



The influence of glycosylation on the antigenicity, allergenicity, and structural properties of 11S-lactose conjugates



Guanhao Bu, Nan Zhang, Fusheng Chen *

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

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ABSTRACT

Soybean is nutritious and is an excellent source of high-quality protein for human food and animal feed. However, glycinin (11S) is considered as the major allergenic protein that causes soybean allergies. Glycosylation is widely used to remove food protein allergens. In this study, soybean 11S was isolated and used in a glycation reaction with lactose at a weight ratio of 4:1 at 55 °C and 79% relative humidity for different periods of time. The effects of glycosylation on the antigenicity and residual allergenicity of 11S were investigated, using the specific IgG polyclonal antibodies for glycinin and soy-allergic patient sera by indirect competitive, enzyme-linked immunosorbent assay (indirect competitive ELISA). Meanwhile, the degree of glycation was determined by the trinitrobenzene sulfonic acid (TNBS) method. The structural properties of 11S-lactose conjugates were characterized by SDS-PAGE, Fourier transform infrared spectrum (FTIR), and ultraviolet spectroscopy. Glycosylation effectively decreased the antigenicity and allergenicity of 11S if we increased the reaction time. The antigenicity of 11S after glycosylation was reduced by approximately 30% compared with raw 11S, while allergenicity of 11S was reduced by 9%. The changes in secondary structures of glycosylated 11S may have influenced the allergic epitopes of protein. Therefore, we suggest that introducing lactose in 11S is an effective method to remove the antigenicity and allergenicity of glycinin.

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1. Introduction

Soybeans have a high nutritional value and are one of the major vegetable protein sources used in the food and feed industries (Hei, Li, Ma, & He, 2012). However, soybeans rank among the eight most significant food allergens, and soybeans have been identified as the most frequent source of human food allergens, accounting for over 90% of the documented food allergies worldwide (Batista, Martins, Jenó, Ricardo, & Oliveira, 2007; Van Boxtel, Van den Broek, Koppelman, & Gruppen, 2008). IgE-mediated food allergies are classified as a type-I immediate hypersensitivity reaction; 1–6% of young children and 2–4% of adults suffer from soybean allergies (Herian, Taylor, & Bush, 1990; Mills & Breiteneder, 2005; Tryphonas, Arvanitakis, Vavasour, & Bondy, 2003).

Allergenic reactions are mainly caused by antigenic proteins in soybean, which can interfere with the digestion and absorption of nutrients, disturb normal metabolism, cause adverse physiological responses, and result in hypersensitivity and even death (Hei et al., 2012; Liu, Teng, Wang, & Wang, 2013; Mills & Breiteneder, 2005). One of the major components in soybean protein is 11S, and it accounts for about 40% of total soybean proteins (Poysa, Woodrow, & Yu, 2006). It is reported that 11S is a major soybean allergen with a molecular mass of 350 kDa (Ma, He, Sun, & Han, 2010; Wang et al., 2015). Each of the 11S subunits can be dissociated under reducing conditions into acidic (A, 31–45 kDa) and basic (B, 18–20 kDa) polypeptide chains. Five major subunits of 11S have been characterized, namely, A1aB2 (G1), A1bB1b (G2), A2B1a (G3), A3B4 (G4), and A5A4B3 (G5) (Ma et al., 2010).

With the increasing consumption of soybean products, the incidence of soybean-induced allergies is also increasing (L'Hocine & Boye, 2007). Most of the 11S is digested and degraded to peptides and amino acids,

* Corresponding author.

E-mail address: fushengc@aliyun.com (F. Chen).

which serve as a source of nutrients, especially for infants, and 11S is difficult to eliminate from the diet. But, undigested 11S can play a negative role by depressing intestinal cell growth, damaging the cytoskeleton, and inducing allergic symptoms by entering the blood through gaps between the intestinal epithelial cells (Chen, Wang, Song, & Ma, 2014). Therefore, it is necessary to reduce or remove the antigenicity and allergenicity of 11S. There are many studies on how to reduce soybean protein antigenicity and allergenicity, including enzymatic hydrolysis, fermentation, heat treatment, and glycation (Serimarco, Battagliin, Ricardo de, & Maria, 2012; Song, Frias, Martinez-Villaluenga, Vidal-Valverde, & De Mejia, 2008; Sun & Qin, 2006; Usui, Tamura, & Nakamura, 2004). Although these methods could reduce the antigenicity and allergenicity of soybean protein, they also have some deficiencies. For example, using enzymatic hydrolysis often produces a bitter peptide that ruins the taste of soybeans; at high temperatures, proteins can become denatured and aggregate.

Considering all these processes, conjugation with reducing saccharides through the Maillard reaction seems to be a promising and safe method for masking soybean protein antigenicity and allergenicity. The Maillard reaction, which is also called the glycation reaction, is a non-enzymatic browning reaction formed between the ϵ -amino groups of a protein and the reducing end carbonyl group of a polysaccharide through an Amadori-type linkage; this is the most common reaction used in the food industry (Zhang et al., 2014). Different factors such as temperature, reaction time, relative humidity (RH), and the molar ratio of the reactants affect the rate and extent of the glycation. Glycation reaction has been proposed as a way to enhance functional properties of proteins and mask allergenicity. Li and Tang (2013) researched the influence of glycation on microencapsulating properties of soy protein isolate-lactose conjugates, finding that the encapsulation efficiency and dissolution properties of soy protein isolate-lactose products were improved after glycation. Babiker et al. (1998) studied the effect of the Maillard reaction on the antigenicity of soybean, and detected the greatest reduction in binding ability to rabbit polyclonal antibodies by means of an indirect ELISA in raw soybeans. Van de Lagemaat, Manuel Silván, Javier Moreno, Olano, and Dolores del Castillo (2007) studied the conjugation reaction between soy protein isolate and fructose or fructo-oligosaccharides via the Maillard reaction in powder and liquid systems; the antigenicity of this glycated protein was also largely reduced (up to 90%) compared with that of the unglycated form. In addition, Hattori et al. (2004) demonstrated that through shielding the B cell epitopes, β -LG-acidic oligosaccharide conjugates exhibited reduced immunogenicity. Most research has focused on glycation of proteins with saccharides at high temperatures for an extended period of time, which could lead to browning and irreversible loss of the protein's structural integrity (Gu et al., 2010; Wang & Zhong, 2014; Zhang et al., 2012). However, this relationship between the protein's structural changes and its effects on antigenicity and allergenicity has not been reported.

The nature of sugars, including the structure and the reactivity, could alter the structure of protein and further affect the functional properties of glycated proteins, and different reducing sugars could differ dramatically in the reaction rate of glycation (Chen, Chen, Guo, & Zhou, 2015). Lactose is a disaccharide present in milk and other dairy products. It was more reactive than polysaccharide, leading to better heat stability of glycated protein (Liu & Zhong, 2015). Moreover, lactose made up of glucose and galactose has many physiological functions. For example, lactose can provide energy for the body and promote the absorption of calcium, phosphorus and magnesium. Additionally, lactose can promote the growth of lactic acid bacteria in the intestinal of children. But lactose can lead to lactose intolerance in some population. Lactose intolerance is common food sensitivity due to the inability to digest lactose into glucose and galactose because of low levels of lactase. An effective treatment of lactose intolerance is to use exogenous-galactosidase (Nichele, Signoretto, & Ghedini, 2011). Industrially, lactose is widely used as an ingredient in foods, beverages and

confectionery products in view of its physiological functions and low sweetness.

In this study lactose is used as a glycosyl donor of glycation. The aim of the present paper is to investigate the glycation of 11S with lactose and the effect of glycosylation on the antigenicity and residual allergenicity of 11S. Moreover, the degree of glycation and the structural properties of glycated protein were determined by SDS-PAGE, Fourier-transform infrared spectrum (FTIR), and ultraviolet spectroscopy to explore the mechanism of glycation modification.

2. Materials and methods

2.1. Materials

Defatted soybean flour was obtained from Henan Kunhua Biological Technology Group Co., Ltd (Anyang, China). Lactose was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). HRP-labeled goat anti-human IgE (A9667), HRP-labeled goat anti-rabbit IgG (A6154), and glycinin (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 rabbits was generated by our own laboratory. Twenty five sera from soybean patients presenting various symptoms were selected from patients referred to the Children's Hospital affiliated with Zhejiang University (Zhejiang, China). All patients had specific IgE antibodies toward soybean from 0.70 to 41.0 KU/L. A pool containing the 25 sera was constituted. A serum pool from four nonallergic people was used as a negative control. All chemicals were analytical grade.

2.2. Preparation of polyclonal antibody

Three New Zealand White rabbits weighing an average of 2.5 kg, which were fed and treated according to principles of the Experimental Animal Center, Zhengzhou University (Zhengzhou, China), were used to produce polyclonal antibodies of glycinin (G3171). Blood samples were collected before immunization as negative serum. The immunization procedure was performed by the method of Bu et al. (2015). The titers of antibodies generated were 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 heart and were incubated at room temperature for 1 h, then stored at 4 °C overnight (Bu et al., 2015). The antiserum was separated by centrifuging at 3000 r/min for 20 min at 4 °C and then all sera was stored at –20 °C until used.

2.3. Preparation of soybean 11S

11S (protein content 94.6%) was purified from defatted soybean flour. It was obtained following the procedure described by Puppo et al. (2011). Defatted soybean flour was dispersed in distilled water (1:15 w/w), adjusted to pH 8.0 with 2 mol/L NaOH, stirred for 1 h in a water bath at 45 °C, and centrifuged at 10,000 $\times$ g for 20 min at 4 °C. Dry NaHSO₃ was added to the supernatant (0.98 g NaHSO₃/L), the pH was adjusted to pH 6.4 with 2 mol/L HCl, and the mixture was kept overnight at 4 °C. The resulting dispersion was centrifuged at 6500 $\times$ g for 15 min at 4 °C. The precipitate (11S fraction) was suspended in distilled water, adjusted to pH 7.8 with 2 mol/L NaOH, dialyzed against distilled water, and freeze dried.

2.4. Production of 11S-lactose conjugates

11S-lactose conjugates were prepared according to the method of Martinez-Alvarenga et al. (2014). Mixtures of 11S-lactose in specific ratios (11S: lactose = 4:1, by weight) were dissolved in deionized water. The protein concentration of dilute solution samples was 6%. The

sample solutions were stirred on a magnetic stirrer at room temperature for approximately 1 h to completely dissolve the mixture. Mixtures were then lyophilized, and the powders were kept at 55 °C and 79% relative humidity (RH) in desiccators equilibrated for different times (sampled at an interval of 24 h). As controls, 11S was treated under the same conditions. The resulting samples were stored at –20 °C prior to analysis.

2.5. Measurement of degree of glycation (DG) and free amino groups

The degree of glycation was measured for unreacted amines using the 2, 4, 6-trinitrobenzene sulfonic acid (TNBS) method (Bu et al., 2015), with some modifications. The glycated sample was diluted in 1% sodium dodecyl sulfate (SDS) to ensure a final protein concentration of 3 mg/mL. Each 0.25 mL conjugate solution was added to the burette, and then mixed with 2 mL 0.1% TNBS working solution and 2 mL 0.2 mol/L phosphate buffers (pH 8.2). Subsequently, the mixture was incubated at 50 °C in a water bath for 1 h, and the reaction was terminated by adding 4 mL 0.1 mol/L HCl. 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 leucine as a standard. The degree of glycation (DG) was calculated based on the loss of amino groups compared to untreated 11S.

$$DG(\%) = (C_0 - C_1) / C_0 \times 100$$

where C_0 was the free amino group content of the untreated 11S and C_1 was the free amino group content of the glycated samples.

2.6. SDS-PAGE analysis

SDS-PAGE was performed according to the method of Laemmli (1970) in a discontinuous buffered system. 5% stacking gels and 12% separating gels were used in the SDS-PAGE analysis. 10 µL samples (protein concentration 2 mg/mL) were added to 10 µL Tris–HCl buffer, containing 1% (w/v) SDS, 2% (v/v) 2-ME, 20% (v/v) glycerol, and 0.025% (w/v) bromophenol blue, and heated for 4 min in boiling water before electrophoresis. 10 µL of sample solution was loaded onto the gels. Electrophoresis was carried out for 40 min at 20 mA in the stacking gels and for 1.5 h at 40 mA in the separating gels. After electrophoresis, gels were stained for protein with Coomassie brilliant blue R-250 for 1.5 h. Finally, the gels were put into a destaining solution and decolorized so that the electrophoresis banding pattern was clear.

2.7. Fourier-transform infrared spectrum measurement

All the 11S-lactose glycated products were analyzed by Fourier-transform infrared spectroscopy (FTIR) using a WQF-510 Fourier-transform infrared spectrum equipped with a computer (Beijing Ruili Analytical Instruments Co. Ltd., Beijing, China). For each sample, an average of 32 scans was recorded at 4 cm^{−1} resolution in the range of 4000–400 cm^{−1} after atmospheric and background subtraction. Spectra of Amide I was deconvoluted using the proprietary software PeakFit V4.12 to obtain the characteristic signals that represented the secondary conformation of the protein (Savadkoobi, Bannikova, Mantri, & Kasapis, 2014).

2.8. Ultraviolet (UV) absorption spectroscopy

UV absorption spectra analysis was performed by a UV spectrophotometer (UV-VS 1901 UC, Beijing Purkinje General Instrument Co., Ltd., Beijing, China) at room temperature (25 °C). All samples were dissolved in deionized water. The protein concentration of the diluted solution samples was 1 mg/mL. The mixtures were homogeneously mixed (vortexed) and scanned from 190 nm to 350 nm (Luo et al., 2013).

2.9. Antigenicity assay of conjugates

An indirect competitive ELISA method was used as previously described to determine the antigenicity of 11S-lactose conjugates (Bu et al., 2015). The coating concentration of antigens was 0.025 µg/mL and the dilution of rabbit anti-glycinin serum was 1:3200. The dilution of the second antibody HRP-labeled goat anti-rabbit IgG (A6154, Sigma-Aldrich, St. Louis, USA) was 1:10,000. All samples were diluted to 2 mg/mL (protein concentration) directly and analyzed in triplicate. The antigenicity of 11S and the glycated products were expressed as inhibition percentages (%), which was calculated as: inhibition rate (%) = $(B_0 - B) / B_0 \times 100$, where B is the OD of glycated samples and B_0 is the OD of native 11S.

2.10. Allergenicity assay

The allergenicity of glycated 11S was analyzed by indirect competitive ELISA (Shi et al., 2014). The 96-well microtitre plate (Costar 3590 High Binding, Corning, New York, USA) was coated with 100 µL of antigen proteins glycinin in 50 mmol/L carbonate buffer and incubated at 4 °C overnight. The antigen concentration in buffer solution was determined earlier by ELISA (10 µg/mL for glycinin). All glycated samples and 11S from the experiments were diluted to 2 mg/mL (protein concentration) with phosphate buffered saline solution (PBS, pH 7.4), and then incubated separately with the selected pool sera (1:2) diluted in 0.01 mol/L phosphate-buffered saline (PBS, pH 7.4) containing 1% (w/v) BSA and 0.1% Tween-20, at a ratio of 1:1 (v/v) at 4 °C overnight. After four washes with 250 µL/well of 0.01 mol/L PBST (0.01 mol/L PBS containing 0.05% Tween-20, pH 7.4), the plate was blocked for 1 h at 37 °C by adding 100 µL per well of 0.01 mol/L PBST containing 5% BSA, and washed four times. After washing, 100 µL of each mixture (glycated 11S and pool of sera) was added and incubated for 1 h at 37 °C and washed. Subsequently, 100 µL/well of HRP-labeled goat anti-human IgE (A 9667, Sigma-Aldrich, St. Louis, USA, 1:1000) was added to each well and the plate was incubated at 37 °C for 1 h. Finally, 100 µL of 3, 3', 5, 5'-Tetramethylbenzidine (TMB) substrate solutions was added to each well and incubated at 37 °C for 10 min. The color reaction was stopped by the addition of 50 µL of 2 mol/L H₂SO₄ to each well. Uninhibited serum sample (no sample) was used as a control. The absorbance was determined at dual wavelengths of 450 nm and 630 nm by a microplate reader (Thermo Fisher Scientific Instrument Co. Ltd., New York, USA). The IgE-binding was represented by the inhibition percentages (%), which was calculated as follows:

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

where B was the absorbance measured in the presence of different glycated 11S samples from the experiments and B_0 was the absorbance

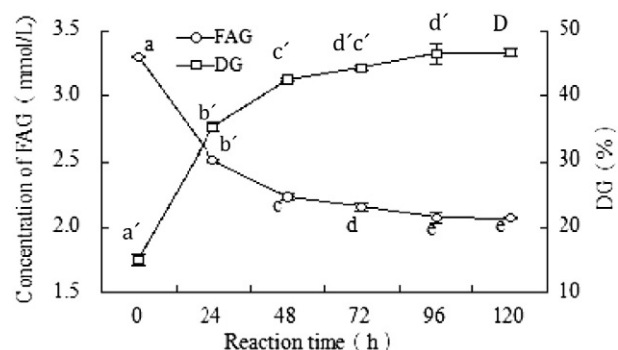


Fig. 1. The degree of glycation (DG) and concentration of free amino groups (FAG) in 11S-lactose conjugates after incubation at 55 °C and 79% relative humidity for different times. The values shown are the mean of three replications $\pm$ SD. Values of FAG and DG followed by the same letter are not significantly different ($P > 0.05$).

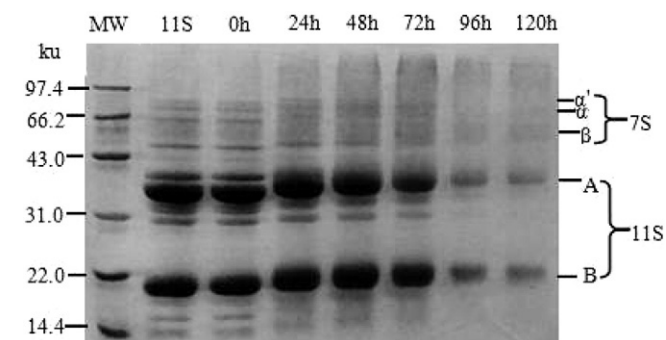


Fig. 2. SDS-PAGE of 11S-lactose conjugates after incubation at 55 °C and 79% relative humidity for different times. MW: molecular weight markers. 0–120 h: 11S-lactose conjugates incubated for 0, 24, 48, 72, 96, and 120 h, respectively.

obtained with the uninhibited serum sample control. A low percentage of inhibition reflects a low allergenicity of samples for glycinin.

2.11. Statistical analysis

All experiments were replicated three times, and values given in the tables and figures are the means of these triplicates. Statistical significance of differences among means was performed by SPSS 16.0. Univariate analysis of variance (ANOVA) and the significance level was established for Duncan's test at $P < 0.05$.

3. Results and discussion

3.1. DG and FAG analysis

In the present study, the degree of glycation (DG) was measured based on the reaction of free amines with TNBS. Glycation occurs by a covalent attachment of the carbonyl group in reducing sugars with free amino groups in proteins to form Schiff bases, and DG is often used to evaluate the extent of Maillard reactions in glycated products (Zhang et al., 2012). The content of free amino groups (FAG) in glycated products decreased gradually and the degree of glycation was enhanced gradually with an increase in incubation time (Fig. 1). With increased reaction times, DG of 11S-lactose increased from 14.97% to 46.68%. The largest increase of DG in the 11S-lactose product was found from 0 h to 48 h (Fig. 1), however, DG did not increase significantly

($P > 0.05$) in the late stage of glycation (72–120 h). This observation was consistent with the study of Van de Lagemaat et al. (2007) who demonstrated that loss of free amino groups occurred in heated SPI-FOS model systems at 95 °C. Approximately 30% of the free amino groups of SPI were lost during heat treatment and glycation with FOS for up to 1 h, but this loss did not change significantly during the last stage of the Maillard reactions. These results indicated that at the beginning of the glycosylation reaction, free amino groups exposed at the surface of the protein molecules were more prone to start Maillard reactions. Glycation slowed during the last stage of reaction. We also studied the effect of heating time on the loss of free amino groups of 11S and found a reduction of only 4% after 120 h of incubation.

3.2. SDS-PAGE analysis

To further confirm the covalent conjugation of lactose to 11S, we analyzed the SDS-PAGE patterns of native 11S and glycated samples with different incubation times (Fig. 2). On visualizing the gel, the characteristic protein bands (A, B) of 11S were visible under protein staining. At the top of the separating gel (Fig. 2), polydisperse bands were observed for the 11S-lactose mixtures with different reaction times, while such dense polydisperse bands at the top of the separating gel were not observed for the native 11S and the mixture after 0 h. In addition, the mobilities of the monomers in the glycated samples decreased compared to native 11S, while the molecular mass of proteins increased. This indicated that some lactose bound to 11S proteins during the reaction. The intensity of the characteristic protein bands was almost the same as the native 11S and mixture after 0 h. However, the intensity of the characteristic protein bands of 11S protein weakened gradually with an increase in glycation time, and new compounds with higher molecular weight began to form as the degree of glycation increased. This suggests that 11S proteins and lactose were covalently crosslinked. In addition, the forming of new polymers may lead to the changes in the spatial structure of the proteins, causing the changes in the antigenicity and allergenicity of glycated protein. Zhang et al. (2012) also reported that covalent conjugating compounds were produced in a 7S-dextran model system treated by heating at 95 °C.

3.3. Fourier transforms infrared spectrum measurement

FTIR is a useful technique for characterizing the conformation of protein structure (β -sheets, α -helices, β -turns, and random coils) and can

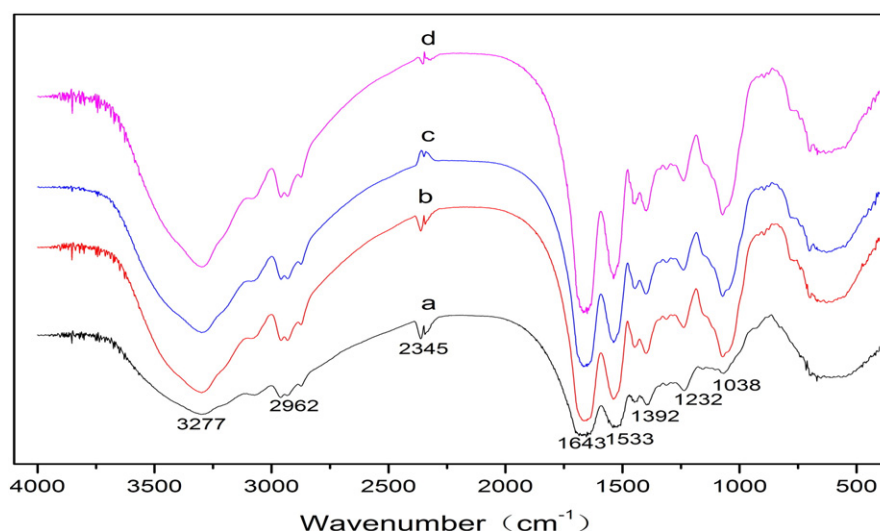


Fig. 3. Fourier transforms infrared spectrum of native and glycated 11S products after incubation at 55 °C and 79% relative humidity for different times. a: 11S; b: 11S-lactose incubated for 48 h; c: 11S-lactose incubated for 96 h; and d: 11S-lactose incubated for 120 h.

Table 1

Secondary structure of the glycosylated 11S measured by Fourier transform infrared spectrum and analyzed by PeakFit V4.12.

Samples	Content of secondary structure (%)			
	α -Helix	β -Sheet	β -Turns	Random coil
11S	12.29 $\pm$ 0.03 ^a	43.14 $\pm$ 0.46 ^a	31.43 $\pm$ 0.57 ^e	12.82 $\pm$ 0.36 ^a
11S-lactose (0 h)	12.62 $\pm$ 0.04 ^a	37.10 $\pm$ 0.33 ^c	37.74 $\pm$ 0.27 ^c	12.54 $\pm$ 0.03 ^a
11S-lactose (24 h)	12.44 $\pm$ 0.02 ^a	38.93 $\pm$ 0.11 ^b	35.99 $\pm$ 0.18 ^d	12.64 $\pm$ 0.05 ^a
11S-lactose (48 h)	12.65 $\pm$ 0.29 ^a	35.46 $\pm$ 0.42 ^d	39.34 $\pm$ 0.23 ^b	12.54 $\pm$ 0.11 ^a
11S-lactose (72 h)	12.70 $\pm$ 0.2 ^a	36.84 $\pm$ 0.95 ^c	37.72 $\pm$ 0.84 ^c	12.74 $\pm$ 0.09 ^a
11S-lactose (96 h)	12.98 $\pm$ 0.08 ^a	32.12 $\pm$ 0.51 ^e	42.57 $\pm$ 0.52 ^a	12.33 $\pm$ 0.09 ^a
11S-lactose (120 h)	12.78 $\pm$ 0.72 ^a	31.20 $\pm$ 0.51 ^e	43.55 $\pm$ 0.29 ^a	12.48 $\pm$ 0.50 ^a

Means $\pm$ standard deviations of triplicate analyses are given. Different superscript letters (a–e) indicate significant difference within the same column ($P < 0.05$).

reflect the changes in the structure of the peptide chain (Savadkoobi et al., 2014). The Amide I and II bands are the most prominent vibrational bands of the protein. The corresponding FTIR spectra for selected soy 11S samples obtained by glycation showed that the intensity of glycosylated products decreased compared to 11S in the region 1700–1600 cm^{-1} (Fig. 3). This may have been caused by the C=O stretching vibrations of the peptide group, whereas the latter absorption (1575–1480 cm^{-1}) arose principally from N–H bending with a contribution from C–N stretching vibrations (Savadkoobi et al., 2014). The band at 1038 cm^{-1} was attributed to vibrations, such as out-of-plane C–H bending. Based on these groups of peaks, we confirmed the presence of glycosylated carbohydrate complexes. Clearly, glycation has an effect on the secondary structure of 11S.

To further investigate changes in the secondary structure of 11S, the fundamental secondary elements of a specific band were deconvoluted by the analysis using the PeakFit Version 4.12; specifically, we examined Amide I (1600–1700 cm^{-1}), which is the characteristic absorption band of protein. Amide I reflected the secondary structure of the protein. The major elements in the Amide I region were assigned as follows: 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} (Chen et al., 2013). The estimated secondary structure of 11S-lactose under various reaction times in this study showed that the secondary structures of the protein in the glycosylated samples were primarily β -sheet and β -turn (Table 1). Moreover, the glycation of soy 11S changed the contents of β -sheet and β -turn mainly. With an increase in reaction time, the portion of the β -turn and α -helix structures in protein increased, and the β -sheet and random coil structure decreased compared with the native 11S. Conflicting results have been reported in other studies on the effect of glycation on secondary structures of proteins. Wang and Zhong (2014) observed that glycation resulted in an increase

in the random coil component and a decrease in the β -sheet and α -helix components of whey protein isolate. The discrepancy between our studies might be due to the differences in the proteins and glycation conditions used. These results implied that the secondary structures of 11S were altered by the Maillard reaction.

3.4. Ultraviolet spectrum analysis

The UV absorbance of native and glycosylated 11S were assayed at a wavelength of 190–350 nm. UV intensity of glycosylated mixtures was remarkably lower than that of 11S and gradually decreased with an increase in incubation time (Fig. 4). Both native 11S and glycosylated samples exhibited maximum absorption at 190–220 and 260–280. In addition, the location of the absorption peak in glycosylated-treated samples underwent a blue shift compared with the control sample.

The proteins absorb certain wavelengths of ultraviolet light, mainly due to the aromatic amino acids such as tyrosine (Tyr), tryptophan (Trp), and phenylalanine (Phe) residue. In this study, the results indicated that the amino residues in the conjugates were more surrounded to the hydrophobic environment than in 11S (Liu, Zhao, Zhao, Ren, & Yang, 2012). In addition, the decrease of UV absorption intensity may also be due to the glycosylation crosslinking reaction of aromatic amino acids. That is to say, the introduction of sugar chain molecules led to the changes in the spatial structure of the protein.

3.5. Changes in the antigenicity (IgG binding inhibition) of glycinin during glycosylation

The changes of the antigenicity of 11S in glycosylated products with reaction time were determined. As shown in Fig. 5, overall, antigen inhibition of the glycosylated products significantly ($P < 0.05$) decreased with reaction time. In the glycosylated products, the initial antigen inhibition rate of 11S was 83.16%. After 120 h, the antigen inhibition rate of 11S decreased to 54.67%, and reduced 28.49%. In this study, during glycosylation, 11S proteins were heated at 55 °C for different times. Compared with the untreated 11S, heat treatment reduced the antigenicity of 11S only from 85.14% to 76.23%. This is presumably caused by the high thermal stability of soy proteins (Van de Lagemaat et al., 2007). In addition, Van de Lagemaat et al. (2007) reported that the antigenicity of glycinin in SPI-fructose solutions at 95 °C was largely reduced (up to 90%) compared with that of the unglycosylated form. This may be related to a difference in binding specificity of the sugar to the allergenic sites of the protein under different temperatures.

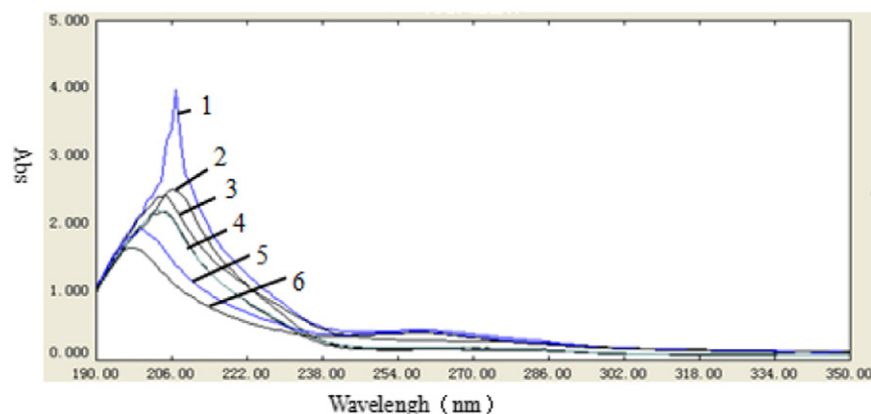


Fig. 4. UV absorption spectra of native and glycosylated 11S products after incubation at 55 °C and 79% relative humidity for different times. 1–6: 11S, 11S-lactose incubated for 24 h, 11S-lactose incubated for 48 h, 11S-lactose incubated for 72 h, 11S-lactose incubated for 96 h, and 11S-lactose incubated for 120 h, respectively.

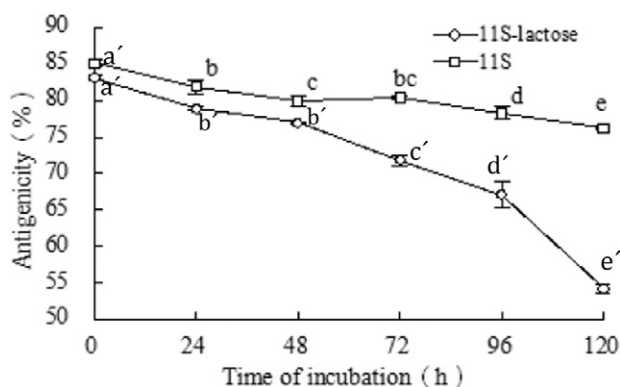


Fig. 5. Effects of incubation for different times at 55 °C and 79% relative humidity on the antigenicity of 11S-lactose conjugates. The values shown are the mean of three replications $\pm$ SD. Values of antigenicity followed by the same letter are not significantly different ($P > 0.05$).

3.6. Changes in the allergenicity (IgE binding inhibition) of glycinin during glycosylation

Fig. 6 shows the variations in IgE-binding inhibition of glycinin during glycosylation. After the glycosylated treatment of 11S, the IgE-binding property of glycinin was reduced by 9.55% at 120 h, while the untreated 11S was reduced by 3.08% (Fig. 6). Previous studies showed that heat-induced and glycosylation of oval proteins were associated with weaker binding of IgE by patients, and that considerably reduced their allergenicity (Huang et al., 2009).

During the glycation with lactose, changes in the IgE-binding inhibition of glycinin showed some fluctuations. Overall, after glycosylation the IgE-binding inhibition of glycinin in glycosylated samples decreased compared with unglycosylated 11S (Fig. 6). As for naive 11S, the IgE-binding inhibition did not decrease significantly ($P > 0.05$), while the IgE-binding inhibition of glycinin was reduced significantly ($P < 0.05$) during glycosylation. It is apparent that the allergenicity and antigenicity of glycosylated 11S have the same downward trend, and the reduction in antigenicity of 11S was greater than that of the IgE-binding ability after glycosylation.

4. Conclusions

The results demonstrated that glycation with lactose could significantly alter the structure of 11S and decrease the antigenicity and residual allergenicity. The reduction in protein antigenicity was greater than

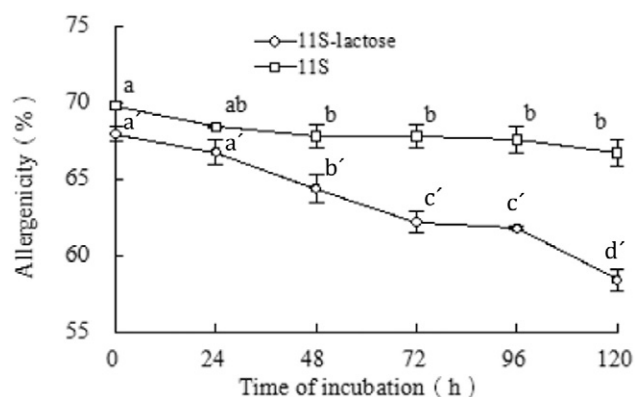


Fig. 6. Effects of incubation for different times at 55 °C and 79% relative humidity on the allergenicity of 11S-lactose conjugates. The values shown are the mean of three replications $\pm$ SD. Values of allergenicity followed by the same letter are not significantly different ($P > 0.05$).

that of the IgE-binding ability in all glycosylated products. With an increase in the incubation times, the degree of glycation increased, suggesting that the extent of glycation might be an important factor in the decrease of antigenicity and allergenicity. In addition, SDS-PAGE also confirmed the occurrence of a Maillard reaction. Moreover, with the increase in reaction time, the content of β -sheet and random coils in the proteins decreased while α -helix and β -turns increased after glycosylation. The changes in these secondary structures may have influenced the allergic epitopes of protein. Therefore, glycosylation is an effective method to reduce the antigenicity and allergenicity of soybean 11S.

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