digestion of β -conglycinin. The band intensity of the β subunit and the core region decreased with increasing pressure, especially at 400 MPa, and almost all proteins were digested (Fig. 5, lane 400).

For the degree of digestion, temperature did not show a synergistic effect, and the increased temperature did not facilitate further enzymatic hydrolysis of β -conglycinin. This may be due to the thermal stability of β -conglycinin, and a higher temperature was needed to change its structure. In addition, the result of SDS-PAGE showed that the IgG and IgE binding capacity of HHP-treated β -conglycinin was inhibited after digestion, which was related to enzymolysis (Fig. 3).

The HHP-treated protein was easily attacked by proteases, so the epitope was cleaved.²⁰ However, the HHP-treated β -conglycinin bound strongly with epitope antibodies after digestion (Fig. 4). We speculate that the conformational epitopes of β -conglycinin were destroyed by proteases, although more linear epitopes that were resistant to digestion were exposed and retained. Therefore, epitope polyclonal antibodies were used to explore the effect of HHP treatment on linear epitopes of β -conglycinin during digestion by using western blotting (Fig. 5 (B)). Strong immunoreactive bands appeared on the PVDF membrane, which indicated that three subunits of β -conglycinin bound effectively with epitope antibodies (lane B). Similar to the results of SDS-PAGE, only the α and α' subunits of β -conglycinin

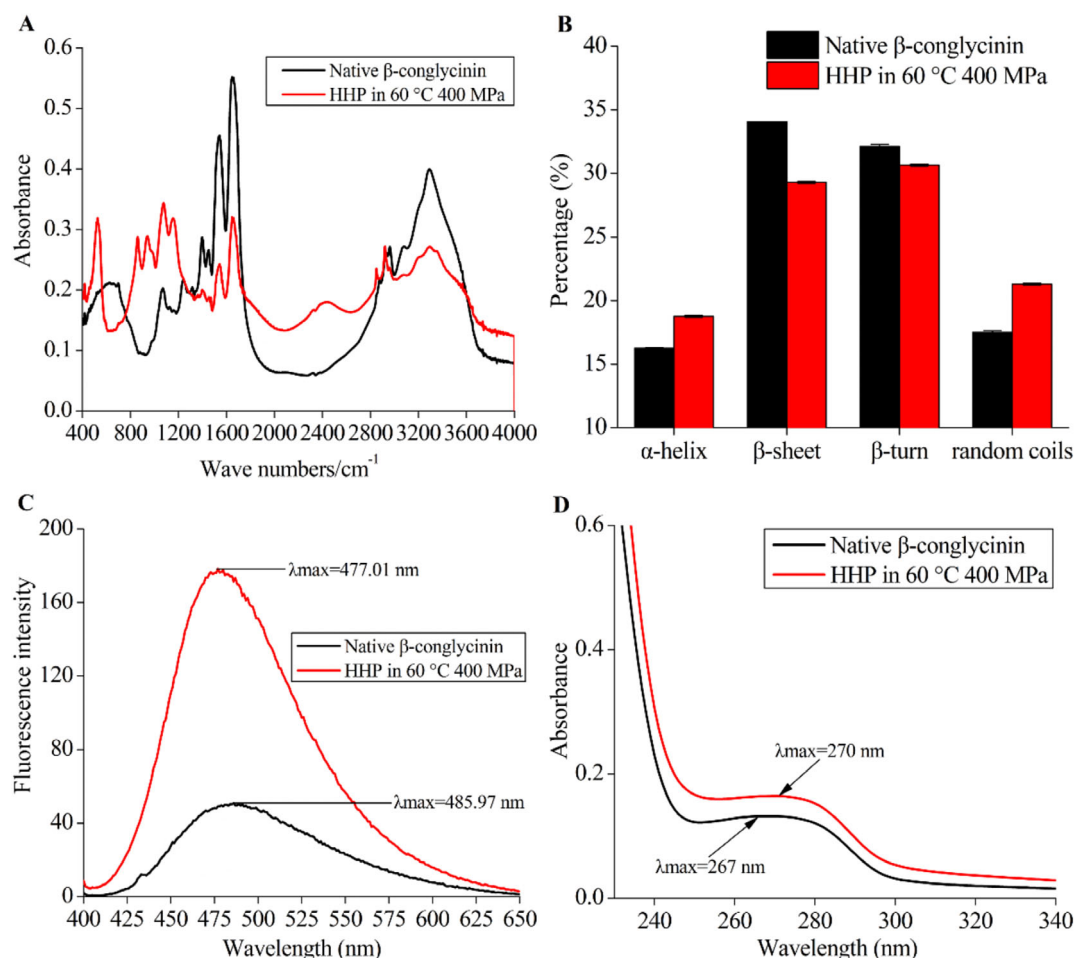


Figure 7. Structure of β -conglycinin after HHP treatment. (A) FT-IR spectroscopy; (B) secondary structural content; (C) emission fluorescence spectrum; (D) UV spectrum.

bound slightly with epitope antibodies after digestion, while the band intensities at the core region and the β subunit were enhanced significantly. Also, a new immune response band appeared in H₁ (lane U). After digestion by protease, the α and α' subunits of β -conglycinin were divided into an expansion region and a core region, which corresponded to the result of SDS-PAGE. Moreover, the result of western blotting showed that the band at H₁ might be derived from the extension regions of the α and α' subunits of the protein because there were obvious immune response bands. As the pressure increased, the intensity of the band produced by the protein bound with the epitope antibodies was weakened after HHP treatment, especially at 400 MPa; the protein band was the weakest, and the band at H₁ disappeared almost completely at 400 MPa. This was mainly due to the fact that HHP treatment promoted the enzymatic hydrolysis of the protein, and a large number of epitopes was destroyed, so that the epitope antibodies did not recognize it. Of note, the obvious immune response band appeared at a molecular weight < 14.4 kDa, because the core region and the extension region could be hydrolyzed further by proteases into small peptides. These peptides could bind specifically to epitope antibodies.¹⁴

Overall, HHP treatment alleviated the potential sensitization of β -conglycinin. After HHP treatment, β -conglycinin was more easily hydrolyzed by protease; thus, linear epitopes were destroyed, and their IgG and IgE binding abilities were inhibited. However, the

potential sensitization of HHP-treated β -conglycinin was low, but it still did not disappear completely because some linear epitopes were retained after *in vitro* digestion and were recognized specifically by antibodies. In our previous published study, the result showed that the structural changes of heated β -conglycinin were closely related to their antigenicity and epitope changes.¹⁴ The structure of processed proteins unfolded and exposed more enzymatic hydrolysis sites, which improved its potential sensitization. Therefore, the study of the structural changes of HHP-treated β -conglycinin was helpful in revealing its antigenicity and epitope changes.

Conformational changes of HHP-treated β -conglycinin

The distribution of molecular weights of β -conglycinin before and after HHP treatment was shown by SDS-PAGE (Fig. 6). The β -conglycinin was clearly separated into three subunits (lane B), and with the increase in pressure the band intensity of the three subunits did not change significantly after HHP treatment; there was also no obvious difference between different temperatures (Fig. 6). This indicated that the distribution of molecular weights after HHP treatment was not affected. No β -mercaptoethanol was added for non-reduced SDS-PAGE, so a dimer that consisted of α' and α subunits appeared (Fig. 6(B)).²⁹ There was no significant change in the band intensity of subunits after HHP treatment

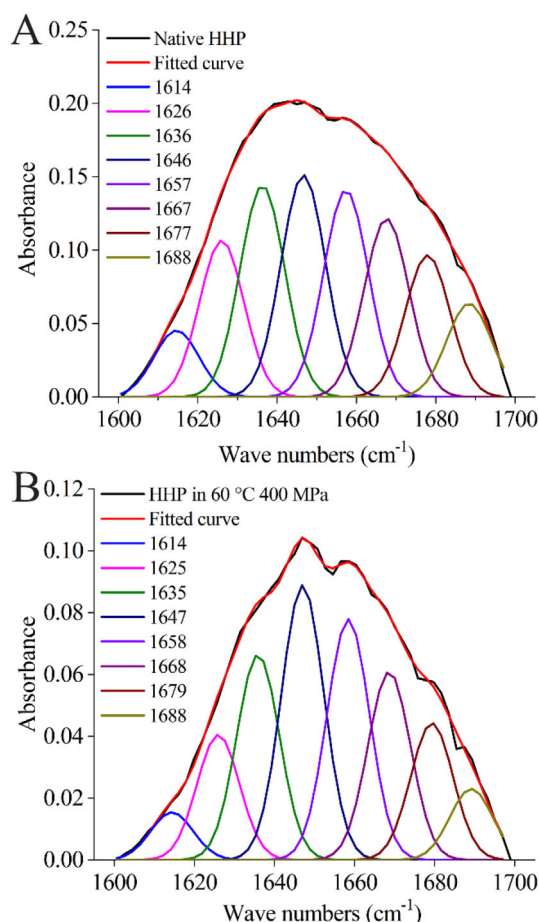


Figure 8. Second-derivative Fourier transform infrared spectra in the amide I region and fitted curve of native (A) and HHP-treated (B) β -conglycinin.

at different temperatures, which indicated that the protein structure was rigid and was related to disulfide bonds.³⁰

The structure of HHP treated β -conglycinin changed significantly, so the infrared spectrum was significantly different (Fig. 7(A)). The procedures of second derivation, deconvolution and multi-peak fitting of the amide I band were used to reveal the secondary structure of β -conglycinin (Figs 7(B) and 8). After HHP treatment, the contents of β -turn and β -sheet decreased, while the random coils and α -helix increased. The secondary structure of HHP-treated β -conglycinin became disordered and was digested by proteases easily because β -sheets and β -turns resisted attack from the enzyme.^{30,31}

Emission fluorescence spectroscopy and UV absorption spectroscopy were used to characterize the conformational structure of β -conglycinin.¹⁴ Compared with the untreated protein, HHP treatment enhanced greatly the fluorescence intensity of β -conglycinin (Fig. 7(C)). Also, after HHP treatment, the maximum absorption peak wavelength ($\lambda_{\max}$) of the protein fluorescence spectrum showed a blue shift of 8.96 nm. This shift was due to the protein structure unfolding after HHP treatment, and abundant hydrophobic groups were exposed; thus the non-polar microenvironment on the protein surface was enhanced.^{11,20} In addition, compared with the native form, the $\lambda_{\max}$ of the UV absorption spectrum of HHP-treated β -conglycinin showed a 3 nm blue shift, and its absorption intensity was enhanced significantly (Fig. 7(D)). This indicated that HHP treatment changed the

conformation structure of β -conglycinin significantly, and the Trp and Tyr residues that were located in the protein were exposed.³² Therefore, the tertiary structure of the HHP-treated β -conglycinin unfolded, plenty of hydrophobic groups were exposed, and Trp and Tyr residues were exposed, which improved the digestive properties and allergenicity of β -conglycinin.

CONCLUSIONS

HHP treatment can alleviate the sensitization of β -conglycinin. In this study, HHP treatment at 300 and 400 MPa reduced the IgG binding of β -conglycinin, while its binding capacity with IgE and epitope antibodies was not significantly affected. After *in vitro* simulated digestion, IgG and IgE binding capacity of HHP-treated β -conglycinin was lower than before. However, the binding capacity of β -conglycinin with epitope antibodies was significantly enhanced after digestion. The results of ELISA and western blotting indicated that HHP promoted proteolysis, conformational epitopes were destroyed, but linear epitopes were retained. In addition, HHP treatment did not affect the distribution of molecular weights of β -conglycinin. The contents of β -turn and β -sheet of HHP-treated β -conglycinin decreased, while the random coils and α -helix increased, so the structure of HHP-treated proteins became disordered. Furthermore, the fluorescence spectroscopy and UV absorption spectroscopy revealed that HHP treatment unfolded the tertiary structure of β -conglycinin; thus, more hydrophobic groups and Trp and Tyr residues were exposed, which facilitated digestion and destroyed epitopes. The present study evaluated the effect of HHP treatment on the antigenicity, antigen epitopes and structural properties of β -conglycinin, and clarified that the mechanism of HHP treatment reduced the sensitization of β -conglycinin to a certain extent.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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