



Establishment of DAS-ELISA for the detection of antigenic changes in glycinin after heat processing

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ABSTRACT

In this study, a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) method was established to detect the antigenic changes of thermally processed products containing glycinin. The proposed DAS-ELISA method used heat-treated antigen-absorbing antiserum as the coating antibody, and horseradish peroxidase (HRP)-labeled rabbit anti-glycinin polyclonal antibody as the detection antibody. The specificity test results which were obtained using the proposed method indicated that good specificity had been achieved. The cut-off value was 0.388, and the LOD was determined to be 19.53 ng/mL. The coefficient of variation was less than 5.25% (intra-day) and 9.50% (inter-day). In this study's milk powder addition test, the recovery rate of the glycinin ranged between 83.65% and 90.13%. The established DAS-ELISA method was also used to detect soybean thermal processing products, such as soy sauce, steamed fish and soy sauce, soybean paste, beef sauce, soy milk powder, and tofu. The results showed that the OD₄₅₀ values of the aforementioned products were lower than the OD₄₅₀ values of the glycinin in defatted soybean flour. Therefore, it was indicated that the above products have undergone different degrees of thermal processing. In other words, the majority of the epitopes of glycinin in the products had been destroyed by the thermal processing and could not be combined with heat-treated antigen-absorbing antiserum.

1. Introduction

At the present time, food allergies are a growing problem throughout the world, and the incidence of food allergies is increasing in many regions [1–3]. Food allergies are essentially abnormal immune responses to food antigens, which may in some cases have the potential to endanger lives [4]. Food allergic reactions can range in severity from mild to severe, and severe cases can be life-threatening [5]. When an individual ingests a food containing allergenic proteins, it triggers the release of related factors which may then lead to the occurrence of an allergic food reaction [6,7].

Soy protein is one of the top eight known allergens. As increasing amount of soy products are used as raw materials in food production, the incidence of soybean allergic reactions continues to rise [8]. Glycinin is one of the main antigenic proteins in soybean. Glycinin has a molecular weight ranging between 300 and 380 kDa, and the content of this protein accounts for approximately 40% of the total protein in seeds [9,10]. Glycinin consists of five subunits: A1aB2 (G1), A1bB1b (G2), A2B1a (G3), A3B4 (G4), and A5A4B3 (G5), which constitute its quad structure

[11]. The results of previous studies have shown that all five subunits are sensitized [12,13]. When young animals consume food containing soy protein, the antigen-active macromolecule protein will enter the lymph and blood systems, stimulate the immune system, and lead to the occurrence of allergic reactions [14]. Glycinin has thermal instability, and modern food industry often reduces its sensitization by thermal processing, so it is very important to detect the degree of desensitization in the process of thermal processing.

In everyday life, the avoidance of certain food intake is the only way to prevent consumers from experiencing food allergies. Therefore, complete and accurate information regarding food ingredients must be provided to consumers. A precise, fast, and cost-effective method is needed in order to screen specific allergens in food products [15]. Allergen detection methods can be further divided into immunoassay-based methods, DNA-based methods, and mass spectrometry-based methods [16–19]. Among the immunoassay methods for detecting allergens in soybeans, the ELISA method has become the most widely used method in routine allergen detections due to its accuracy, sensitivity, simple operation, and high application potential [20]. During the past

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decade, studies have established indirect competitive ELISA methods in order to detect Gly m Bd 28K [21], glycinin [22], α subunit of β -conglycinin [23], and β -conglycinin [24,25]. In addition, DAS-ELISA methods have also been widely applied in the detection of soybean allergens. DAS-ELISA methods can more effectively reduce the matrix effects caused by the fat, cellulose, minerals, or other proteins in samples. When using DAS-ELISA methods, the colors of the response values are directly related to the allergen content levels in the extracted food samples. These methods have been successfully applied to detect soy allergens in food in recent years [26]. Hei et al. proposed a DAS-ELISA method in which mouse anti- β -conglycinin monoclonal antibody (mAb 5C5) was used as the coating antibody, and rabbit anti- β -conglycinin polyclonal antibody (pAb) was used to detect the antibodies. The detection limit of β -conglycinin in soybean and soybean products reached 1.63 ng/mL [27]. Morishita et al. described a new type of DAS-ELISA method with a detection line of 0.47 ng/mL. The method was used to detect and quantify the main soy allergen protein Gly m Bd 30K, which is the main soybean allergen protein in processed foods [28]. There have also been studies conducted to establish various DAS-ELISA methods for the detection of 2S (Gly m 8) in soy protein [29], as well as Gly m 4 [30], and Gly m 6 [31]. Those studies were all aimed at the establishment of certain types of soy protein allergen ELISA detection methods. However, it has not yet been reported if those methods have accurately detected the antigenicity of soybean allergen protein after product processing. It has been well documented that there are many types of heat-processed foods containing soy glycinin on the market today, such as tofu, soy milk powder, soy sauce, soybean paste, and so on. When such products are ingested, there is a strong likelihood that glycinin sensitive allergy patients will have allergic reactions. The aforementioned processed soy products generally must undergo high-temperature cooking processes. After such products have been heat-treated, there is still a lack of available effective detection methods which can determine whether or not the glycinin allergen epitopes have been destroyed, or to identify the extent of the damages.

Therefore, in response to the above-mentioned problems, this study used heat-treated antigen-absorbing antiserum as the coating antibody, and HRP-labeled rabbit anti-glycinin polyclonal antibody was used as the detection antibody, in order to establish a novel DAS-ELISA method. The DAS-ELISA established in this research investigation was adopted for the detection glycinin in soy protein. The innovation of the proposed method was to determine whether or not products containing glycinin have been effectively heat-processed. This study's exploration of the changes in antigenicity caused by thermal processing will provide a guide for the safe production of soybean thermally processed products in the future.

2. Materials and methods

2.1. Preparation of the heat-treated antigen-absorbing antiserum

The preparation procedure of the heat-treated antigen-absorbing antiserum was the same as that applied in our previous research [32]. The excess heat-treated glycinin antigen (optimum heat treatment conditions: 110 °C, 50 min, 10 mg/mL) and antibody were incubated at 37 °C for 1 h with a mass ratio of 5:1 until being fully absorbed. The precipitation was discarded by centrifugation and the supernatant was retained. Finally, the heat-treated antigen-absorbing antiserum was stored at −20 °C for later use.

2.2. Preparation of the HRP-labeled IgG

This study referred to sodium periodate method presented by Guo Chunxiang for the labeling process [33].

Step 1: 5 mg of HRP was dissolved in 0.5 mL of distilled water. Then, 0.5 mL of sodium periodate solution were added, and the solution was mixed well and stored at 4 °C for 30 min. Step 2: 0.5 mL of ethylene

glycol were added to terminate the oxidation reaction, and the solution was mixed well and placed at room temperature for 30 min. Step 3: 1 mL (5 mg/mL) of purified anti-glycinin rabbit polyclonal antibody was added. The solution was stirred continuously during the polyclonal addition, and then placed into a dialysis bag. Next, 0.05 mol/L pH 9.5 CBS (carbonate buffer solution) was used to dialyze the solution overnight at 4 °C. Step 4: 0.2 mL of sodium borohydride (5 mg/mL) was added at 4 °C for 2 h. Step 5: An equal volume of saturated ammonium sulfate was added, and the solution was stored at 4 °C for 30 min. Then, the solution was centrifuged at 3000 r/min for 30 min in order to obtain a precipitate. Step 6: The precipitate was dissolved in 1 mL of 0.02 M pH 7.4 PBS solution (0.2 g KCl, 1.15 g Na₂HPO₄, 2.9 g Na₂HPO₄·12H₂O, 8 g NaCl, 0.2 g KH₂PO₄, 1000 mL double distilled water), mixes well, and placed into a dialysis bag for dialysis overnight. The solution was then centrifuged in order to remove the precipitate and obtain the enzyme-antibody conjugate. Finally, 5 mL of PBS was added.

2.3. Establishment and optimization of this study's DAS-ELISA method

The two types of antibodies were the heat-treated antigen-absorbing antiserum and the HRP-labeled IgG. The heat-treated antigen-absorbing antiserum was used as the coating antibodies. The HRP-labeled IgG was used as the detection antibodies. First of all, CBS (2.93 g NaHCO₃, 1.59 g NaCO₃, 1000 mL double distilled water) was used to dilute the coating antibodies to final concentrations of 20 µg/mL, 10 µg/mL, 5 µg/mL, 2.5 µg/mL, and 1.25 µg/mL, respectively, and coated overnight at 4 °C. Then, 5% BSA block (PBS + 0.05% Tween 20 + 5% Skimmed milk powder) was applied in 200 µL/wells at 37 °C for 2 h. The glycinin antigen was diluted with PBS to a final concentration of 5 µg/mL. The glycinin antigen was then used under optimal heat treatment conditions as a negative control. The HRP-labeled IgG was utilized according to 1:30, 1:60, 1:120, and 1:240 dilution rates, and incubated at 37 °C for 1 h. TMB was added for the color development process, and 2 M sulfuric acid was used to terminate the reaction. There were three replicates performed under each condition, and the antibody combination with the largest P/N was selected after the results were detected. Then, after selecting the most preferable antibody combination conditions, a series of optimizations were carried out, including the following coating times. The specific procedural design is detailed in Table 1.

2.4. Determination of the cut-off value and specificity

The glycinin was selected under the optimal heat treatment conditions, and nine replicates were set up according to the explored DAS-ELISA optimal reaction conditions. The average values and standard deviations (SD) of the sample were calculated according to the obtained OD₄₅₀ values. Then, in accordance with the cut-off value judging standards, the cut-off value was equal to (mean + 3SD); OD₄₅₀ values greater than the cut-off value were judged to be positive; and the OD₄₅₀ values equal to or less than the cut-off value were judged to be negative. Subsequently, using the DAS-ELISA method established in this study, the glycinin, β -conglycinin, peanut protein, glutenin, and BSA were tested to

Table 1
The optimization of DAS-ELISA for glycinin.

Optimization of DAS-ELISA	Options
Coating condition	4 °C 14 h; 4 °C for 16 h; 37 °C for 2 h; 37 °C for 4 h
Blocking buffer	1% BSA; 2% BSA; 3%BSA; 1% skimmed milk powder; 3% skimmed milk powder; 5% BSA
Blocking time	60 min; 90 min; 120 min; 150 min
Incubating time (antigen)	30 min; 45 min; 60 min; 90 min; 120 min
Incubating time (HRP-labeled IgG)	30 min; 45 min; 60 min; 90 min; 120 min
Incubating time (Colorant)	3 min; 5 min; 7 min; 10 min; 12 min; 15 min

determine the specificity of the proposed method.

2.5. Sensitivity

Using the established DAS-ELISA, the glycinin was diluted with PBS to 20,000.00, 10,000.00, 5000.00, 2500.00, 1250.00, 625.00, 312.50, 156.25, 78.13, 39.06, 19.53, and 9.77 ng/mL, with three replicates for each dilution. The data were then processed and the sensitivity of the proposed method was calculated.

2.6. Repeatability

Operations were carried out according to the DAS-ELISA method established in this study as follows:

Intra-day variations: Five different concentrations of glycinin were selected, with five replicates for each concentration, and the coefficient of variation was calculated.

Inter-day variations: Under the same conditions, five different concentrations of glycinin solution were selected for the DAS-ELISA detection method analysis. Each concentration was analyzed three times per day, and measured for five days. Finally, the coefficient of variation of the established DAS-ELISA method was calculated.

2.7. Recovery

Commercial skimmed milk powder without soybean protein was used for the addition and recovery tests. The skimmed milk powder was dissolved in 0.01 mol/L of NaHSO_3 solution, and the ratio of skimmed milk powder to the solution was 1:15. Then, after stirring for 1 h, the supernatant was extracted by centrifugation. The obtained supernatant was diluted to 2.5, 1.25, and 0.625 $\mu\text{g/mL}$ of glycinin. The OD_{450} values were finally determined in order to analyze the recovery rates.

2.8. Detection of glycinin in heat-processed food systems

Soy sauce, steamed fish soy sauce, soybean paste, beef sauce, soy

milk powder, and tofu were selected for pre-treatment of the sample to be tested. The untreated glycinin was dilute with PBS to 5 $\mu\text{g/mL}$, and the protein was dissolved in the thermally processed products containing glycinin. For example, the soy sauce, steamed fish soy sauce, soybean paste, beef sauce, soy milk powder and tofu were dissolved in Tris-HCl (pH 8.0) for 1 h at room temperature and centrifuged at 3500 r/min for 30 min. The precipitate was then discarded, and the supernatant was filtered with a 0.45 μm filter membrane. The defatted soybean flour was treated in the same way. In order to determine the protein concentration, the protein concentration was diluted to 25 $\mu\text{g/mL}$ with PBS. Considering that the product system was soy protein, the glycinin was known to account for approximately 19.5 to 23.1% of the soy protein content. Glycinin under optimal heat treatment conditions was used as a negative control. Each sample was set with four replicates.

3. Results

3.1. Determination of a novel DAS-ELISA detection method

The DAS-ELISA process was established according to the optimal conditions, as shown in Fig. 1 and Table 2.

The concentrations of the coated antibodies were 5 $\mu\text{g/mL}$. The coated antibodies placed at 100 $\mu\text{L/well}$, at 37 °C for 2 h, and the plate was washed four times using PBST (phosphate buffer solution of tween), at 5 min per time. Then, 200 μL of 3% skim milk powder were added to each well. The plate was sealed and placed at 37 °C for 90 min. The plate was then washed four times again with PBST, at 5 min per time. The sample to be tested and the negative and positive controls were added at 100 $\mu\text{L/well}$, under 37 °C temperature conditioned for 1 h. The plate was washed five times with PBST, at 5 min per time. Then, a 1:60 times diluted detection antibody was added at 100 $\mu\text{L/well}$, under temperature conditions of 37 °C for 45 min. The plate was washed with PBST four times, at 5 min per time. At that point, TMB color solution was added at 100 $\mu\text{L/well}$ under dark conditions at room temperature for 18 min. Finally, 50 μL of 2 M concentrated sulfuric acid was added to each well to terminate the reactions. The OD values at 450 nm were determined using an enzyme plate analyzer.

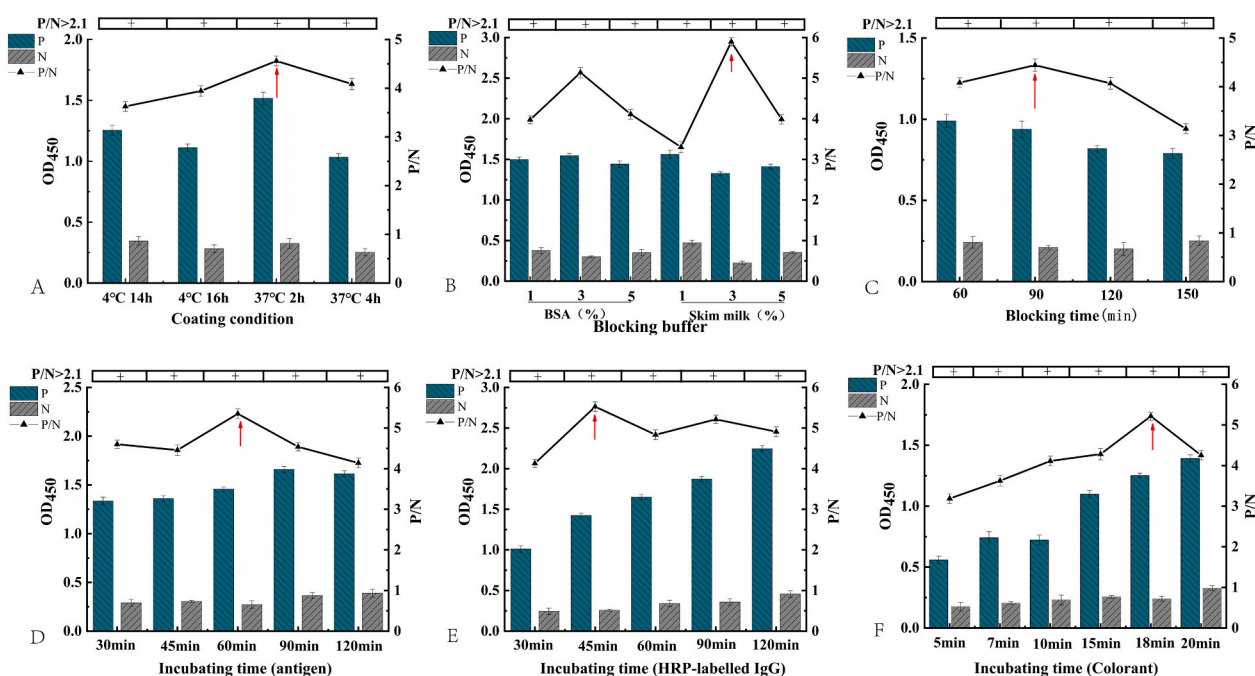


Fig. 1. DAS-ELISA condition optimization: A. Coating condition; B. blocking buffer; C. selection of the blocking time; D. incubating time (antigen); E. incubating time (anti-capture antibody); F. incubating time (colorant). In the figure, the P value represents positive and the N value represents negative; $P/N > 2.1$ represents positive results (+), and the optimal condition is indicated by the red arrow (†); each condition was repeated three times.

Table 2

The optimization on the dilution of coated antibody and HRP-labeled IgG.

Dilution of coated antibody (μg/mL)	Dilution of HRP-labeled IgG				OD ₄₅₀
	1:30	1:60	1:120	1:240	
20	2.061	1.791	0.752	0.481	P
	0.641	0.340	0.151	0.372	N
	3.218	5.275	4.997	1.295	P/N
10	2.023	1.721	0.731	0.384	P
	0.508	0.359	0.232	0.132	N
	3.985	4.801	3.151	2.920	P/N
5	1.830	1.689	0.592	0.443	P
	0.456	0.235	0.199	0.128	N
	3.935	7.187 ^a	2.975	3.475	P/N
2.5	1.371	1.003	0.519	0.404	P
	0.453	0.163	0.148	0.102	N
	2.907	6.800	3.519	3.961	P/N
1.25	0.929	0.623	0.405	0.638	P
	0.367	0.175	0.121	0.245	N
	2.531	3.56	3.347	2.609	P/N

Note: In the table, P indicates positive and N indicates negative. The maximum P/N values were selected as the optimal dilution times of the coated antibodies and HRP-labeled IgG using the proposed DAS-ELISA method. The data represent the means of three replicate subsamples. a: The optimal on the dilution of coated antibody and HRP-labeled IgG

3.2. Specificity test results

According to the procedure of the DAS-ELISA method established in this study, the determination of the glycinin under optimal heat treatment conditions was repeated nine times. Then, following the completion of the statistical analysis, the calculated cut-off value was determined to be 0.388. That is to say, when the OD₄₅₀ value of the glycinin sample was lower than 0.388, it was confirmed that the majority of the epitopes of the thermally processed products containing glycinin had been destroyed following the heat treatments, indicating that the heat treatment effects were quite good. As shown in Fig. 2, the specificity test results revealed that the proposed method had good specificity for β-conglycinin, peanut protein, wheat protein, and BSA.

3.3. Sensitivity test results

As shown in Fig. 3, this study's experimental tests were carried out according to the established DAS-ELISA method, and the results indicated that the method had displayed the ability to detect 19.53 ng/mL of

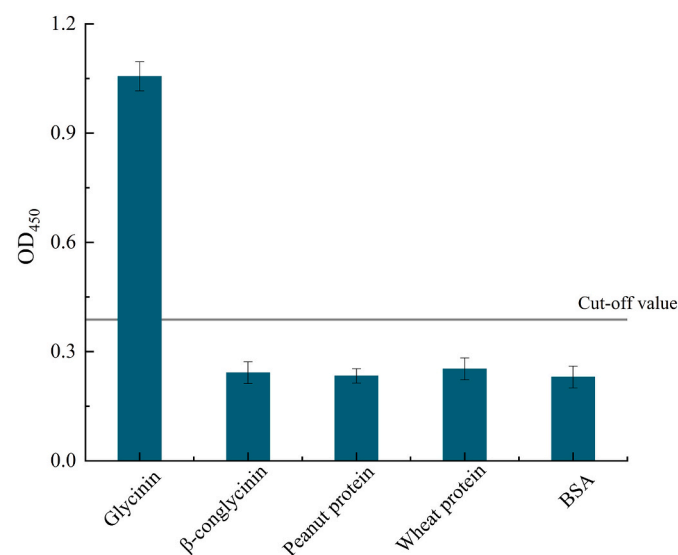


Fig. 2. DAS-ELISA specificity. In the figure, the measurement process was repeated three times for each protein sample.

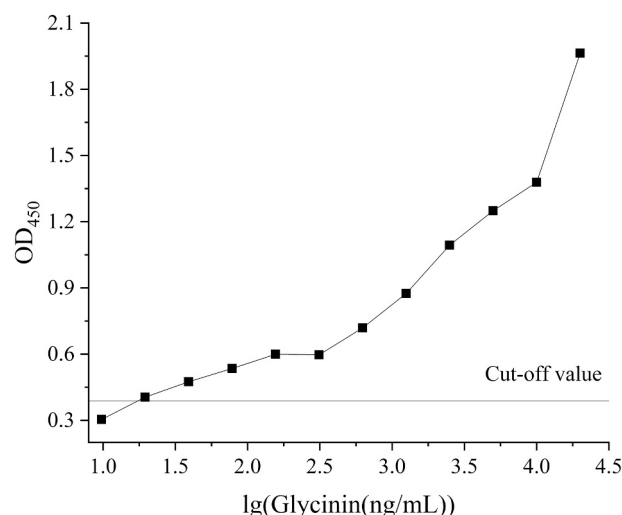


Fig. 3. DAS-ELISA sensitivity. In the figure, the determination of each protein concentration was repeated three times; different concentrations of purified glycinin were used; At least 19.53 ng/mL of the glycinin were detected.

glycinin.

3.4. Repeatability test results

As detailed in Table 3, the coefficients of variation for the intra-day and inner-day tests were 3.33% to 5.25% and 5.20% to 9.50%, respectively, and both were less than 15%. Therefore, the results confirmed that the DAS-ELISA method established in this study had good repeatability.

3.5. Recovery test results

In order to simulate real food systems, commercial skim milk powder without glycinin was used in order to conduct glycinin addition and recovery tests. As can be seen in Table 4, following the milk powder addition and recovery test, the DAS-ELISA method established in this study had achieved additive recovery rates of 90.13% and 83.65%.

3.6. Detection of thermally processed products

The soy sauce, steamed fish soy sauce, soybean paste, beef sauce, soy milk powder, and tofu used for this study's tests were generally processed by high-temperature cooking. This study's established DAS-ELISA method was used to analyze the antigenic changes of glycinin

Table 3

Intra and inter plate variation of DAS-ELISA for detection of glycinin.

Concentration of glycinin (ng/mL)	DAS-ELISA method of glycinin		
	A450	SD	CV (%)
A; Intra plate			
5000	1.125	0.059	5.25
2500	1.008	0.042	4.20
1250	0.843	0.037	4.36
625	0.623	0.021	3.33
312.5	0.537	0.025	4.71
B; Inter plate			
5000	1.196	0.114	9.50
2500	1.052	0.082	7.77
1250	0.919	0.078	8.44
625	0.686	0.064	9.30
312.5	0.592	0.031	5.20

Note: In the table, the data represent the means of three replicate subsamples.

Table 4

Recovery analysis of the DAS-ELISA.

Glycinin ($\mu\text{g/mL}$)	Mean ($\mu\text{g/mL}$)	SD	Recovery (%)
2.5	2.250	0.045	90.13
1.25	1.050	0.030	83.65
0.625	0.550	0.016	85.15

Note: In the table, the data represent the means of three replicate subsamples.

in the aforementioned heat-processed products containing glycinin. The test results are shown in Fig. 4. It was revealed that the OD_{450} of the soy sauce, steamed fish soy sauce, beef sauce, soy milk powder, and tofu were all lower than the defatted soybean flour. These findings indicated that the above-mentioned types of thermally processed products containing glycinin had undergone different degrees of thermal processing. The analysis results revealed that the majority of the epitopes of soybean glycinin contained in the products had destroyed by thermal processing and could not be combined with the heat-treated antigen-absorbing antiserum. This resulted in decreases in OD_{450} value. Therefore, antigenicity of the glycinin allergen contained in the above-mentioned products had also decreased. The OD_{450} value of defatted soybean meal was higher than that of the glycinin, which may have been due to the non-specific binding of non-glycinin allergens contained in the defatted soybean powder to antibodies, which increased the antigenicity. Therefore, the method proposed in this study had displayed the potential to be initially used to detect the antigenic changes of thermally processed products containing glycinin after heat treatments. The method was able to detect the degrees of the thermal processing of the products containing glycinin, and thereby could be used in the future to effectively guide the thermal processing of products containing glycinin under safe production conditions.

4. Discussion

Glycinin is one of the main allergens in soybean. The tests conducted in this study optimized and established a novel DAS-ELISA method for the detection of the antigenic changes of heat-processed products containing glycinin after heat treatments. The proposed DAS-ELISA detection method was established through optimization experiments, and the results are shown in Fig. 1 and Tables 1 and 2. The specificity tests of the

proposed detection method confirmed that the specificity of the method was good. The cut-off value was 0.388, and results which were higher than that value were considered to be positive results, as detailed in Fig. 2. The sensitivity test results showed that the LOD of the glycinin was 19.53 ng/mL (Fig. 3). Then we found a competitive ELISA based on monoclonal antibody 4B2 to measure glycine, with a detection limit of 0.3 ng/mL [22]. The sensitivity of the method established in our study is not as high as the above sensitivity, which is largely due to the use of monoclonal antibody, which makes it more sensitive. The coefficient of variation of the proposed method was less than 5.25% (intra-day) and 9.50% (inter-day), as detailed in Table 3. In this study's milk powder addition test, the recovery rate of the glycinin had ranged from 83.65% to 90.13% (Table 4). The DAS-ELISA method established in this study was used to detect thermally processed products containing glycinin. The results showed that the OD_{450} values of the tested soy sauce, steamed fish soy sauce, soybean paste, beef sauce, soy milk powder, and tofu were all lower than that of the defatted soybean flour. The OD_{450} value of the defatted soybean flour indicates that the above-mentioned types of products containing glycinin had undergone different degrees of thermal processing. It was found that after thermal processing, the majority of the epitopes of the glycinin contained in the products had been destroyed by thermal processing and could not be combined with the heat-treated antigen-absorbing antiserum. Therefore, the detection values of OD_{450} were reduced, as illustrated in Fig. 4.

The detection method established in this experiment was a double-antibody sandwich direct ELISA method. The two antibodies were homologous. The differences between the two antibodies were the coating antibody used in the experiment was a heat-treated antigen-absorbing antiserum, in which the supernatant after the polyclonal antibody was incubated with glycinin under optimal heat treatment conditions. The antibody contained in the supernatant refers to the antibody that specifically binds to the glycinin epitopes, which could be destroyed by thermal processing. In other words, the antibody contained the binding site of the antigen destroyed by thermal processing, and the HRP-labeled rabbit anti-glycinin polyclonal antibody was used to detect the antigenic changes of glycinin in the thermally processed products. In the detection of thermally processed products containing glycinin, if the epitopes are destroyed by thermal processing, they will basically not be able to bind to the coating antibodies. Therefore, after adding the HRP-labeled rabbit anti-glycinin polyclonal antibodies, it will not be able to bind to the coating antibodies. As a result, the OD_{450} values of glycinin will be reduced. In addition, if the degree of thermal processing damage is low, part of the glycinin epitopes which have not been destroyed will bind to the coating antibodies, and the glycinin epitopes will be combined with the HRP-labeled rabbit anti-glycinin polyclonal antibodies to cause the final OD_{450} values to increase.

Abbreviations

DAS-ELISA	Double antibody sandwich enzyme-linked immunosorbent assay
PBS	Phosphate-buffered saline
CBS	Carbonate-buffered saline
TMB	3, 3', 5, 5'-Tetramethylbenzidine liquid substrate
LOD	Limit of detection

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Declaration of competing interest

The authors declare that there were no competing financial interests

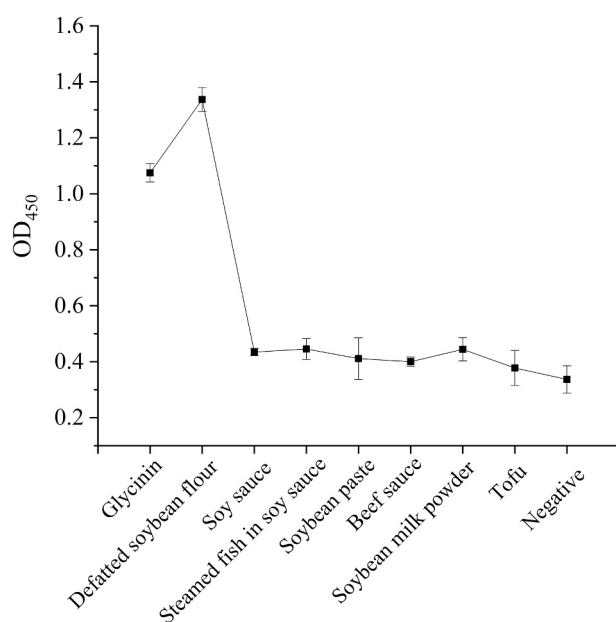


Fig. 4. Detection results of the thermal processed soybean products using the established DAS-ELISA method. Each sample test was repeated three times.

in this study.

References

- [1] L. Monaci, R. Pilolli, E. De Angelis, J.F. Crespo, N. Novak, B. Cabanillas, Food allergens: classification, molecular properties, characterization, and detection in food sources, *Adv. Food Nutr. Res.* 93 (2020) 113–146.
- [2] A. Rulff, B.S. Stahlman, Increasing incidence and prevalence of food allergies in the US military, 2000–2017, *J. Allergy Clin. Immunol.* 8 (1) (2020) 361–363. In Practice.
- [3] S. Rostam, Prevalence of common food allergies in Erbil Province, Kurdistan Region of Iraq, *AIMS AllergyImmunol.* 4 (4) (2020) 117–127.
- [4] F. Onyimba, S.E. Crowe, S. Johnson, J. Leung, Food allergies and intolerances: a clinical approach to the diagnosis and management of adverse reactions to food, *Clin. Gastroenterol. Hepatol.* 19 (11) (2021) 2230–2240.
- [5] S.H. Sicherer, H.A. Sampson, Food allergy: A?review and update on epidemiology, pathogenesis, diagnosis, prevention, and management, *J. Allergy Clin. Immunol.* 141 (1) (2018) 41–58.
- [6] K.P. Walsh, K.H.G. Mills, Dendritic cells and other innate determinants of T helper cell polarisation, *Trends Immunol.* 34 (11) (2013) 521–530.
- [7] K.D. Stone, C. Prussin, D.D. Metcalfe, IgE, mast cells, basophils, and eosinophils, *J. Allergy Clin. Immunol.* 125 (2) (2010) S350.
- [8] S. Wilson, K. Blaschek, E.G. Mejia, Allergenic proteins in soybean: processing and reduction of P34 allergenicity, *Nutr. Rev.* 63 (2) (2005) 47–58.
- [9] M. Adachi, J. Kanamori, T. Masuda, K. Yagasaki, S. Utsumi, Crystal structure of soybean 11S globulin: glycinin A3B4 HOMOhexamer, *Proc.Natl.Acad.Sci.* 100 (12) (2003) 7395–7400.
- [10] Y. Zhao, D. Liu, R. Han, X. Zhang, S. Zhang, G. Qin, Soybean allergen glycinin induced the destruction of the mechanical barrier function in IPEC-J2, *FoodAgric. Immunol.* 26 (4) (2015) 601–609.
- [11] K. Prak, K. Nakatani, T. Katsube-Tanaka, M. Adachi, N. Maruyama, S. Utsumi, Structure-function relationships of soybean proglycinins at subunit levels, *J. Agric. Food Chem.* 53 (9) (2005) 3650–3657.
- [12] Tandang-Silvas, MRG, Fukuda, Prak, Cabanos, Kimura, Itoh, Mikami, Utsumi, Maruyama, Conservation and divergence on plant seed 11S globulins based on crystal structures, *BBA-Proteins Proteom.* 1804 (7) (2010) 1432–1442.
- [13] R.M. Helm, G. Cockrell, C. Connaughton, H.A. Sampson, A.W. Burks, A soybean G2 glycinin allergen. 1. Identification and characterization, *Int.Arch.AllergyImmunol.* 123 (3) (2000) 205–212.
- [14] F. Chen, Y. Hao, X.S. Piao, X. Ma, J.J. Wang, Soybean-derived beta-conglycinin affects proteome expression in pig intestinal cells in vivo and in vitro, *J. Anim. Sci.* 89 (3) (2011) 743–753.
- [15] A. Sena-Torralba, Y. Pallás-Tamarit, S. Morais, Á. Maquieira, Recent advances and challenges in food-borne allergen detection, *TrAC Trends Anal. Chem.* 132 (2020), 116050.
- [16] L. Monaci, A. Visconti, Immunochemical and DNA-based methods in food allergen analysis and quality assurance perspectives, *Trends Food Sci. Technol.* 21 (6) (2010) 272–283.
- [17] R. Pilolli, L. Monaci, A. Visconti, Advances in biosensor development based on integrating nanotechnology and applied to food-allergen management, *TrAC Trends Anal. Chem.* 47 (2013) 12–26.
- [18] L. Monaci, E. De Angelis, N. Montemurro, R. Pilolli, Comprehensive overview and recent advances in proteomics MS based methods for food allergens analysis, *TrAC Trends Anal. Chem.* 106 (2018) 21–36.
- [19] H. Hu, W. Zeng, Y. Wang, Q. Wang, C. Shi, Development and application of a recombinant protein-based indirect ELISA for detection of anti-tilapia lake virus IgM in sera from tilapia, *Aquaculture* 734756 (2019).
- [20] S. Jayasena, M. Smits, D.L. Fiechter, A. De Jong, J. Nordlee, J. Baumert, S. L. Taylor, R.H. Pieters, S.J. Koppelman, Comparison of six commercial ELISA kits for their specificity and sensitivity in detecting different major peanut allergens, *J. Agric.Food Chem.* 63 (6) (2015) 1849–1855.
- [21] J. Xi, Q.Q. Shi, Development of an indirect competitive ELISA kit for the detection of soybean allergenic protein Gly m Bd 28K, *Food Anal. Methods* 9 (11) (2016) 2998–3005.
- [22] X. Ma, P. Sun, P. He, P. Han, J. Wang, S. Qiao, D. Li, Development of monoclonal antibodies and a competitive ELISA detection method for glycinin, an allergen in soybean, *Food Chem.* 121 (2) (2010) 546–551.
- [23] B. Liu, D. Teng, Y. Yang, X. Wang, J. Wang, Development of a competitive ELISA for the detection of soybean α subunit of β -conglycinin, *Process Biochem.* 47 (2) (2012) 280–287.
- [24] J.M. You, D. Li, S.Y. Qiao, Z.R. Wang, P.L. He, D.Y. Ou, B. Dong, Development of a monoclonal antibody-based competitive ELISA for detection of beta-conglycinin, an allergen from soybean, *Food Chem.* 106 (1) (2008) 352–360.
- [25] I. Segura-Gil, A. Blázquez-Soro, P. Galán-Malo, L. Mata, A.P. Tobajas, L. Sánchez, M. Pérez, Development of sandwich and competitive ELISA formats to determine β -conglycinin: evaluation of their performance to detect soy in processed food, *Food Control* 103 (2019) 78–85.
- [26] A. St, A. Pt, B. Sma, B. Nma, Recent advances and challenges in food-borne allergen detection, *TrAC Trends Anal. Chem.* 132 (2020), 116050.
- [27] W. Hei, Z. Li, X. Ma, P. He, Determination of beta-conglycinin in soybean and soybean products using a sandwich enzyme-linked immunosorbent assay, *Anal. Chim. Acta* 734 (1) (2012) 62–68.
- [28] N. Morishita, K. Kamiya, T. Matsumoto, S. Sakai, R. Teshima, A. Urisu, T. Moriyama, T. Ogawa, H. Akiyama, F. Morimatsu, Reliable enzyme-linked immunosorbent assay for the determination of soybean proteins in processed foods, *J. Agric. Food Chem.* 56 (16) (2008) 6818–6824.
- [29] E. Ueberham, H. Spiegel, H. Havenith, P. Rautenberger, N. Lidzba, S. Schillberg, J. Lehmann, Simplified tracking of a soy allergen in processed food using a monoclonal antibody-based sandwich ELISA targeting the soybean 2S albumin gly m 8, *J. Agric. Food Chem.* 67 (31) (2019) 8660–8667.
- [30] T. Geng, K. Liu, R. Frazier, L. Shi, E. Bell, K. Glenn, J.M. Ward, Development of a Sandwich ELISA for quantification of gly m 4, a soybean allergen, *J. Agric. Food Chem.* 63 (20) (2015) 4947–4953.
- [31] J. Chen, J. Wang, P. Song, X. Ma, Determination of glycinin in soybean and soybean products using a sandwich enzyme-linked immunosorbent assay, *Food Chem.* 162 (2014) 27–33.
- [32] J. Xi, M.X. He, J.Y. Pi, Identification of antigenic sites destructed by high hydrostatic pressure (HHP) of the β subunit of β -conglycinin, *Int. J. Biol. Macromol.* 141 (1) (2019) 1287–1492.
- [33] W.H. Li, Q.H. Zhang, J.D. Li, S.C. Cao, Detection of IgM antibody against hantavirus by chemiluminescent enzyme-linked immunosorbent assay, *Chin. J. Exp. Clin. Virol.* 21 (2) (2007) 171–173.