

The structural properties and antigenicity of soybean glycinin by glycation with xylose

Running title: Structure and antigenicity of soybean glycinin by glycation

Guanhao Bu,* Tingwei Zhu and Fusheng Chen*

College of Food Science and Technology, Henan University of Technology,
Zhengzhou 450001, China

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/

*Correspondence to: Guanhao Bu and Fusheng Chen, College of Food Science and Technology, Henan University of Technology, Zhengzhou 450001, China, E-mail: buguanhao2008@126.com (Guanhao Bu), fushengc@aliyun.com (Fusheng Chen).

Abstract:

BACKGROUND: Soybean glycinin is considered as a major allergenic protein, and glycation is widely used to reduce allergenic potential of present allergens. Glycation of soybean glycinin with xylose at 55 °C for different lengths of time was investigated. The extent of Maillard reaction was reflected through the content changing of free amino groups, color analysis and SDS-PAGE. Alteration in the structural properties of glycinin was characterized by Fourier transform infrared spectrum (FTIR), and the antigenicity was evaluated by indirect competitive ELISA.

RESULTS: The changes in the color of glycinin-xylose samples and the reduction of free amino group content in proteins indicated the Maillard reaction occurred. The degree of glycation increased in glycated samples with the increase of reaction time. Glycation induced the changes in the secondary structure of glycinin and the ordered structure of proteins increased during the glycation reaction. The antigenicity of glycinin was reduced with the increase of reaction time. After glycation for 12 h, the antigenicity of glycinin declined about 18% compared with native glycinin.

CONCLUSION: The application of glycation may be an efficient method to reduce the antigenicity of soybean glycinin.

Keywords: Soybean allergen; Glycinin; Glycation; Antigenicity; Structure

INTRODUCTION

Soybean is one of the most valuable sources of protein, and its by-products are commonly used in the food and feed industries because of their high nutritional value.^{1,2} However, soybean is one of eight common allergenic foods. Soybean allergens induced allergic reaction can decrease digestibility, cause respiratory, cutaneous, and gastrointestinal symptoms, and even result in death.^{3,4} Soybean allergens affected about 1~6% of infants and 2~4% of adults, who exhibited a variety of clinical symptoms.⁵

Glycinin also known as 11S seed storage protein is one of the major components in soybean protein with a molecular mass of 300~380 kDa.^{6,7} Glycinin has five different subunits, G1 (A1aB1b, 53.6 kDa), G2 (A2B1a, 52.4 kDa), G3 (A1bB2, 52.2 kDa), G4 (A5A4B3, 61.2 kDa) and G5 (A3B4, 55.4 kDa), which accounts for about 40% of the total soybean globulins.^{1,8,9} Each of the glycinin subunits can be dissociated into acidic (A, 31~45 kDa) and basic (B, 18~20 kDa) polypeptide chains under reducing conditions.¹⁰ Glycinin can harm intestinal morphology, and be responsible for disorders of the immune system, depress growth, and cause diarrhea.¹¹ The higher the doses of glycinin are, the more severe are the immune reaction. Therefore, it is necessary to search for methods to reduce the antigenicity of glycinin. Several processing methods have been studied to reduce protein antigenicity, including heat treatment, enzymatic hydrolysis, fermentation, and glycation.¹²⁻¹⁷ Since glycinin has thermal stability, this characteristic increases the difficulty of removing its antigenicity. Therefore, glycinin cannot be degraded by heating or extruding, which limits the use of soybean in the food and feed industries.³

The Maillard reaction is a complex form of nonenzymatic browning between free amino groups in protein and reducing sugars. The Maillard reaction is a

promising approach for shielding food allergens and also seems to be an effective approach to improve the functional properties of proteins, because of mild reaction and no extraneous chemicals.¹⁸ It revealed that β -LG-acidic-oligosaccharide conjugates reduced the immunogenicity through shielding the B-cell epitopes.¹⁵ Bielikowicz *et al.*¹⁹ found that the pea globulin 7S conjugated to glucose had a lowered affinity to antibodies.

It was reported that the trace amounts of glycinin in soybean products could be detected by a sandwich ELISA. The developed assay provides a specific and sensitive method for the screening of glycinin.³ In another study, the physicochemical properties of casein-glucose were measured using SEC, SDS-PAGE, PR-HPLC, and then the structure was observed by FTIR. Experiments investigated the effects of Maillard reaction on the antioxidant activities of glycated proteins and changes in the structure of protein.²⁰ Currently, many studies have focused on the effect of glycation on shielding protein allergens with different saccharides and the changes between functional properties and structural properties. The glycation induced changes in the antigenicity of soybean glycinin and the alteration of its structure have not been well understood yet. Xylooligosaccharides have immunomodulatory activity inhibitory effect on precancerous colon lesions and inhibitory effects on the growth of sulphite-reducing clostridia.²¹ Using the monomer, Xylose can accelerate the reaction. Therefore, the current study was to investigate the antigenicity of glycinin by glycation with xylose. At the same time, changes in protein content and structural properties were determined by SDS-PAGE and FTIR, respectively. This study may provide a method to reduce the antigenicity of soy protein products.

MATERIALS AND METHODS

Materials

Defatted soybean flour (protein content 556.2 g kg⁻¹) was obtained from Gushen Biological Technology Group Co., Ltd. (Shandong, China). Xylose was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). HRP-labeled goat anti-rabbit IgG (A6154) and glycinin standards (G3171) were obtained from Sigma Chemical Co. Ltd (St. Louis, MO, USA). TMB chromogenic single-component liquid was obtained from Beijing Solarbio Science and Technology Co. Ltd. (Beijing, China). The anti-glycinin serum from rabbit was generated by our own laboratory. All other reagents were of analytical grade.

Preparation of soybean glycinin

Soybean glycinin was obtained following the procedure described by Puppo *et al.*²² Defatted soybean flour (protein content 556.2 g kg⁻¹) was dispersed with ratio 1:15 w/w in distilled water, adjusted to pH 8.0 with 2 mol L⁻¹ NaOH, stirred for 1 h in a 45 °C water bath, and centrifuged at 10,000 × g for 20 min at 4 °C. NaHSO₃ (0.01 mol L⁻¹) was added to the supernatant and adjusted to pH 6.4 with 2 mol L⁻¹ HCl, and kept overnight at 4 °C. The dispersion was centrifuged at 6500 × g for 20 min at 4 °C. The precipitate was dissolved with distilled water, adjusted to pH 7.5, and freeze-dried, to obtain soybean glycinin.

Protein content

Protein content was determined by the Kjeldahl nitrogen method. The protein content of extracted glycinin was 934.4 g kg⁻¹ and the quantitative analysis of glycinin by

BandScan 5.0 software was 926.0 g kg⁻¹.

Preparation of polyclonal antibody

Polyclonal antibody against glycinin (G3171) was produced in three young male New Zealand rabbits, weighing an average of 2.5 kg, which were obtained from the Experimental Animal Center, Zhengzhou University (Zhengzhou, China). Before immunizing the rabbits, blood from an ear vein was collected as a negative control serum. The immunization procedure was performed as we previously described.²³ The titers of antibodies generated was controlled by indirect ELISA method with blood drawn from the rabbit marginal vein 3 days before the subsequent immunization. Four days after the sixth sensitization, blood samples were collected from the rabbit hearts and stored at 4 °C overnight. The antiserum was separated by centrifuging at 3000 r min⁻¹ for 20 min at 4 °C and then stored at -20 °C for the further analysis of the antigenicity of glycinin by indirect competitive ELISA. Titers of rabbit sera obtained are above 1:10000 in this experiment.

Preparation of glycinin-xylose conjugates

Glycinin and xylose mixtures (weight ratio 3:1) were dissolved in distilled water until the solution's ultimate solubility was 60 g L⁻¹. After that the solution was freeze-dried and then incubated at 55 °C and 79% relative humidity controlled by saturated solution of KBr at different times (sampled at an interval of 3 h). These samples were stored at -20 °C before analysis.

Measurement of color

The evaluation of color of the glycinin-xylose samples was carried out using a

CR-400 tristimulus colorimeter (KonikaMinolta, Tokyo, Japan). The system expressed the values of three-color components in L (black-white component), a (+red to -green component), and b (+yellow to -blue component) according to the CIE definition. The E index was calculated from the equation: $E = (L^2 + a^2 + b^2)^{1/2}$. Chroma value was calculated by the equation: $C = (a^2 + b^2)^{1/2}$.²⁴

Determination of free amino groups and the degree of glycation

The content of free amino groups (FAG) was determined by the trinitrobenzene sulfonic acid (TNBS) method.²⁵ The sample was dissolved with 10 g L⁻¹ sodium dodecyl sulfate (SDS) to ensure the final protein concentration of 5 mg mL⁻¹. The TNBS reagent was freshly prepared each time. Two mL 1 g L⁻¹ TNBS reagent was added to 0.25 mL sample solution with 2 mL of 0.2 mol L⁻¹ phosphate buffers (pH 8.2). After incubating at 50 °C for 60 min, 4 mL 0.1 mol L⁻¹ HCl was added to end the reaction. The mixtures were placed in the dark for 30 min. The final mixture solution was measured for absorbance at 420 nm using the UV spectrophotometer. The calibration was made by using 0-3.5 mmol L⁻¹ leucine as a standard. The degree of glycation (DG) was calculated based on the loss of amino groups compared to untreated glycinin.

$$DG (\%) = (C_0 - C) / C_0 \times 100$$

where C_0 was the free amino group content of the untreated glycinin and C was the free amino group content of the glycated samples.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted on a discontinuous buffered system using a 120 g L⁻¹ separating gel and a

40 g L⁻¹ stacking gel.²⁶ Five μ L of a sample buffer (containing 0.5 mol L⁻¹ Tris-HCl buffer, 100 g L⁻¹ SDS, 20 g L⁻¹ β -mercaptoethanol, 200 g L⁻¹ glycerol, and 5 g L⁻¹ bromophenol blue) was added to a 5 μ L protein sample (protein concentration 2 mg mL⁻¹) and heated for 4 min in boiling water before electrophoresis. Gels were run at a constant current of 25 mA (lasting about 45 min) before changing to 40 mA when the bromophenol blue indicator ran into the separating gel (lasting about 90 min). After electrophoresis, proteins in gels were stained with Coomassie brilliant blue R-250.

Fourier transform infrared spectrum measurement

All infrared spectra of the glycinin-xylose samples were obtained by a WQF-510 Fourier transform infrared spectrum (FTIR) equipped with a computer (Beijing Ruili Analytical Instruments Co. Ltd. Beijing, China). Samples were mixed with potassium bromide (KBr) (1:100, w/w), ground with a mortar to form a homogeneous powder, and then pressed into a sheet, and scanned from 4000 to 400 cm⁻¹ within 32 scans using the Fourier transform infrared spectrophotometer.²⁷

Antigenicity assay by indirect competitive ELISA

The antigen inhibition rate of glycinin in the glycated protein products was tested by indirect competitive ELISA.^{23,28} Before determining the antigenicity of glycinin, working conditions about the ELISA method were optimized in detail, including working concentrations of antigen and antibody, reproducibility, sensitivity and specificity of ELISA method. The operating procedures of the method are as follows. Microtiter plates with 96 wells (Costar, Corning, New York, USA) were coated with 100 μ L per well of glycinin antigen (0.025 μ g mL⁻¹, G3171) diluted in 50 mmol L⁻¹ carbonate buffer and incubated at 4 °C overnight. The sample solution (2 mg mL⁻¹)

was mixed with an equivalent volume of rabbit anti-glycinin serum (1:3200) diluted in 0.01 mol L⁻¹ phosphate-buffered saline (PBS, pH 7.4) containing 10 g L⁻¹ BSA and 1 g L⁻¹ Tween-20 (PBST-BSA) and then they were incubated, respectively, in test tubes overnight at 4 °C. The wells were washed four times (250 µL per well) the next day with PBST buffer (0.01 mol L⁻¹ PBS, pH 7.4, 0.5 g L⁻¹ Tween-20). The washing procedure was repeated after each analytical step. The plate was filled with 100 µL per well of PBST-BSA to block residual free binding sites and incubated at 37 °C for 1 h after washing. The plate was washed before adding the reactive mixtures of glycated samples and polyclonal rabbit antibodies (100 µL per well), and then it was incubated for 1 h at 37 °C. After plate washing, HRP-labeled goats anti-rabbit IgG (100 µL, A6154, 1:10000) was added to each well and was incubated at 37 °C for 1 h. After the plate was washed again before adding the substrate 3, 3', 5, 5'-Tetramethylbenzidine (TMB) (100 µL per well) and incubating at 37 °C for 10 min. The reaction was stopped by the addition of 2 mol L⁻¹ H₂SO₄ (50 µL per well). Uninhibited serum sample (no sample) was used as a control. The optical density (OD) was detected at the dual wavelengths of 450 nm and 620 nm by using a microplate reader (Thermo Fisher Scientific Instrument Co. Ltd. New York, USA). The value of OD was obtained by subtracting OD₆₂₀ from OD₄₅₀.

The antigenicity of glycated samples was represented by the inhibition rate of glycinin, which was calculated as follows: Inhibition rate (%) = $[(B_0 - B)/B_0] \times 100$, where B is the OD of the glycated samples and B_0 is the OD of the uninhibited serum sample control. A low percentage of inhibition reflects a low antigenicity of samples for glycinin.

Statistical analysis

Three independent experiments were performed for each parameter. One-way analysis of variance (ANOVA) was performed using the SPSS 16.0 statistical analysis system. The significance level was established for Duncan's test at $P < 0.05$. Secondary structure of proteins from FTIR was analyzed using the PeakFit V4.12 software. The protein percent from SDS-PAGE was analyzed by BandScan 5.0 software.

RESULTS AND DISCUSSION

Color analysis

The color measurement is often used to analyze the degree of Maillard reaction. The relative visual color with browning was shown in Table 1. There was a significant increase ($P < 0.05$) in a value of glycinin-xylose samples with the increase in reaction times. Meanwhile, the b value also showed the same trend. The values changed from yellow-brown to orange-red during the Maillard reaction. The glycinin-xylose sample showed the darkest color at 12 h. The value of E is mostly affected by the color lightness (L), and the value of C is defined as the degree of departure from grey to a pure chromatic color.²¹ As shown in Table 1, the value of E decreased and the value of C had an opposite trend, this indicates that the glycated samples increased in darkness and exhibited more red and yellow during the Maillard reaction. The color was more intense with increasing Maillard reaction time, which suggested the increase of the extent of glycation. The color formation is mainly due to the presence of melanoidins, which have a high molecular weight.²⁹

Changes in free amino groups and glycation degree

The free amino groups (FAG) in the glycinin-xylose samples and the degree of glycation (DG) are depicted in Figure 1. With the increase of reaction time, FAG

content of glycated samples gradually reduced and that is to say DG increased.

DG is often used to evaluate the Maillard reaction. The reaction time was controlled to explore the effects of glycinin-xylose interactions on DG. DG increased in the glycinin-xylose samples from 0 h to 12 h (Figure 1). At the beginning of the Maillard reaction, FAG exposed at the surface of the protein molecules were more prone to start reactions than were those that were masked inside the proteins. Reaction occurred in the FAG inside the protein molecules after the protein structure changed. The decrease in the content of FAG indicated that the Maillard reaction occurred between FAG of protein and saccharides,³⁰ which could also increase the color intensity.

SDS-PAGE analysis

SDS-PAGE patterns of glycinin-xylose samples, at various reaction times, are depicted in Figure 2. Lighter colour of electrophoretic bands in Figure 2 indicated glycation could cause a decrease in the intensity of the protein subunits (~40 kDa, ~30 kDa). Meanwhile, the migration rate of main primary protein fractions slowed down and the high molecular mass aggregates increased (the top of the electrophoresis gel). The glycated samples incubated for different lengths of time also had a similar band pattern. These results suggested that xylose bound to glycinin during the dry-heat treatment through the Maillard reaction, and that high molecular weight products formed (Figure 2, No. 0).

Additionally, the protein bands (Figure 2) corresponding to protein subunit content listed in Table 2. Glycinin (11S) is a heterogeneous protein and each of the glycinin subunits can be dissociated into acidic subunits (A, 31~45 kDa) and basic subunits (B, 18~20 kDa) under reducing conditions.³ As the reaction proceeded, the

content of glycinin was reduced from 92.60% to 70.15% within 12 h. A smear band with high molecular weight appeared at the top of the stacking gel (Figure 2, No. 0), which occupied 16.51% and 27.50% of the content after reaction for 6 h and 12 h, respectively. The SDS-PAGE indicated that high molecular weight conjugated proteins were generated from native glycinin when incubated with xylose, which confirms probable the formation of a complex polymer.^{20,31} It may be that polymerization occurred during glycation between protein and monosaccharide, and the polymerization caused by covalent bonds other than the disulphide bonds, and the sulphydryl-disulphide interchange reaction caused the polymerization between proteins and polysaccharides.³² With the formation of numerous polymers, the antigenicity of glycated samples might be altered, because these generated conjugates may mask IgE binding epitopes.³³

FTIR analysis

The FTIR spectroscopy is an especially useful method for studying protein-saccharide systems, as there are several easily discernable regions of the mid-infrared spectrum where the chemical fingerprints of carbohydrates and proteins do not overlap significantly.^{20,34} Figure 3 shows that absorption of glycinin in the region of 1041.37cm^{-1} appeared after a reaction time of 6 h or more, while the absorption at the same region was weak at 0 h, indicating that there seemed to be some saccharides cross-linked to glycinin. In the region of $953\sim 1180\text{cm}^{-1}$, there were overlapping peaks for carbohydrates, which results from the stretching of C-C and C-O and the bending mode of C-H bonds. These bonds are often referred to as the “saccharide” bands; these absorptions are weak in the spectra of most proteins.^{20,35} Compared to native glycinin, the glycated glycinin's peak intensity in 1533.13cm^{-1} and 1643.05cm^{-1}

decreased. It reflects C-N and C=O stretching. The Maillard reaction in glycinin could lead to several changes in the spectrum as a result of the depletion of some functional groups such as NH₂ Lysine; while the quantity of the Amadori compound (C=O), Schiff base (C=N), and pyrazines (C-N) may be changed by the Maillard reaction.^{20, 36} In addition, the absorption peak in the region of 3250 cm⁻¹ changed was related to hydrogen bond stretching and the vibration of OH groups. An important change in the glycosylated samples was the broadening of the absorption peak in the region of 3288.04 cm⁻¹ due to the hydrogen bond in the OH stretching vibration. In addition, the peak in the region of 1553 cm⁻¹ showed a slight change, indicating that the hydrogen bond changed, which may be related to the β -structure changes.

The protein secondary structure is reflected in the 1600~1700 cm⁻¹ range, which is the characteristic absorption band for protein amino I. The following parameters were used in the study: β -sheet, 1600~1640 cm⁻¹; disordered structure, 1640~1650 cm⁻¹; α -helix structure, 1650~1660 cm⁻¹; and β -turn structure, 1660~1700 cm⁻¹.³⁷ The data were analyzed by the PeakFit V4.12 software, which depicted the secondary structure as shown in Table 3. The glycinin-xylose samples lost random coil structure with a concomitant increase in β -sheet structure. The structure of α -Helix and β -Turns were essentially unchanged. With the increase in reaction time, the portion of the ordered structure (β -sheet) in proteins increased, and the disordered structure decreased. In sum, the ordered structure of proteins increased during the glycation reaction. These results implied that the secondary structures of glycinin could be changed by the Maillard reaction. The β -sheet structure of proteins is usually buried in the interior of polypeptide chains. The cross-linking of saccharides to proteins led to an unfolding of protein molecules and changes in spatial structure.³⁸

The difference between glycosylated products and the native glycinin was manifested

in the secondary structure of the proteins defined by FTIR spectra. Prolonged high temperatures may denature proteins through folding and subsequently aggregation. Glycated samples could be converted to high molecular weight conjugates due to the generation of protein-saccharide cross-linking reactions.^{39,40} The subunits of glycinin or β -conglycinin could participate in changes in the β -sheet by disulfide bonds.⁴¹

Antigenicity of the glycated products

The changes in electrophoretic patterns and secondary structure of glycated proteins may result in the alteration of their antigenicity. In order to confirm this, an indirect competitive ELISA was developed to detect the antigenicity of glycated proteins and the native form. The indirect competitive ELISA method was established by coating an antigen with glycinin, using the rabbit anti-glycinin polyclonal antibody as the primary antibody and using HRP (horse radish peroxidase) -marked goat anti rabbit IgG as IgG-HRP. The coefficients of variation of intra-plate error and inter-plates error were 3.82% and 10.22% separately, and detecting the linear range was 0.01~0.05 $\mu\text{g/mL}$. The indirect competitive ELISA method established in the study has good reproducibility, sensitivity and specificity, and can be used for the detection of soybean glycinin.⁴² In another study, Chen *et al.*³ performed a sandwich ELISA for detection of trace amounts of glycinin in soybean products. Using anti-glycinin monoclonal antibody as coating antibody, with the rabbit anti-glycinin polyclonal antibody as a detected antibody, the developed assay showed high sensitivity and specificity for the detection and screening of glycinin. So, the above two immunological methods can be all applied to effectively detect soybean glycinin.

Glycinin in glycated products was detected by the established indirect competitive ELISA. In the glycated samples, the initial antigen inhibition rate of

glycinin (0 h) was 85.89% (Figure 4). After 12 h, the antigen inhibition rate of glycinin decreased by 18%. In the above study, SDS-PAGE analysis showed that the content of glycinin was reduced from 92.60% to 70.15% within 12 h with the increasing reaction time (see Figure 2 and Table 2). This indicates that the content of glycinin may have a positive correlation with its antigenicity. The antigenicity and structural properties of glycinin were changed significantly during the Maillard reaction depending on the reaction time. Glycation treatment for 6 h had an important impact on the secondary structures (see Figure 3 and Table 3) and the antigenicity of glycinin. Moreover, longer the reaction time, the greater the protein antigenicity and structures changed.

Glycation could reduce the antigenicity of soybean protein. However, changes in the antigenicity of proteins might depend on types of sugar used and Maillard reaction conditions. Bieliowicz *et al.*¹⁹ found that the immunoreactivity of pea globulin 7S after glycation with glucose at 60 °C for 3 days was reduced about 30%. The reduced antigenicity in our work could have been achieved after a 12 h reaction time, because xylose is a pentose sugar; the reaction rate of glycation is faster than the rate of glycation with glucose, which is a hexose sugar. In addition, previous study reported that the antigenicity of SPI glycated with fructose at 95 °C was largely reduced (up to 90%) compared with the native protein. The changes of antigenicity may be related to a difference in binding specificity of the sugar to the allergenic sites of the protein under certain reaction temperatures.²⁸ Moreover, the antigenicity of glycated products was reduced with the increase of reaction time, suggesting that the extent of glycation might be a factor leading to decreased antigenicity. This phenomenon can be explained by formation of a different conformational structure during the binding specificity of saccharides to the antigenic sites of proteins.^{19,43}

The changes of protein antigenicity are closely related to the structural changes caused by glycation. The secondary structures (β -structure, random coil) of glycinin due to glycation were unfolded, which were confirmed in the SDS-PAGE and FTIR patterns. This change presumably caused partial shielding of the antigenic epitopes of glycinin, which resulted in the decrease of antigen and antibody binding specificity, thereby reducing the antigenicity. In addition, soybean glycinin used in this research should be further purified to obtain the high-purity glycinin by an immunoaffinity chromatographic method,⁴⁴ which is useful for future investigation to reveal how glycinin functions in anaphylaxis and to explore the mechanism of reducing glycinin antigenicity during food processing.

CONCLUSIONS

Glycinin-xylose samples were prepared by a dry-heated Maillard reaction. The changes in the color of the samples and the reduction of free amino group content in proteins indicate the Maillard reaction occurred. At the same time, the antigenicity inhibition rate of glycinin in glycinin-xylose samples was reduced up to 18% compared with native glycinin. The increase in β -sheet provided important insights into how glycation affects the antigenicity through the changes in structure. The decrease in antigenicity with the increasing in Maillard reaction time suggests that glycation might be a promising method to reduce antigenicity. Further research could be carried out on the spatial structures of glycated proteins and the glycated effective sites. Furthermore, the influences of glycation on the gastrointestinal digestibility of soybean proteins also need to be explored in the future studies.

ACKNOWLEDGEMENTS

This work was supported financially by the National Natural Science Foundation of China (31201293), the National High Technology Research and Development Program of China (863 Program) (2013AA102208-5), Foundation of Henan Educational Committee (14B550013), and the Fundamental Research Funds for the Henan Provincial Colleges and Universities in Henan University of Technology (2015RCJH02).

REFERENCES

- 1 Ma X, Sun P, He PL, Han PF, Wang JJ, Qiao SY *et al.*, Development of monoclonal antibodies and a competitive ELISA detection method for glycinin, an allergen in soybean. *Food Chem* **121**: 546-551 (2010).
- 2 Friedman M and Brandon DL, Nutritional and health benefits of soy proteins. *J Agric Food Chem* **49**: 1069-1086 (2001).
- 3 Chen JS, Wang J, Song PX and Ma X, Determination of glycinin in soybean and soybean products using a sandwich enzyme-linked immunosorbent assay. *Food Chem* **162**: 27-33 (2014).
- 4 Sampson HA, Food anaphylaxis. *Brit Med Bull* **56**: 925-935 (2000).
- 5 Tryphonas H, Arvanitakis G, Vavasour E and Bondy G, Animal models to detect allergenicity to foods and genetically modified products: workshop summary. *Environ Health Persp* **111**: 221-222 (2003).
- 6 Maruyama N, Katsube T, Wada Y, Oh MH, Barba De La Rosa AP, Okuda E *et al.*, The roles of the N-linked glycans and extension regions of soybean β -conglycinin in folding, assembly and structural features. *Eur J Biochem* **258**:

- 854-862 (1998).
- 7 Mujoo R, Trinh DT and Ng PKW, Characterization of storage proteins in different soybean varieties and their relationship to tofu yield and texture. *Food Chem* **82**: 265-273 (2003).
- 8 Prak K, Nakatani K, Katsube-Tanaka T, Adachi M, Maruyama N and Utsumi S, Structure-function relationships of soybean proglycinins at subunit levels. *J Agric Food Chem* **53**: 3650-3657 (2005).
- 9 Ma X, He P, Sun P and He P, Lipoic Acid: an immunomodulator that attenuates glycinin-induced anaphylactic reactions in a rat model. *J Agric Food Chem* **58**: 5086-5092 (2010).
- 10 Maruyama N, Prak K, Motoyama S, Choi SK, Yagasaki K, Ishimoto M *et al.*, Structure physicochemical function relationships of soybean glycinin at subunit levels assessed by using mutant lines. *J Agric Food Chem* **52**: 8197-8201 (2004).
- 11 Liu X, Feng J, Xu ZR, Wang YZ and Liu JX, Oral allergy syndrome and anaphylactic reactions in BALB/c mice caused by soybean glycinin and betaconglycinin. *Clin Exp Allergy* **38**: 350-356 (2008).
- 12 Gomaa A and Boye JI, Impact of thermal processing time and cookie size on the detection of casein, egg, gluten and soy allergens in food. *Food Res Int* **52**: 483-489 (2013).
- 13 Cucu T, Platteau C, Taverniers I, Devreese B, De Loose M and De Meulenaer B, Effect of partial hydrolysis on the hazelnut and soybean protein detectability by ELISA. *Food Control* **30**: 497-503 (2013).
- 14 Song YS, Frias J, Martinez-Villaluenga C, Vidal-Valverde C and De Mejia EG, Immunoreactivity reduction of soybean meal by fermentation, effect on amino acid composition and antigenicity of commercial soy products. *Food Chem* **108**:

- 571-581 (2008).
- 15 Hattori M, Miyakawa S, Ohama Y, Kawamura H, Yoshida T, To-o K *et al.*, Reduced immunogenicity of β -lactoglobulin by conjugation with acidic oligosaccharides. *J Agric Food Chem* **52**: 4546-4553 (2004).
- 16 Teng D, Gao MY, Yang YL, Liu B, Tian ZG and Wang JH, Bio-modification of soybean meal with *Bacillus subtilis* or *Aspergillus oryzae*. *Biocatal Agric Biotechnol* **1**: 32-38 (2012).
- 17 He L, Han M, Qiao SY, He PL, Li DF, Li N *et al.*, Soybean antigen proteins and their intestinal sensitization activities. *Curr Protein Pept Sci* **16**: 613-621 (2015).
- 18 Liu JH and Ru QM, Physicochemical properties and structure, a review, *Food Res Int* **49**: 170-183 (2012).
- 19 Bielikowicz K, Kostyra H, Kostyra E, Teodorowicz M, Rigby N and Wojtacha P, The influence of non-enzymatic glycosylation on physicochemical and biological properties of pea globulin 7S. *Food Res Int* **48**: 831-838 (2012).
- 20 Gu FL, Kim JM, Abbas S, Zhang XM, Xia SQ and Chen ZX. Structure and antioxidant activity of high molecular weight Maillard reaction products from casein-glucose. *Food Chem* **120**: 505-511 (2010).
- 21 Davide AC, Régis F, Elisabeth L and Gabrielle PV, Biochemical identification of the catalytic residues of a glycoside hydrolase family 120 β -xylosidase, involved in xylooligosaccharide metabolism by gut bacteria. *FEBS Lett* **589**: 3098-3106 (2015).
- 22 Puppo MC, Beaumal V, Speroni F, de Lamballerie M, Añón MC and Anton M, β -Conglycinin and glycinin soybean protein emulsions treated by combined temperature-high-pressure treatment. *Food Hydrocolloid* **25**: 389-397 (2011).
- 23 Bu GH, Zhu TW, Chen FS, Zhang N, Liu KL, Zhang LF *et al.*, Effects of

- saccharide on the structure and antigenicity of β -conglycinin in soybean protein isolate by glycation. *Eur Food Res Technol* **240**: 285-293 (2015).
- 24 Morales FJ and Jiménez-Pérez S, Free radical scavenging capacity of Maillard reaction products as related to colour and fluorescence. *Food Chem* **72**: 119-125 (2001).
- 25 Nissen JN, Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. *J Agric Food Chem* **27**: 1256-1263 (1979).
- 26 Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680-685 (1970).
- 27 Zhao XY, Chen FS, Xue WT and Lee L, FTIR spectra studies on the secondary structures of 7S and 11S globulins from soybean proteins using AOT reverse micellar extraction. *Food Hydrocolloid* **22**: 568-575 (2008).
- 28 Van de Lagemaat J, Manuel Silván J, Javier Moreno F, Olano A and Dolores del Castillo M, In vitro glycation and antigenicity of soy proteins. *Food Res Int* **40**: 153-160 (2007).
- 29 Adams A, Borrelli RC, Flogiano V and De Kimpe N, Thermal degradation studies of foods melanoidins. *J Agric Food Chem* **53**: 4136-4142 (2005).
- 30 Bu GH, Luo YK, Lu J and Zhang Y, Reduced antigenicity of β -lactoglobulin by conjugation with glucose through controlled Maillard reaction conditions. *Food Agr Immunol* **21**: 143-156 (2010).
- 31 Jiang SJ and Zhao XH, Transglutaminase-induced cross-linking and glucosamine conjugation in soybean protein isolates and its impacts on some functional properties of the products. *Eur Food Res Technol* **231**: 679-689 (2010).
- 32 Taheri-Kafrani A, Gaudin JC, Rabesona H, Nioi C, Agarwal D, Drouet M *et al.*, Effects of heating and glycation of β -lactoglobulin on its recognition by IgE of

- sera from cow milk allergy patients. *J Agric Food Chem* **57**: 4974-4982 (2009).
- 33 Shriver SK and Yang WW, Thermal and nonthermal methods for food allergen control. *Food Eng Rev* **3**: 26-43 (2011).
- 34 Turner JA, Sivasundaram LR, Ottenhof MA, Farhat IA, Linforth RST and Taylor AJ, Monitoring chemical and physical changes during thermal flavor generation. *J Agric Food Chem* **50**: 5406–5411 (2002).
- 35 Oliver CM, Kher A, McNaughton D and Augustin MA, Use of FTIR and mass spectrometry for characterization of glycated caseins. *J Dairy Res* **76**: 105–110 (2009).
- 36 Farhat IA, Orset S, Moreau P and Blanshard JMV, FTIR study of hydration phenomena in protein–sugar systems. *J Colloid Interf Sci* **207**: 200-208 (1998).
- 37 Chen XY, Ru Y, Chen FL, Wang XC, Zhao XY and Ao Q, FTIR spectroscopic characterization of soy proteins obtained through AOT reverse micelles. *Food Hydrocolloid* **31**: 435-437 (2013).
- 38 Xue F, Li C, Zhu XW, Wang LF and Pan SY, Comparative studies on the physicochemical properties of soy protein isolate-maltodextrin and soy protein isolate-gum acacia conjugate prepared through Maillard reaction. *Food Res Int* **51**: 490-495 (2013).
- 39 Kleber N, Maier S and Hinrichs J, Antigenic response of bovine β -lactoglobulin influenced by ultra-high pressure treatment and temperature. *Innov Food Sci Emerg* **8**: 39-45 (2007).
- 40 Shannon W, Kristen B and Elvira G, Allergenic Proteins in Soybean: Processing and Reduction of P34 Allergenicity. *Nutr Rev* **63**: 47-48 (2005).
- 41 Wang WQ, Bao YH and Chen Y, Characteristics and antioxidant activity of water-soluble Maillard reaction products from interactions in a whey protein

- isolate and sugars system. *Food Chem* **139**: 355-361 (2013).
- 42 Bu GH, Zhu TW, Chen FS, Zhang N, Liu KL and Zhang LF, Construction of indirect competitive enzyme-linked immunosorbent assay for soybean glycinin detection. *Journal of Henan University of Technology (Natural Science Edition)* **35**: 1-5+11 (2014).
- 43 Zhang M, Zheng JY, Ge KS, Zhang H, Fang B, Jiang L *et al.*, Glycation of α -lactalbumin with different size saccharides: Effect on protein structure and antigenicity. *Int Dairy J* **34**: 220-228 (2014).
- 44 Hu S, Liu H, Qiao S, He PL, Ma X and Lu WQ, Development of immunoaffinity chromatographic method for isolating glycinin (11S) from soybean proteins. *J Agric Food Chem* **61**: 4406-4410 (2013).

Figure Captions

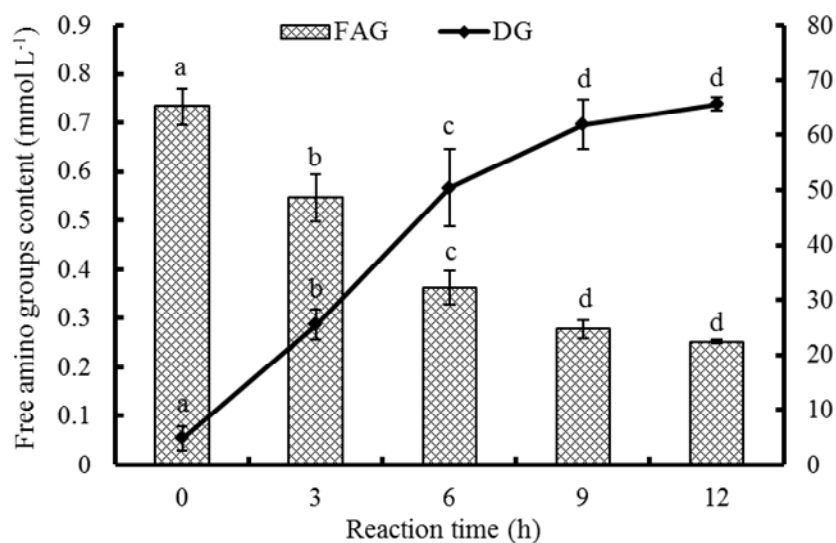


Figure 1.

Figure 1. Concentration of free amino groups (FAG) and the degree of glycation (DG) in glycinin-xylose samples incubated for different times. The values shown are the mean of three replications $\pm$ SD. Values of FAG and DG followed by the same letter are not significantly different ($P > 0.05$).

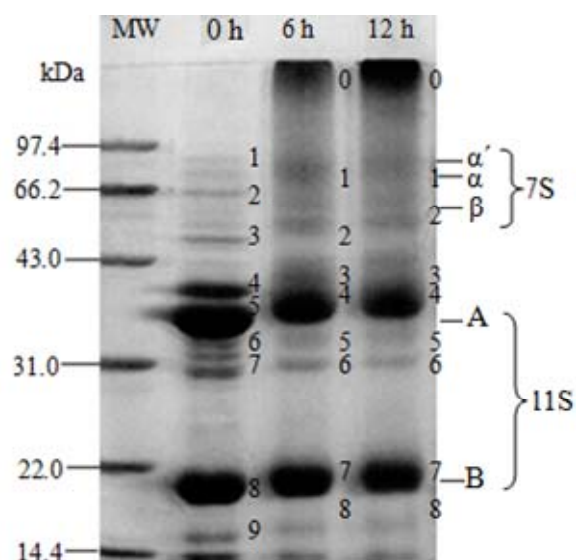


Figure 2.

Figure 2. SDS-PAGE of glycinin-xylose samples incubated for 0 h, 6 h, and 12 h.

MW, molecular weight marker. No.1~9 means the different subunits of proteins.

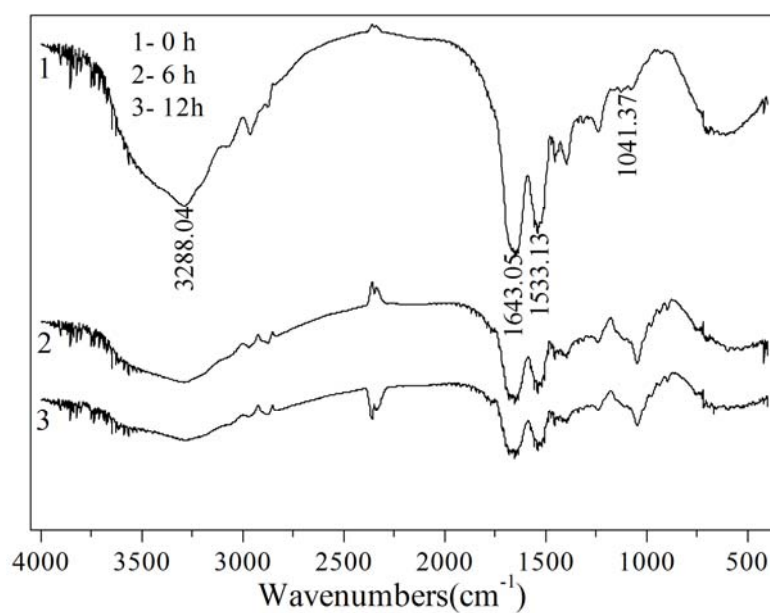


Figure 3.

Figure 3. Fourier transform infrared spectrum of glycinin-xylose samples at 0 h,

6 h, and 12h.

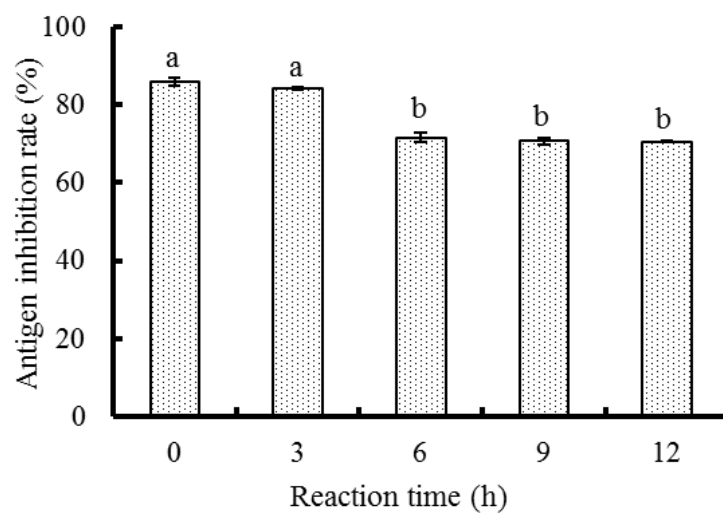


Figure 4.

Figure 4. The antigenicity of glycinin-xylose samples at different reaction times.

Table 1. The color measurement of glycinin-xylose samples at different reaction times.
(Weight ratio 3:1 of glycinin and xylose, at 55 °C, 79% relative humidity)

Reaction time (h)	<i>L</i>	<i>a</i>	<i>b</i>	<i>E</i>	<i>C</i>
0	84.65±0.31 ^a	-0.91±0.03 ^c	15.46±0.20 ^d	86.05±0.28 ^a	15.46±0.20 ^d
3	82.07±0.20 ^b	-0.37±0.14 ^d	23.24±0.32 ^c	85.30±0.21 ^b	23.26±0.32 ^c
6	81.37±0.35 ^c	0.63±0.09 ^c	27.75±0.21 ^b	85.99±0.10 ^a	27.81±0.21 ^b
9	79.63±0.10 ^d	2.26±0.11 ^b	30.23±0.05 ^a	85.25±0.08 ^b	30.43±0.05 ^a
12	78.13±0.09 ^e	3.09±0.11 ^a	30.35±0.02 ^a	83.93±0.07 ^c	30.65±0.04 ^a

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

Table 2. The protein subunit content of the glycated glycinin detected by SDS-PAGE and analyzed by BandScan 5.0 software.

Reaction time (h)	Glycinin (11S)		Conglycinin (7S)	New image
	(%)		(%)	(%)
	A	B	($\alpha+\alpha'+\beta$)	
0	35.35±0.78 ^a (No. 4~7)	57.25±0.49 ^a (No. 8~9)	8.05±0.49 ^a (No. 1~3)	—
6	33.15±0.78 ^b (No. 3~6)	45.05±0.64 ^b (No. 7~8)	5.25±0.78 ^b (No. 1~2)	16.51±0.57 ^a (No. 0)
12	29.00±0.42 ^c (No. 3~6)	41.15±0.64 ^c (No. 7~8)	4.35±0.21 ^b (No. 1~2)	27.50±3.39 ^b (No. 0)

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

Table 3. Secondary structure of the glycated glycinin measured by FTIR and analyzed by PeakFit V4.12.

Samples	Content of secondary structure (%)			
	α -Helix	β -Sheet	β -Turns	Random coil
Glycinin-xylose (0 h)	12.81 $\pm$ 0.05 ^a	34.70 $\pm$ 0.10 ^b	40.09 $\pm$ 0.80 ^a	12.40 $\pm$ 0.05 ^a
Glycinin-xylose (3 h)	12.88 $\pm$ 0.05 ^a	35.21 $\pm$ 0.58 ^{ab}	40.40 $\pm$ 0.57 ^a	11.51 $\pm$ 0.03 ^b
Glycinin-xylose (6 h)	12.81 $\pm$ 0.02 ^a	35.43 $\pm$ 0.63 ^{ab}	40.27 $\pm$ 0.66 ^a	11.49 $\pm$ 0.06 ^b
Glycinin-xylose (9 h)	12.84 $\pm$ 0.08 ^a	35.37 $\pm$ 0.04 ^{ab}	40.41 $\pm$ 0.01 ^a	11.39 $\pm$ 0.12 ^b
Glycinin-xylose (12 h)	12.79 $\pm$ 0.01 ^a	36.03 $\pm$ 0.09 ^a	40.11 $\pm$ 0.13 ^a	11.36 $\pm$ 0.23 ^b

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