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Identification of β -conglycinin α' Subunit Antigenic Epitopes Destroyed by Thermal Treatments

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Research highlights

The C1-Y fragments ($^{488}\text{PHFNSKAIVVLVINEGEANIELVG}^{511}$) were identified as α' subunit damaged epitopes by Thermal Treatments.

The coupling peptide iELISA results showed that the linear epitope $^{488}\text{PHFNSKAIVVLV}^{499}$ was observed in the fragment C1-Y.

Heat treatments can reduce not only the antigenicity, but also the allergenicity, of α' subunits in β -conglycinin.

Abstract

In the present study, phage display technology (PDT) was used to examine β -conglycinin α' subunit epitopes destroyed by heat treatments. The overlapping gene fragments of the α' subunits were amplified using a polymerase chain reaction (PCR) method, and then the recombinant T7 phages containing the target genes were expressed on the phage surface. In addition, an indirect enzyme-linked immunosorbent assay (iELISA) method was adopted to screen the α' subunit epitopes which had been damaged by the heat treatments. Then, after three rounds of the expression and screening processes, the C1-Y fragments ($^{488}\text{PHFNSKAIVVLVINEGEANIELVG}^{511}$) were identified as α' subunit damaged epitopes. In order to confirm the types of epitopes, three peptides of the C1-Y fragments were synthesized using a solid-phase peptide synthesis method. The results of the iELISA showed that there was a linear epitope of $^{488}\text{PHFNSKAIVVLV}^{499}$ in the C1-Y fragments. The results obtained in this study confirmed that the C1-Y fragments contained both linear and conformational epitopes, and that the conformational epitopes could be destroyed by thermal treatments. However, it was found that the

linear epitopes could not be destroyed. The phage display protein Pt-C1-Y, coupling peptide BSA-Pep-Y, BSA-Pep-1, BSA-Pep-2, and soybean allergy patients' serum reaction results showed that only the Pt-C1-Y had reacted positively with the soybean allergy patients' serum. These findings further indicated that heat treatments can reduce not only the antigenicity, but also the allergenicity, of α' subunits in β -conglycinin.

Keywords: β -conglycinin α' subunits; PDT; heat treatments; IELISA; damaged antigenic epitopes.

1. Introduction

Food allergies are defined as immune responses triggered by food proteins, and account for approximately 50% of all known types of allergies. Therefore, public concern regarding food allergies and related allergic reactions has been increasing (Untersmayr, & Jensenjarolim, 2008; Muraro, & Hernandez, 2020). Studies have shown that 8% of children and 1 to 2% of adults have food allergies (Tordesillas, Berin, & Sampson, 2017; Li, Chen, Tong, & Wen, 2012). As important food sources for human, soybean and its products are widely used in food industries due to its rich protein, vitamin, and mineral content (Gagnon, et al., 2010; Angelis, Pilolli, Bavaro, & Monaci, 2017; Zheng, Qin, Chen, & Zhang, 2018). However, soybean is considered to be one of the eight main food allergens. These antigenic proteins not only affect intestinal digestion and nutrition absorption, but also cause adverse reactions, such as allergic reactions (Sun, et al., 2013; He, Han, Qiao, & He, 2015).

β -conglycinin is one of the major soy allergenic proteins (Huang, Bu, & Chen, 2018; Lu, et al., 2018; Murakami, et al., 2018; Li, et al., 2018). Conjugated glycoproteins with molecular weights of approximately 180 kDa are formed by linking α (68 kDa), α' (72 kDa), and β (52 kDa) subunits, and these three subunits are similar in gene sequences (Campàs, Prieto-Simón, & Rouillon, 2010; Zheng, et al., 2012; Picariello, Amigo, Castillo, & Ferranti, 2013). Studies have shown that α , α' , and β

subunits of β -conglycinin are potential allergens (Krishnan, Kim, Jang, & Kerley, 2009). Guo et al., (2008) found that the β -conglycinin α' subunit possesses an intrinsic immune-stimulating capacity and that it can induce an allergic reaction. However, the sensitization of soybean protein can be reduced by means of heat treatment, ultra-high pressure, glycosylation, and enzymatic methods (Plaimein, & Elviragonzalez, 2010; Xi, & He, 2017; Norihiro, Naoya, Makio, & Shoichiro, 2017; Qing, 2013). It is generally believed that heat treatments can alter the conformation and molecular sizes of proteins, thereby changing the antigenicity of the proteins. The structural changes induced by heating food proteins have been widely studied (Blanc, Vissers, Adel-Patient, & Rigby, 2011; Rahaman, Vasiljevic, & Ramchandran, 2016; Stănciuc et al., 2018). However, the structural immunological essence of these antigenic changes has not yet been fully revealed.

The essence of antigenic changes is the inactivation or destruction of antigenic epitopes. At the present time, many researchers locate epitopes using phage display technology, peptide synthesis technology, bioinformatics methods, and so on (Zhu, et al., 2012; Shan, et al., 2012; Yang, et al., 2020). Phage display technology (PDT) can be utilized to make the target genes of protein connect with phage complementary DNA (cDNA) and then be expressed on phage surfaces. The expressed protein have the ability to maintain relatively independent spatial structures and biological activities. Therefore, the recognition and binding of target molecules continues to be promoted. It is believed that the application of this type of technology will further increase the current understanding of the mechanisms of allergies (Johns, 2000; Li, 2012; Chen, & Dreskin, 2017). Bazan and co-workers described the antigenic epitopes of pollen allergens and amygdaloid allergens using PDT (Bazan, Cał kosiński, & Gamian, 2012). It was revealed that the antigenic determinant clusters of fish albumin could also be located by PDT (Untersmayr, Szalai, & Riemer, 2006). Helm and co-workers discovered

5 major antigenic epitopes of Gly m Bd 30K by PDT (Helm, Cockrell, & Herman, 1998). T7 phage display technology is one of the most commonly used phage display technology methods. The system has the advantages of expressing different molecular weight proteins, high stability, short replication cycles, and so on (Mi, Ma, & Zhao, 2012). Previous studies have examined the antigenic sites of β and α subunits of process damaged β -conglycinin using T7 phage display technology (Xi, He, & Pi, 2019; .Xi, & He, 2020). Pablo et al. (2018) have combined T7 phage display and protein microarrays for the identification of allergenic peptides from olive pollen and mustard seeds. However, effective localization methods for antigenic epitopes disrupted by processing procedures in the β -conglycinin α' subunits have not yet been reported. Therefore, the purpose of this study was to use T7 phage display technology to precisely locate the epitopes of β -conglycinin α' subunits destroyed by heat treatments. The results obtained in this study not only provide a theoretical basis for screening processing methods in food industries, but will also assist in developing application products for the rapid detections of the desensitization effects of processed food.

2. Materials and methods

2.1 Materials

The β -conglycinin α' subunits were synthesized by the Sangon Biotech Co., Ltd. (Shanghai, China). The Ex-Taq deoxyribonucleic acid (DNA) premix, *E. coli* JM109, pMD18-T vector, and the restriction enzymes *Eco*R I and *Hind* III were procured from the Takara Bio Co., Ltd. (Dalian, China). The *E. coli* BLT5615 and the T7Select10-3b Cloning Kit utilized in this study were purchased from the Novagen Company (USA).

2.2 Heat treatment conditions for the β -conglycinin

This study improved the heat treatment conditions based on the research findings (Shan,et al.,

2012). Heat treatment temperatures (60, 80 and 100°C); heat treatment durations (10-60 minutes); and protein solution concentrations 10 mg/mL were the factors examined in this study. The response values were the antigenicity inhibition rates of the β -conglycinin. The box Behnken included in the Design Expert 8.0 software package was used to optimize the heat treatment conditions. Finally, the optimal process parameters were determined.

2.3 Preparation of the heat-treated antigen-absorbing antiserum

The preparation procedure of heat-treated antigen-absorbing antiserum was the same as our previous work (Xi, He, & Pi, 2019). The excess heat-treated antigen was added into rabbit antiserum against β -conglycinin and incubated at room temperature for 1h. The supernatant was then used in this study as a heat-treated antigen-absorbing antiserum.

2.4 Construction of the cloning vectors of the β -conglycinin α' subunits and their overlapping fragments

The α' subunits were divided into A, B, and C overlapping fragments. The primers were synthesized by the Sangon Biotech Co., Ltd. (Shanghai, China). The experiments were carried out on a PCR instrument (nexus GSX1, EPPENDORF, Germany). PCR was performed using the primers and α' subunits as templates, and PCR system was 25 μ L. The target fragments were detected using a 2% agarose gel electrophoresis method. The products of the PCR were ligated into a pMD18-T vector and transformed into *E. coli* JM109 in order to construct the cloning vectors. The recombinant plasmids were evaluated by PCR and double enzyme digestion.

2.5 Phage packaging of the β -conglycinin α' subunits and their overlapping fragments

The purified α' subunit gene and its overlapping fragments were ligated to the T7 Select vector

arms. In this study, the following quantities were used: ligation system: 1 μL ; target fragment: 0.3 μL ; T7 Vector arms: 0.2 μL ; $10 \times$ ligation buffer: 0.1 μL ; T4 DNA ligase: 0.2 μL ; 10 mM ATP: 0.1 μL ; and 10 mM DTT: 0.1 μL . Five times the volume of Packaging Extract 5 was added to the ligation product. The reaction was carried out for a period of two hours, and was ceased by adding nine volumes of the packaging mixture Luria-Bertani (LB) liquid medium to 54 μL .

The processes of culturing and identifying the recombinant phages were as follows: When the BLT5615 was expanded in an M9TB medium (Amp⁺) to $\text{OD}_{600} = 0.5$, 500 μL of 100 mg/mL isopropyl β -D-thiogalactoside (IPTG) were added. The specimens were then shaken for 30 minutes until $\text{OD}_{600} = 0.6$ to 1.0. The bacteriophage packaging products were serially diluted, mixed with the top agarose, and then poured into pre-warmed (37°C) LB or LB/carbenicillin (carb⁺) agar plates. The plates were then allowed to culture at 37°C for more than 3 hours for the purpose of observing the plaque formation. The plaques were randomly selected from different recombinant phages, and then placed in an EP tube containing 100 μL of TE. The specimens were then placed in warm water at 65°C for 10 minutes and centrifuged (D3024R high speed frozen micro centrifuge, SCIOLOGEX, America) at 12,000 rpm for two minutes. The supernatant was used as a template in this study, and PCR amplification was performed using T7Select UP Primer and T7Select DOWN Primer as the adopted primers. The following conditions were applied in this study: Reaction system: 25 μL ; template: 1 μL ; Ex-Taq premix: 12.5 μL ; T7Select UP Primer: 1 μL ; T7Select DOWN Primer: 1 μL ; and sterile ultrapure water: 10.5 μL . Recombinant plaques were identified using a 2% agarose gel electrophoresis method. The recombinant phages were subsequently referred to in this study as S-Q, S-A, S-B, and S-C, respectively.

2.6 Purification and preservation of the phage lysate

The recombinant phage was inoculated into BLT5615 cultures with an OD_{600} ranging between

approximately 0.6 and 1.0. Expansion was performed until the culture became clear and the culturing process was terminated. The lysate was centrifuged at 8,000 g for 10 minutes, and the supernatant was then stored for future use. The purification method used for the recombinant phage was PEG precipitation. DNase I and RNase were added to the final 1 $\mu\text{L}/\text{mL}$ solution and digested at room temperature for 30 minutes. Then, NaCl with a final concentration of 1 M and PEG 8000 with a final concentration of 10% (m/v) were added. The phage particles were precipitated using ice water for 3 to 5 hours, and then centrifuged for the purpose of obtaining the phage precipitate. The centrifuge tube was washed thoroughly, and the phage particles in the supernatant were then lysed using a sterile SM suspension at room temperature for a period of one hour. The PEG 8000 and cell fragments in the supernatant were extracted using chloroform of equal volume, and then slowly rotated up and down for 30 seconds. The specimen was centrifuged at 3,000 g at 4°C for 15 minutes, and the supernatant was stored at -20°C. The phage display proteins were referred to in this study as Pt-Q, Pt-A, Pt-B, and Pt-C, respectively.

2.7 Initial locations of the destroyed antigen epitopes

In the present study, iELISA was used to identify the destroyed antigen epitopes. The protein concentrations of the Pt-Q, Pt-A, Pt-B, and Pt-C were measured and then diluted to 20 $\mu\text{g}/\text{mL}$ for the coating solution. The specimens were placed in a 200 $\mu\text{L}/\text{well}$ plate and maintained overnight at 4°C. Then, the plate was washed four times with the PBST (0.05% Tween-20 in phosphate buffered saline (PBS, pH 7.2, 0.01 M)). The blocking buffer utilized in this study was 5% defatted milk powder administered at 200 $\mu\text{L}/\text{well}$, and the blocking process was conducted at 37°C for a two-hour period. At this point in the experiment, PBST was used to wash the plate. The heat-treated antigen-absorbing antiserum was diluted to 1:8,000; incubated at 37°C for one hour at 100 $\mu\text{L}/\text{well}$; and then washed with

PBST. Goat anti-rabbit IgG HRP diluted 1:2,000 was added at 250 $\mu\text{L}/\text{well}$; incubated at 37°C for one hour; and then washed with PBST five times. Substrate TMB (3, 3', 5, 5'-tetramethylbenzidine) was added at 100 $\mu\text{L}/\text{well}$, and incubated at room temperature for 10 minutes. The reaction was stopped with 2 mol/L of H_2SO_4 at 50 $\mu\text{L}/\text{well}$. Then, the OD_{450} was determined. Each experiment was repeated three times.

2.8 Precise locations of the potentially destroyed antigen epitopes

In order to accurately locate the epitopes of the damaged antigen, the most damaged fragments (The greater the OD_{450} value, the stronger the antigenicity of the fragment) were divided into three overlapping fragment groups. Then, after three rounds of overlapping segments were obtained, the precise locations of the α' subunits which had been destroyed during the heat treatments were identified.

2.9 Confirmations of the epitope types by peptide synthesis

The overlapping peptide design was in accordance with the amino acid sequences of the fragmented C1-Y. For example, peptide C1-Y: $^{488}\text{PHFNSKAIVVLVINEGEANIELVG}^{511}$; peptide 1: $^{488}\text{PHFNSKAIVVLV}^{499}$; and peptide 2: $^{500}\text{INEGEANIELVG}^{511}$. These peptides were synthesized using a solid-phase peptide synthesis method (Zhu, Wu, Xiao, & Cheng, 2012). The purity and the molecular masses were assessed using high-performance liquid chromatography (HPLC) and mass spectrometry (MS) method. The peptides were then conjugated to the carrier protein of bovine serum albumin (BSA, Sigma), and were referred to in this study as BSA-Pep-Y, BSA-Pep-1, and BSA-Pep-2, respectively. The types of coupling peptides were identified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and stored at -20°C. IELISA was used to detect the reactions of the coupling peptides with antiserum.

2.10 Statistical analysis

All experiments were performed three times. SPSS 16.0 software (IBM, New York, USA) was used to analyze the obtained data, and a significance level of $P < 0.05$ was established. A Duncan Multi-range Test Method was used to detect the differences between the averages.

3. Results and discussion

3.1 Heat treatment conditions

The results showed that the best conditions were a protein concentration of 10 mg/ mL; heating temperature of 100°C; heating duration of 60 minutes; and an antigenicity inhibition rate of 38.4%.

3.2 Amplification of the α' subunits and fragmented genes

The α' subunits and their overlapping segments A (I44-D289), B (T279-M480), and C (Q467-Y640) were amplified by the primers. Primers were designed using Edit Seq and Primer Premier 5.0 software. The validation of the PCR by agarose gel electrophoresis indicated that the corresponding bands were the same as the expected bands, and the sizes of fragments A, B, and C were 738 bp, 606 bp, and 525 bp, respectively (Fig. 1a). The double-enzyme digestion and PCR of the α' subunits and their overlapping segments A, B, and C showed the same size bands at 1791, 738, 606, and 525 bp, respectively (Figs. 1b and 1c). The results showed that the β -conglycinin α' subunits and their overlapping fragments were successfully inserted into the pMD18-T, and the sequences of the inserted fragments were consistent with its gene sequences.

3.3 Identification of the recombinant phages

The aforementioned DNA solution of randomly selected plaques was amplified by PCR, and the PCR results of the S-Q, S-A, S-B, and S-C were detected using a 2% agarose gel electrophoresis method. The results showed that the DNA fragments were correct. The DNA bands

of the recombinant bacteriophages S-Q, S-A, S-B, and S-C were found to be in the correct positions (Fig. 2a). An iELISA method was used to identify the antigenicity of phage-expressed proteins and locate the antigenic epitopes which had been destroyed during the heat treatments. The antiserum titer was described in our previous work (Xi, He & Pi, 2019). which also can be used for later analysis. According to the results obtained by the iELISA method (Fig. 2b), the antigenicity of the phage expressed protein Pt-C was the highest. with an OD_{450} of approximately 0.976 ± 0.012 . In addition, it was observed that the heat treatments had damaged the α 'subunits and their A, B and C fragments to different degrees. The most serious damages had occurred to Pt-C. The sequence of the C was as follows: ⁴⁶⁷QLRDL DVFLSVVDMNEGALFLP HFNSKAIVVLVINEGEANIELVGIKEQQQRQQEQPLEVRKYRAELSEQDIFVIPAGYPVVVN ATSDLNFFAFGINAENNQRNFLAGSKDNVISQIPSVQELAFGLGSAKDIENTLIKSQSESYFVDAQ PQKKEEGNKGRKGPLSSILRAFY⁶⁴⁰.

3.4 Expressions and identifications of the C fragment gene and its overlapping segments

The C fragment gene and its overlapping segments C1 (Q467-F541), C2 (R532-D600), and C3 (E591-Y640) were amplified by the primers. The PCR and double enzyme digestion results of the recombinant plasmids showed that the C1, C2, and C3 fragments exhibited the same sized bands at 225 bp, 207 bp, and 153 bp, respectively (Fig. 3a). The sequencing results further indicated that the recombinant plasmid contained the expected gene. The PCR amplified band coincided with the expected target band position, thereby confirming that the recombinant phage contained the target gene and the phage could successfully express the target protein (Fig. 3b). The results obtained using an iELISA method showed that the antigenicity of the phage

expressed protein Pt-C1 was the strongest, and its OD₄₅₀ was determined to be approximately 0.463 ± 0.007 (Fig. 3c). It was observed in this study that the heat treatments had affected the antigenicity of Pt-C1, Pt-C2, and Pt-C3, and the most serious damage was to Pt-C1. The sequence of the C1 was as follows: ⁴⁶⁷QLRDL DVFLSVVDMNEGALFLPHFNSKAIVVLVINEGEANIELVGIKEQQQRQQEEQPLEVRKYRAELSEQDIF⁵⁴¹.

3.5 Expressions and identifications of the C1 fragment gene and its overlapping segments

The C1 gene and its overlapping segments C1-X (Q467-A494), C1-Y (P488-G511), and C1-Z (I507-F541) were amplified by the primers. The PCR amplification band was found to be consistent with the destination band position. The PCR and double enzyme digestion results showed that the C1-X, C1-Y, and C1-Z fragments exhibited the same sized bands at 84 bp, 72 bp, and 105 bp, respectively (Fig. 4a). The sequencing results further indicated that the recombinant plasmid contained the expected gene. The band amplified by PCR was consistent with the expected target band position, thereby confirming that the recombinant phage contained the target gene and the phage could successfully express the target protein (Fig. 4b). The antigenicity of the phage expressed protein Pt-C1-Y was found to be the strongest, and its OD₄₅₀ was approximately 0.429 ± 0.011 (Fig. 4c). It was observed that the heat treatments also affected the antigenicity of the C1-X, C1-Y, and C1-Z fragments, and the most serious damage was to C1-Y. The sequence of the C1-Y was as follows: ⁴⁸⁸PHFNSKAIVVLVINEGEANIELVG⁵¹¹.

3.6 Confirmation of epitope types by peptide synthesis

3.6.1 Identifications of the synthesized peptides

The identifications of the peptide C1-Y, peptide 1, and peptide 2 using HPLC (Figs. 5a1, 5a2 and 5a3) and MS (Figs. 5b1, 5b2 and 5b3) showed that the synthesized peptides were correct, and their purity was 82.69%, 81.63% and 85.10%, respectively. The SDS-PAGE results revealed that the peptide C1-Y, peptide 1, and peptide 2 were successfully coupled with BSA, and the target band positions of the coupled peptides were all above the BSA (Fig. 5c).

3.6.2 IELISA results

The iELISA results showed that the β -conglycinin, Pt-C1-Y, BSA-Pep-Y, and BSA-Pep-1 were all recognized by the antiserum against β -conglycinin. The coupling peptides BSA-Pep-Y and BSA-Pep-1 were found to have not reacted with heat treated antigen-absorbing antiserum. However, it was observed that the β -conglycinin and Pt-C1-Y had reacted with the heat-treated antigen-absorbing antiserum. In addition, the BSA-Pep-2 did not react with either the antiserum against β -conglycinin or the heat-treated antigen-absorbing antiserum (Table 1). Therefore, based on the obtained results, this study speculated that the fragment C1-Y may have contained both linear and conformational epitopes, and the conformational epitopes may have been destroyed after processing. Meanwhile, the linear epitopes were not destroyed. In the fragment C1-Y, the linear epitope referred to as ⁴⁸⁸PHFNSKAIVVLV⁴⁹⁹ was observed. In regard to the β -conglycinin phage display protein Pt-C1-Y, the three coupling peptides and soybean allergy patients' serum reaction results showed that the phage-expressed protein Pt-C1-Y could bind to the soybean allergy patients' serum. These findings suggested that the Pt-C1-Y was also a type of allergenic epitope.

Presently, many methods by which to study the location of allergen epitopes are available. In 2003, Erlandsson et al. used epitope mapping to locate antibody binding sites in staphylococcal enterotoxins A and E. The analysis results of the overlapping polypeptides expressed by *E. coli* showed

that positions 380-461 and 483-565 were antigenic in mice and chickens in the haemagglutinin identification subunit 2 of the virus H5N1 avian influenza (Putri, Wawegama, Ignjatovic, & Noormohammadi. 2018). Zheng et al. (2011) synthesized 10 peptides of tropomyosin by solid combination method, among which eight epitopes were identified with the serum of shrimp allergy patients. In addition, Bu et al. (2020) also synthesized 10 subunits' potential epitopes in the β subunit of β -conglycinin by the solid combination method, and the results showed that all 10 peptides (P1~P10) exhibited IgG binding activity, while P2 and P6 were also confirmed as IgE binding peptides.

In previous studies, much research and many screenings have been performed regarding food allergens and epitopes using PDT. Tordesillas et al. (2009) used a phage display library to screen the total IgE of allergic patients with purified antigen, and identified the simulated epitopes of wheat protein Tria A 4 and peach protein Pru P 3 (conformational epitopes). Li et al. (2012) identified six linear epitopes that could bind to rabbit anti- β -lactoglobulin serum (IgG) by using phage random heptide library. In addition, several conformational epitopes were selected by affinity panning (Li, 2014). In our previous survey, we used PDT to locate the damaged antigenic sites of α subunit and β subunit. We found that the amino acid sequence C2-3(D378-D400) in β subunit is the conformational epitopes of the high hydrostatic pressure (HHP) destructive antigen (Xi, He, & Pi, 2019). We also observed that C2-1(E380-R408) and C2-2(A404-N448) in α subunit were allergic sites. This result prompted us to continue studying the C2-1 and C2-2 fragments in antigenic regions damaged by overlapping peptide synthesis technology, leading to the discovery that C2-2 epitopes can be conformational, while the upper segment of C2-1 fragment may contain both conformational and linear epitopes (Xi, & He, 2020). Finally, the further study of the damaged antigen site of Gly M Bd 60K by overlapping peptide technology proved the presence of an epitopes containing conformation and linear

IgE epitopes with ³⁸⁰EGQQGEQRLQ³⁹⁰ as amino acid sequence and alanine scan confirmed the fact that Q382, Q383, G385 and Q387 were the key amino acids in the epitopes (He, & Xi, 2020). Notwithstanding, the study on the epitope localization of α' subunits has not been found yet. Therefore, we first studied the antigenic epitope of the α' subunit destroyed by heat treatments, successfully located the C1-Y (⁴⁸⁸PHFNSKAIVVLV⁴⁹⁹), and the segment may contain both linear and conformational epitopes.

4. Conclusions

After three rounds of expression and screening using T7 phage display technology and iELISA, the C1-Y fragment (⁴⁸⁸PHFNSKAIVVLVINEGEANIELVG⁵¹¹) was identified as one of the α' subunit antigenic sites destroyed by the application of thermal treatments. The peptide C1-Y, peptide 1, and peptide 2 were synthesized using overlapping peptide synthesis technology and identified by HPLC and MS methods, and determined to have purity percentages of 82.69%, 81.63%, and 85.10%, respectively. In addition, the SDS-PAGE results revealed that the three peptides were successfully coupled with BSA. The coupling peptide iELISA results showed that the linear epitope ⁴⁸⁸PHFNSKAIVVLV⁴⁹⁹ was observed in the fragment C1-Y. Therefore, this study assumed that the C1-Y fragment may have contained both linear and conformational epitopes. It was further speculated that the heat treatments may have destroyed the conformational epitope structure, but not the linear epitope structure. These findings indicated that thermal treatments were capable of destroying the conformational epitopes of the β -conglycinin, as well as reducing the antigenicity. The phage display protein Pt-C1-Y and soybean allergy patients' serum reaction results showed that the Pt-C1-Y reacted positively with the soybean allergy patients' serum, which further indicated that heat treatments were not only able to reduce the antigenicity, but also the allergenicity of α' subunits in the β -conglycinin.

The results obtained in this experimental study will potentially provide valuable references for future investigations of the antigenicity or allergenicity of removing β -conglycinin α' subunits. Moreover, the obtained data will be of significance for the preparations of the test strips in future studies to detect whether or not the antigenic epitopes of β -conglycinin α' subunits have become damaged by processing procedures in order to guide the production of safe food products.

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Conflicts of Interest

The authors state that there was no conflict of interest in the study.

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Figure legends

Fig. 1 Identifications of the recombinant plasmids of β -conglycinin α' subunits: a. PCR amplification of β -conglycinin α' subunits and their fragments; b. Double digestion identification; c. PCR identification (a: M: AL2000 DNA Marker; 1: C; 2: B; 3: A; 4: full length; b: M: AL2000 DNA

Marker; 1: C; 2: B; 3: A; 4: full length; c: M: AL2000 DNA Marker; 1: C; 2: B; 3: A; 4: full length)

Fig. 2 Phage display and identifications of the β -conglycinin α' subunit fragments and their overlapping segments: (M indicates the AL2000 DNA Marker; 1-11 refers to the recombinant phages)

Note: In the figure, the displayed values are the mean $\pm$ standard deviations of the two values, and the different superscripts indicate significant differences at the level of $P < 0.05$ in the comparisons of the same processing method, yet different fragments, as indicated by the same color coding.

Fig. 3 Phage display and identifications of the C fragment and its overlapping segments: a. Identification of recombinant plasmids of the C fragment and its overlapping segments; b. Identification of the recombinant phages by PCR amplification; (a. M: D2000 DNA Ladder; 1:C3; 2:C2; 3:C1; b. M: D2000 DNA Ladder; 1-9: recombinant phages)

Note: In the figure, the displayed values are the mean $\pm$ standard deviations of the two values, and the different superscripts indicate significant differences at the level of $P < 0.05$ in the comparisons of the same processing method, yet different fragments, as indicated by the same color coding.

Fig. 4 Phage display and identifications of the C1 fragment and its overlapping segments: a1 to a3. Identifications of the recombinant plasmids of the C1 fragment and its overlapping segments; b. Identification of recombinant phages by PCR amplification (a1: M: 50 bp DNA Ladder; 1: C1-Z; 2: C1-Y; 3:C1-Z; a2: M: 50 bp DNA Ladder; 1: C1-Z; 2: C1-Y; 3:C1-Z; a3: M: 50 bp DNA Ladder; 1: C1-Z; 2-3: C1-Y; 4-5: C1-Z; b: M: 50 bp DNA Ladder; 1-12: recombinant phages)

Note: In the figure, the displayed values are the mean $\pm$ standard deviations of the two values, and the different superscripts indicate significant differences at the level of $P < 0.05$ in the comparisons of the same processing method, yet different fragments, as indicated by the same color coding.

Fig. 5 Identifications of synthesized peptides: a1 to a3. HPLC of three fragments; b1 to b3. MS of three fragments; c. SDS-PAGE of coupling peptides (a1: HPLC of peptide C1-Y; a2: HPLC of peptide1; a3: HPLC of peptide 2) (b1: MS of peptide C1-Y; b2: MS of peptide1; b3: MS of peptide 2) (c: M: Low molecular weight protein marker; 1: BSA; 2: BSA-Pep-Y; 3: BSA-Pep-1; 4: BSA-Pep-2).