Food Chemistry 111 (2008) 29–37

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Functional properties of protein isolates from soybeans stored under various conditions

Chun Liu [a,b], Xiansheng Wang [a], Hao Ma [a,*], Zhanqin Zhang [a], Wenrui Gao [a], Li Xiao [c]

[a] State Key Laboratory of Crop Genetics and Germplasm Enhancement, National Center for Soybean Improvement, Nanjing Agricultural University, Nanjing 210095, PR China
[b] Department of Life Science, Hengyang Normal University, Hengyang 421008, PR China
[c] College of Agriculture, Hunan Agricultural University, Changsha 410128, PR China

ARTICLE INFO

*Article history:*
Received 11 August 2007
Received in revised form 20 January 2008
Accepted 5 March 2008

*Keywords:*
Soybeans
Protein isolates
Subunits
Storage
Functional properties

ABSTRACT

Soybeans were stored under 88% relative humidity at 30 °C (adverse condition) for 8 months, under 55% relative humidity at 20 °C (mild condition), cold condition (4 °C), and an uncontrolled ambient garage for 12 months, respectively. Protein isolates were prepared from the soybeans stored under various conditions and periods, and their functional properties were characterized. The protein subunits of the isolates prepared under the adverse conditions were degraded slightly after 3 months, degraded significantly after 6 months, and almost all subunits were degraded completely after 8 months; the relative contents of the protein subunits markedly decreased at the 7th or 8th month and some even approached zero. The relative contents of the 7S and 11S fractions began to decrease markedly at the 5th or 6th month, and the 11S/7S ratios seemed to decrease after the initial 6 months, and then increased at the 7th and 8th month. The nitrogen solubility index (NSI), protein disperse index (PDI), emulsifying activity, emulsifying stability, texture index and thermal stability of soybean protein isolates decreased following the degradation of subunits. The functional properties of protein isolates prepared from the other three conditions (mild, cold and ambient) showed almost no significant changes for 12 months of storage when compared with those of the control.



## 1. Introduction

Soybean proteins, due to their good nutritional quality and excellent functionality, have become an ingredient of choice in many diverse food applications, including meat extension, meat and dairy alternatives, noodles, soups and a variety of nutritional foods and supplements. It has been estimated that approximately 60% of processed foods contain ingredients that are from soybeans. Soybean proteins generally constitute about 35–45% of the total seed on a dry basis. Around 90% of total proteins in soybean are globulin and are extractable with dilute salt solutions. Glycinin and β-conglycinin are two major storage globulins. The glycinin, also called soy 11S protein, consists of acidic and a basic polypeptides which are linked by a disulfide bridge. β-Conglycinin, a 7S protein, is a trimeric glycoprotein consisting of three types of subunits (α′, α, and β) (Hou & Chang, 2004a).

Soybean proteins have many functional properties, which have been investigated intensively, including solubility, heat-denaturation, gelling properties, emulsibility, foaming properties, water-binding capacity, surface hydrophobicity, structural characteristics, rheological properties and textural properties (Cai, Mccurdy, & Baik, 2002; Hou & Chang, 2004a, 2004c; Nagano, Hirotsuka, & Mori, 1992; Nir, Feldman, Aserin, & Garti, 1994; Ortiz & Wagner, 2002; Renkema, Knabben, & Van Vliet, 2001; Renkema, Lakemond, De Jongh, Gruppen, & Van Vliet, 2000; Saio & Watanabe, 1978). Proper storage technology is essential to maintain soybean quality and protein functional properties (Narayan, Chauhan, & Verma, 1988a, 1988b). Researchers found that soybean 11S and 7S proteins became difficult to extract from soybean under the adverse condition (85% relative humidity, 35 °C) (Saio, Kobayakawa, & Kito, 1982). After storing 7S and 11S globulins at 50 °C under 96% and 11% relative humidity for up to 45 days, the redispersibility of both proteins under 96% relative humidity drastically decreased within a few hours, whereas it did not decrease under 11% relative humidity for up to 45 days (Hoshi, Yamauchi, & Shibasaki, 1982). The storage of whole soybean under various conditions has negative effects on the physicochemical properties of proteins, including decreases in nitrogen solubility index (NSI), decreases in extractability of glycinin and β-conglycinin, and changes in the subunit compositions of glycinin (Murphy, Chen, Hauck, & Wilson, 1997; Saio et al., 1982). Hou and Chang (2004a, 2004b, 2004c) stored soybeans under four sets of conditions and analyzed the changes in colour, composition and structural characteristics of glycinin and β-conglycinin (including sugar content, sulfhydryl and disulfide contents, amino acid

* Corresponding author. Tel./fax: +86 25 84395324.
*E-mail address:* Lq-ncsi@njau.edu.cn (H. Ma).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.03.040

composition, surface hydrophobicity, secondary structure and differential scanning calorimetry) and their effects on yield and textural properties of tofu.

However, some functional properties of protein isolates (including glycinin and β-conglycinin) prepared from whole soybean stored under various conditions have not been fully investigated, especially textural properties. Therefore, the objective of this study was to provide an understanding of how the protein subunit compositions and some protein functional properties of the soybean seeds change during the four sets of storage conditions. The investigated functional properties, included the nitrogen solubility index (NSI), protein disperse index (PDI), emulsifying activity, emulsifying stability, texture properties and thermal stability.

## 2. Materials and methods

### 2.1. Soybeans and chemicals

The soybean cultivar 'Nannongdahuangdou', which is one of the elite summer cultivars and is extensively planted in the south of China mainly for soymilk and tofu making, was used in this study. The cultivar was planted on June, 16th, and harvested on October, 21st, in 2005, in Jiangpu Agriculture Experiment Station, Nanjing, Jiangsu Province, PR China. All seeds were threshed by hand and the moisture content was controlled to less than 12%. Chemicals used in this study were of reagent grade from Sinopharm Chemical reagent (Shanghai, China) and Sigma Chemical (St. Louis, MO).

### 2.2. Storage of soybean

Soybean seeds were stored under four sets of environmental conditions: adverse conditions (88% relative humidity, 30 °C), mild conditions (55% relative humidity, 20 °C), cold conditions (4 °C in a freezer), and uncontrolled ambient conditions [in a garage in Jiangpu Agriculture Experiment Station, Nanjing Agricultural University, Nanjing, Jiangsu Province, PR China]. The range of relative humidity was 60–88% (average: 72%) and the range of temperature was 7–32 °C (average: 16.7 °C) during the storage time. These conditions were selected to represent an adverse, a mild, a good, and a natural environment, because soybean seeds after harvest might be stored and/or transported under various environmental conditions before processing. Soybean seeds stored in a freezer (−20 °C) served as the control. At the end of each storage period, soybean seeds were stored in a −20 °C freezer prior to analysis.

### 2.3. Preparation of protein isolates

Soybean seeds were peeled, ground in a Straub grinding mill (model 4E, Straub Co., Philadelphia, PA) to pass through a 60-mesh sieve, and defatted by *n*-hexane extraction (soy flour/hexane = 1:5, v/v) for 1 h at room temperature. After centrifugation (8000*g*, 15 min, 4 °C), the supernatant was discarded and the precipitate was extracted at least twice. The defatted flour was collected for preparation of protein isolates. The defatted soybean flour was extracted for 2 h at room temperature with water adjusted to pH 8.0 with 2 N NaOH [water:flour ratio, 10:1(v/w)]. The mixture was centrifuged at 10,000*g* for 15 min at 4 °C. The supernatant was adjusted to pH 4.5 with 1 N HCl, and then kept for 2 h at 4 °C and subsequently centrifuged at 10,000*g* for 20 min at the same temperature. The precipitate was washed with water, resolubilized in water, neutralized to pH 7.0 with 2 N NaOH at room temperature, and then freeze-dried.

### 2.4. Electrophoresis (SDS–PAGE)

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was carried out in a continuous buffer system: 0.375 M Tris–HCl, pH 8.8, 1% (w/v) SDS. Sample buffer used was 0.125 M Tris-HCl, pH 6.8, 20% (v/v) glycerol, 1% (w/v) SDS, and 0.05% (w/v) bromophenol blue; 5% (v/v) 2-mercaptoethanol was added. The gel was prepared with 12% separating gel and 5% stacking gel. Runs were performed in a vertical electrophoresis unit (Beijing Liuyi Instruments Factory, Beijing, China) at 20 mA for 0.5 h, followed by 40 mA for about 3 h, with electrode buffer [0.025 M Tris–HCl, 0.192 M glycine, and 0.1% (w/v) SDS, pH 8.3], until the tracking dye migrated to the bottom edge of the gel. Gels were stained with Coomassie Brilliant Blue R-250 (0.05%, w/v) in methanol–acetic acid–water (25:10:65 v/v/v) and destained in the same solution without the dye. The quantification of protein subunits was performed by using a Bio-Rad imaging scanning densitometer (Versa Dco 3000) and analyzed with Quantity-one software (version 4.6). The relative protein quantity of each subunit (protein band) of 11S and 7S globulins was calculated from its respective percent area on the densitograms against the total area of 11S and 7S fractions.

### 2.5. Determination of protein content

Protein contents of crude soybean flour, defatted soybean flour, and protein isolates were determined according to the microKjeldahl method (AACC, 2000). A nitrogen to protein conversion factor of 6.25 was used.

### 2.6. Determination of protein solubility

#### 2.6.1. Nitrogen solubility index (NSI)

The nitrogen solubility index (NSI) was determined in three replicates according to AACC approved method 46-23 (AACC, 1995) with minor modifications. Samples (1 g) were weighed in 50 ml centrifuge tubes. Twenty milliliters of water was measured; a small portion was used to disperse the sample using the vortex mixer, and then the remainder of the water was added. The mixture was shaken on a mechanical shaker for 1 h at room temperature and centrifuged at 7720*g* for 15 min. The supernatant was collected, and the residue was suspended and centrifuged twice with 10 ml of water. The supernatants were combined and analyzed for nitrogen by the standard Kjeldahl method. NSI values were expressed as the percent of nitrogen soluble in distilled water at room temperature (25 °C).

#### 2.6.2. Protein dispersibility index (PDI)

The protein dispersibility index (PDI) was determined in three replicates according to AACC approved method 46–24 (AACC, 1995).

### 2.7. Emulsifying properties

Emulsifying activity index (EAI) and emulsifying stability index (ESI) were determined by the turbidimetric methods of Pearce and Kinsella (1978) and Molina, Papadopoulou, and Ledward (2001); 3 ml of protein solution (1.0 mg/ml) was dispersed in 0.2 M phosphate buffer, pH 5.8, 7.0, 7.6, 8.0, respectively, with 1 ml of sunflower seed oil in an Omnimixer for 1 min. Emulsion (50 μl) was pipetted from the bottom of the container into 5 ml of 0.1% sodium dodecyl sulfate (SDS) (w/v) solution immediately (0 min) and 10 min after homogenization. Absorbance of the SDS solution was measured at 500 nm. EAI and ESI were calculated by the equation

$$\text{EAI } (\text{m}^2/\text{g}) = \frac{2 \times T \times A_0 \times \text{dilution factor}}{c \times \phi \times 10000}$$

$$\text{ESI } (\text{min}) = \frac{A_0}{A_0 - A_{10}} \times 10$$

where $T = 2.303$, dilution factor = 100, $c$ is the weight of protein per unit volume (g/ml) and $\phi$ is the oil volumetric fraction (0.25).

### 2.8. Texture profile analysis (TPA)

Texture profile analysis (TPA) was performed using a Texture Analyzer (Model TA-XT2, Stable Micro Systems, Surrey, England). Crosshead speed was set to 1.00 mm/s. A cylindrical plunger (5 cm diameter) was used to compress the samples. The sample deformation was set to 30% of the original sample height. A three-second pause was allowed between the first and second compressions. Each treatment was measured 5 times. It was ascertained that the sample did not touch the probe while going back after the first compression as it might alter the values/parameters. The TA-XT2 plus was equipped with a texture exponent software system, which gave the results in 8 parameters and the graph was force vs time.

### 2.9. Differential scanning calorimetry (DSC)

The use of DSC to study thermal denaturation and thermodynamics of proteins has been widely adopted by researchers (Ma, 1990). For soybean proteins, DSC is commonly used as indicator of protein denaturation that significantly affects functionalities and thus their applicability in the food system. The measure method for DSC in this study was the same as that of Arress, Sorgentini, Wagner, and Anon (1991). DSC thermograms were recorded by using a Universal V4.1D TA Instrument. The heating rate was 2 °C/min, from 25 to 110 °C. Protein samples (45 mg) of 10% (w/v) solution in 35 mM potassium phosphate buffer, pH 7.6, were hermetically sealed in aluminium pans; an empty pan was used as reference. Peaks indicating an endothermic heat flow were obtained. The denaturation temperature ($T_d$), obtained from the intercept of the extrapolated slope of the peak and the baseline, was identified. The temperature at the peak maximum ($T_p$), generally used to indicate heat denaturation of proteins, was recorded as well.

### 2.10. Statistical analyses

Soybean samples from all storage conditions were extracted at least in triplicate. All data were analyzed by using analysis of variance (ANOVA) in the general linear models procedure of the Statistical Analysis Systems software package (SAS, version 8.0). Differences between group means were analyzed by Ducan's multiple-range test. Statistical significance was set at a 0.05 probability level.

## 3. Results and discussion

### 3.1. SDS–PAGE profile of the soybean protein isolates

Electrophoretic patterns of protein isolates prepared from soybean seeds stored under various conditions and different storage periods are shown in Fig. 1. The α′-, α-, and β-subunits of β-conglycinin migrated at MW~72,000 Da, 68,000 Da and 52,000 Da, respectively. In the case of the glycinin polypeptides, the acidic polypeptides, including $A_3$, Ax ($A_{1a}$, $A_{1b}$, $A_2$, and $A_4$), and $A_5$, could be identified from the gel. The acidic, Ax, and the basic, Bx, polypeptides are two prominent bands in the pattern of glycinin. $A_3$ migrated slightly more slowly (MW~42,000 Da) than the major group of acidic ones, Ax (MW~37,000 Da). Polypeptide $A_5$ had a molecular mass of ~10,000 Da and was located near the bottom of the gel. The basic polypeptides could be separated into minor $B_3$ and $B_4$ bands and a major Bx band ($B_{1a}$, $B_{1b}$, and $B_2$). The polypeptide Bx migrated in the gel at a position between the $B_3$ and $B_4$ bands. These results were very similar to previous reports (Hou & Chang, 2004a; Kitamura, 1995; Yagasaki, Kaizuma, & Kitamura, 1996).

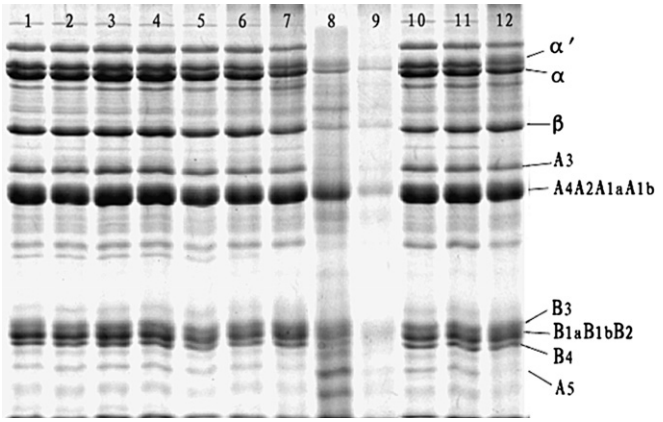


**Fig. 1.** SDS–PAGE analysis of soybean protein isolates prepared from soybean seeds stored under various conditions lane 1: control, 0 months; lane 2–9: 88% relative humidity, 30 °C, 1–8 months, respectively; lane 10: 4 °C, 12 months; lane 11: uncontrolled ambient temperature, 12 months; lane 12: 55% relative humidity, 20 °C, 12 months.

Compared with those of the control (Fig. 1, lane 1), the protein subunits of the isolates prepared from the soybean seeds stored under the three conditions (mild conditions, cold conditions and uncontrolled ambient conditions) showed no significant changes during 12 months of storage (Fig. 1, lanes 10–12). However, the protein subunits of the isolates prepared from the soybean seeds stored under the adverse condition were degraded slightly after 3 months of storage (Fig. 1, lanes 2–7), degraded markedly after 6 months (Fig. 1, lane 8), and almost all subunits were degraded completely after 8 months (Fig. 1, lane 9).

The relative contents of 7S and 11S fractions and their subunits, and the 11S/7S ratios of the protein isolates prepared from the soybeans stored under the various conditions, depicted in Fig. 1, are shown in Fig. 2. Compared with that of the control (Fig. 1, lane 1) and initial 3 months of storage (Fig. 1, lanes 2–3), the relative contents of some subunits of the protein isolates prepared from the soybeans under the adverse condition begun to markedly decrease at the 4th month, and all subunits showed marked decrease at the 7th or 8th month and some even approached zero. The relative contents of 7S and 11S fractions began to decrease markedly at the 5th or 6th month; 11S/7S ratios seemed to decrease after the initial 6 months, and then increased at the 7th and 8th months. However, the relative contents of 7S and 11S and their subunits, and 11S/7S ratios of the protein isolates prepared from the soybeans under the other three conditions (mild conditions, cold conditions and uncontrolled ambient conditions) were not markedly different from each other during 12 months of storage, and also not markedly different from that of the control over the initial 3 months of the adverse condition.

Storage proteins synthesized during seed development are degraded during germination to small peptides or amino acids that are subsequently transported to the growing seedling (Shewry, Napier, & Tatham, 1995; Wilson, Papastoitsis, Hartl, & Tan-Wilson, 1988). The increasing evidence suggests that protein degradation

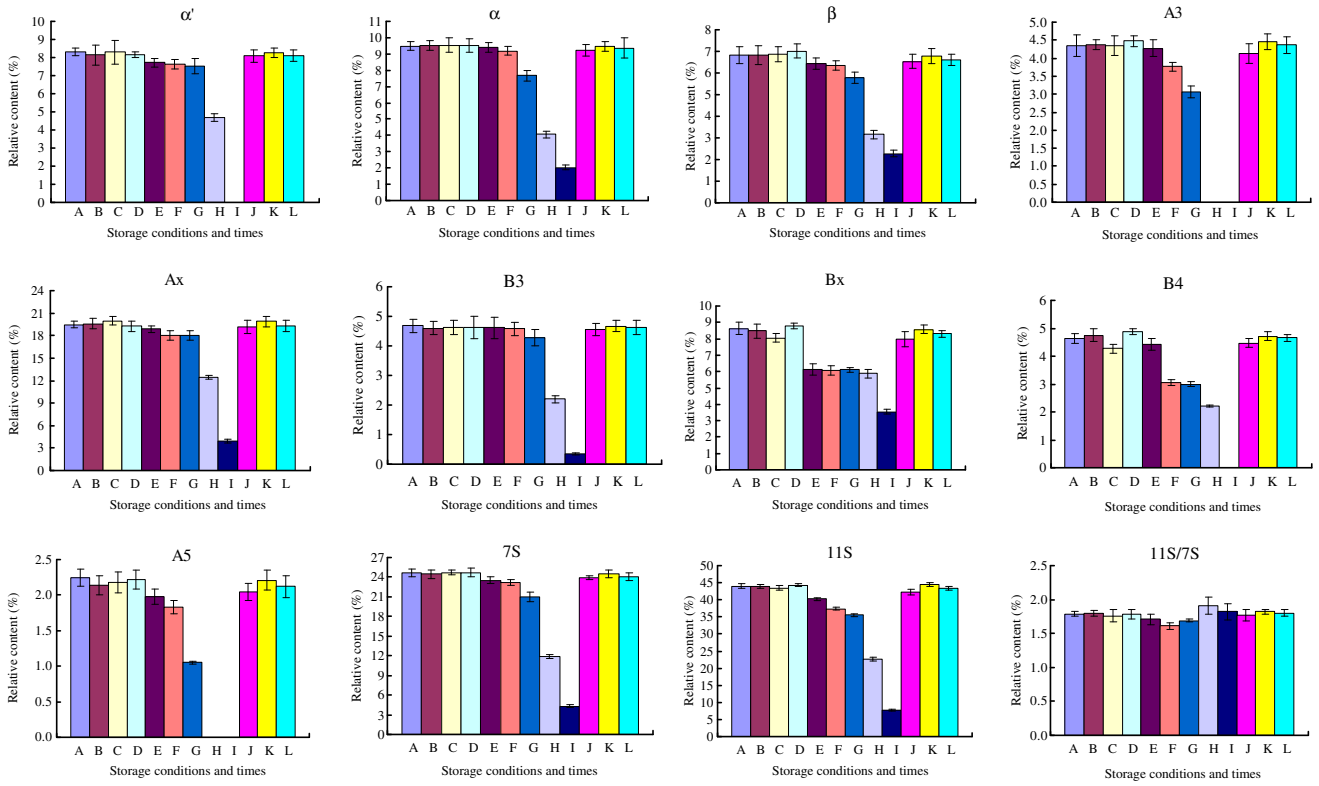


**Fig. 2.** Relative contents of 7S and 11S and their subunits, and 11S/7S ratios of protein isolates prepared from soybeans under various conditions (1) Letters of x axis mean various storage conditions and times: A: control; B: adverse, 1 month; C: adverse, 2 months; D: adverse, 3 months; E: adverse, 4 months; F: adverse, 5 months; G: adverse, 6 months; H: adverse, 7 months; I: adverse, 8 months; J: mild, 12 months; K: cold, 12 months; L: ambient, 12 months. (2) The relative content of 7S fraction was sum of the relative contents of α′-, α-, and β-subunits and the relative content of 11S fraction was sum of the relative contents of $A_3$, Ax ($A_4A_2A_{1a}A_{1b}$), $B_3$, Bx ($B_{1a}$, $B_{1b}$, and $B_2$), $B_4$, and $A_5$ subunits.

is indeed a regulatory mechanism in vivo. But few data are available about the mechanism of breakdown of the subunits in dry soybean seeds. Possibly, bacteria were developed when seeds were under the condition of high temperature and high humidity, and the cell wall was destroyed by the bacteria, which might have resulted in the exposure of the protein to proteinases in cells. These proteinases are thought to be responsible for the initial proteolysis of soybean storage proteins, and finally the subunits of proteins may be broken down and small molecular polypeptides and amino acids produced.

### 3.2. Protein content

The protein contents of crude flours, defatted flours and isolates prepared from the soybeans under the various storage conditions and different storage periods are shown in Fig. 3. No significant differences were found among the protein contents of the crude flours, defatted flours and isolates prepared from the soybeans of the control and stored for 12 months under the three conditions (mild, cold and ambient). However, the protein contents of the crude flour and defatted flour prepared from the soybeans after six months of storage under the adverse condition were significantly lower than that of the control, the initial six-month storage under the same condition and 12th month storage of the other three conditions (mild, cold and ambient). The protein content of the isolates prepared from the soybean stored under the adverse condition was not markedly lower until seven-months of storage. This suggested that measurement of protein content of isolates could not be used to evaluate the stability or fall of protein quality of the soybean seeds stored under good conditions or not.

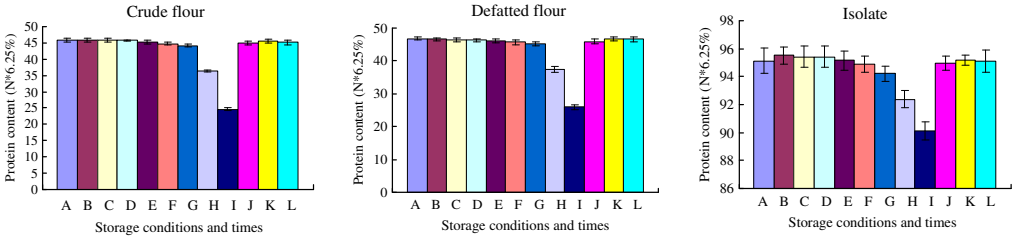


**Fig. 3.** Protein content of crude flour, defatted flour and isolates were prepared from soybeans under various conditions: (1) Letters of *x*-axis mean various storage conditions and times: A: control; B: adverse, 1 month; C: adverse, 2 months; D: adverse, 3 months; E: adverse, 4 months; F: adverse, 5 months; G: adverse, 6 months; H: adverse, 7 months; I: adverse, 8 months; J: mild, 12 months; K: cold, 12 months; L: ambient, 12 months. (2) Soybean seeds were peeled, ground in a Straub grinding mill to pass through a 60-mesh sieve to obtain crude flour.

### 3.3. Protein solubility

Both the protein dispersibility index (PDI) and nitrogen solubility index (NSI) are used to measure protein solubility in water. These assays differ in the speed (and vigor), at which the water/soybean flour mixture is stirred. The water solubility PDI and NSI assays have been extensively used to evaluate soybean quality. High solubility is very important to manufacturers of soymilk and tofu, as their job is to extract as much protein from the soybean as possible. The effect of storage conditions and duration on protein solubility, NSI and PDI of the crude flours, defatted flours and isolates prepared from the soybeans under the various storage conditions and different storage periods are shown in Fig. 4. During the initial six months of storage under the adverse condition, there were very small changes in NSI and PDI values of the crude flours, defatted flours and isolates. However, from the 7th to the 8th months, both solubility indices decreased substantially. No significant differences were found among the NSI and PDI values of the crude flours, defatted flours and isolates prepared from the soybeans of the control and those stored for 12 months under the other three conditions (mild, cold and ambient), except that there were significant differences between the PDI values of the mild storage condition, and of the control and cold condition for 12 month.

### 3.4. Emulsifying properties

Emulsifying properties of food proteins are usually described by: (1) emulsion capacity, or emulsion activity, which reflects the ability of the proteins to aid formation and stabilization of the newly created emulsion, and (2) emulsion stability, which reflects the ability of the proteins to impart strength to emulsion for resistance to stress. The emulsifying capacity (expressed as emulsifying activity index) and emulsion stability (expressed as emulsion stability index) of the protein isolates prepared from the soybeans stored under various conditions and periods are shown in Figs. 5 and 6. Emulsifying properties could be markedly affected by the pH. For all the isolates prepared from soybeans stored under the same conditions and the same storage period, lowest emulsifying activity indices occurred at pH 5.8 and highest emulsifying activity indices occurred at pH 8.0. The results showed that the emulsifying activity could increase as the pH values were enhanced. This phenomenon could be attributed to a greater solubility of proteins as the pH value increased. The relationship between the pH values

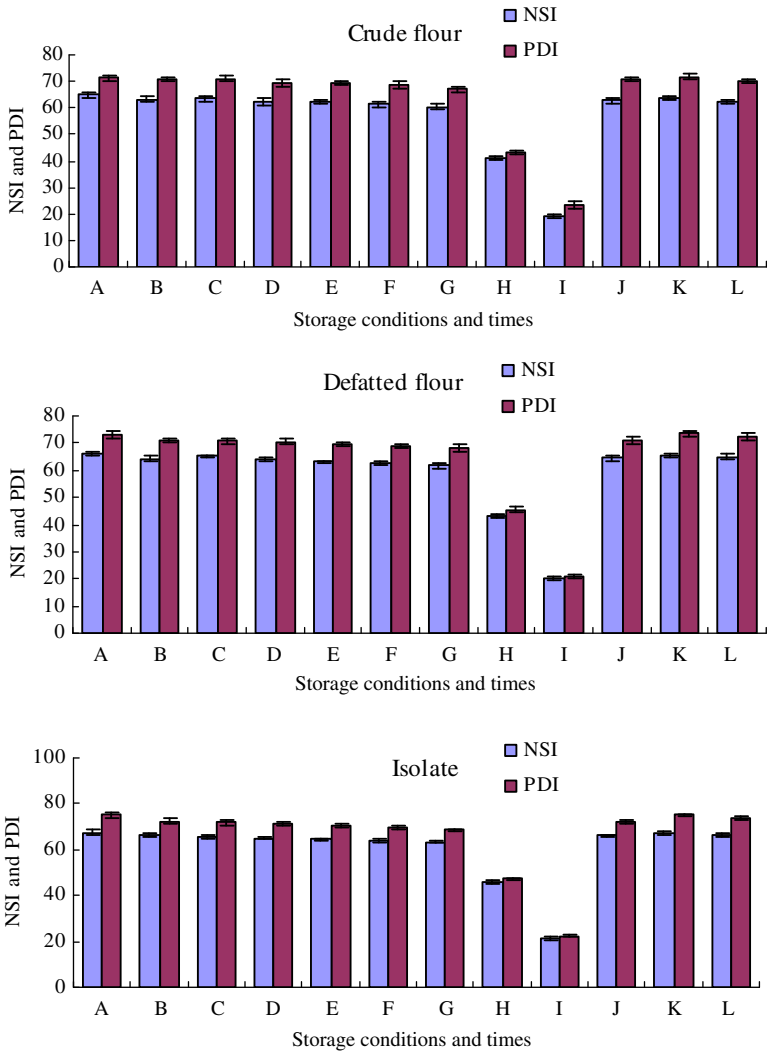


**Fig. 4.** NSI and PDI of crude flour, defatted flour and isolates were prepared from soybeans under various conditions (1) Letters of *x*-axis mean various storage conditions and times: A: control; B: adverse, 1 month; C: adverse, 2 months; D: adverse, 3 months; E: adverse, 4 months; F: adverse, 5 months; G: adverse, 6 months; H: adverse, 7 months; I: adverse, 8 months; J: mild, 12 months; K: cold, 12 months; L: ambient, 12 months. (2) Soybean seeds were peeled, ground in a Straub grinding mill to pass through a 60-mesh sieve to obtain crude flour.

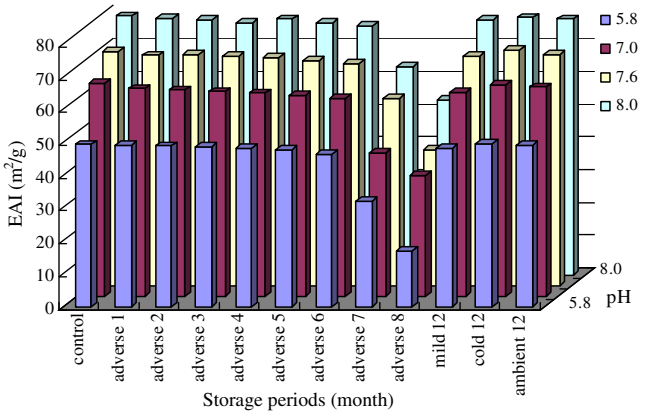


**Fig. 5.** Emulsifying activity index (EAI, $m^2/g$) of the isolates prepared from soybeans stored under various conditions and periods.

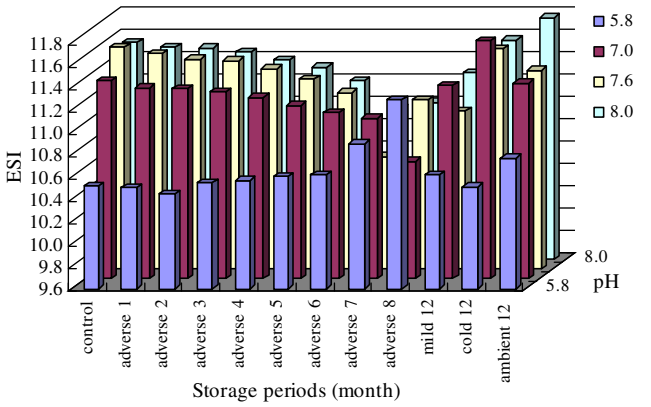


**Fig. 6.** Emulsion stability index (ESI) of the isolates prepared from soybeans stored under various conditions and periods.

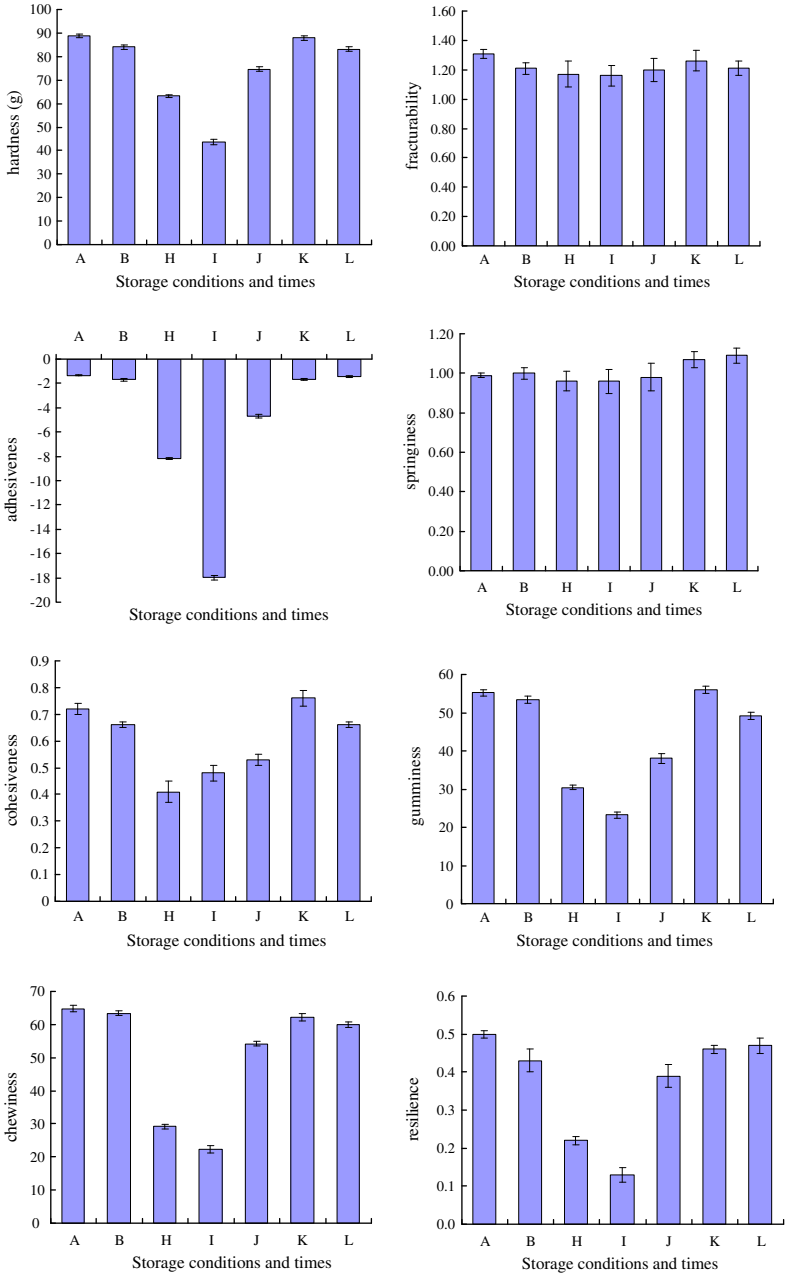


**Fig. 7.** Texture profile analysis of the protein isolates studied Letters of *x*-axis mean various storage conditions and times: A: control; B: adverse, 1 month; H: adverse, 7 months; I: adverse, 8 months; J: mild, 12 months; K: cold, 12 months; L: ambient, 12 months.

and the emulsifying stability indices (ESI) of the isolates did not seem to be so clear, especially for the isolates prepared from the soybeans stored under the adverse condition for 7 and 8 months, mild condition for 12 months, and cold condition for 12 months. The reason for this was not obvious. Ordinarily, protein isolates were more efficient for emulsifying oil under alkaline condition than under acid conditions. Similar pH dependence of emulsification properties has been reported for other proteins (Crenwelge, Dill, Tybor, & Landmann, 1974; Ramanatham, Ran, & Urs, 1978).

Emulsifying properties could also be markedly affected by the storage conditions. The emulsifying capacity of the isolates prepared from the soybeans stored under the adverse condition after 6 months was significantly lower than that of the isolates made from the soybeans stored under the other three conditions ($P < 0.05$) (Fig. 2). However, the effects of the storage conditions on the emulsion stability indices were more complex. The emulsions of the isolates prepared from the soybean stored under the ambient condition for 12 months had the highest stability index (pH 8.0). The emulsions prepared from the soybean stored under the adverse condition for 7 and 8 months were significantly less stable than those prepared from the soybeans stored under the other three conditions ($P < 0.05$) at pH 7.0, 7.6 and 8.0, and the emulsions prepared from the soybean stored under the adverse condition for 7 and 8 months were significantly more stable than those prepared from the soybeans stored under the other three conditions ($P < 0.05$) at pH 5.8 (Fig. 3). This is a very interesting phenomenon, since these isolates have the lowest EAI values.

### 3.5. Texture profile analysis

The TPA results are shown in Fig. 7. The gel made from the protein isolate prepared from the control soybean seed had the highest values for hardness, fracturability, adhesiveness, chewiness and resilience, and higher values for cohesiveness and gumminess. No significant differences were found among the springiness values of the gels prepared from the control and stored soybeans under the four storage conditions and various storage periods ($P < 0.05$). Moreover, no significant differences were found between the eight texture parameters of the gels prepared from the soybeans stored under the control condition and cold condition ($P < 0.05$). However, significant differences were found between the cohesiveness values of the gels prepared from the soybeans under control and cold conditions stored for 12 months, and from the soybeans stored under the control and the ambient condition for 12 months ($P < 0.05$) while, for the gel prepared from the soybeans stored under the mild condition for 12 months, only fracturability value and springiness value showed no significant difference ($P < 0.05$). After the first storage month, the cohesiveness and resilience values of the gel prepared from the soybeans stored under the adverse condition were observed to be markedly changed ($P < 0.05$), while six texture parameters of the gels prepared from the soybeans stored under the adverse condition for 7 and 8 months were significantly lower than those prepared from the control and stored soybeans under the other three conditions ($P < 0.05$), except that only the fracturability values were significant lower than those of the cold

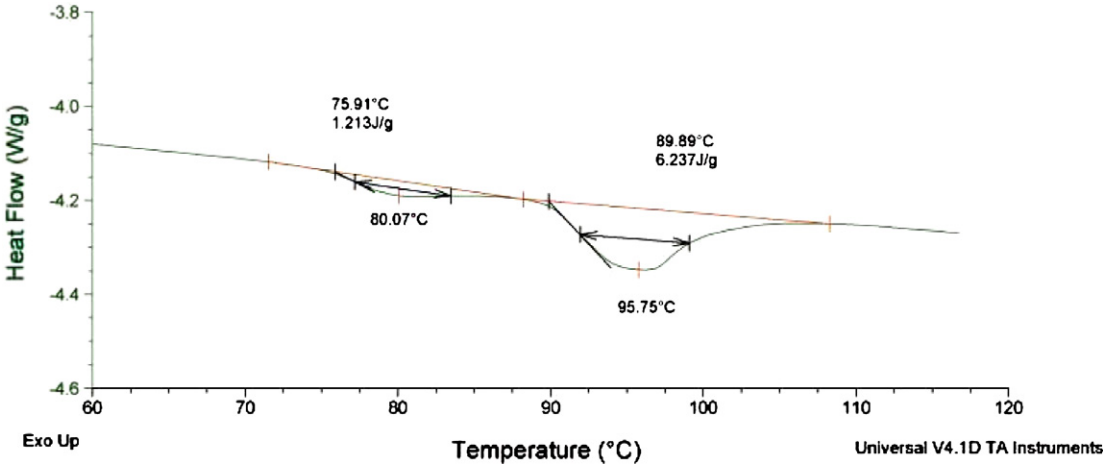


**Fig. 8.** DSC thermogram of protein isolate prepared from soybean stored under the control condition.

**Table 1**
Comparison of thermal properties of isolates prepared from soybeans stored under various conditions[A]

| Storage conditions | 7S | | | 11S | | |
|---|---|---|---|---|---|---|
| | $T_d$ (°C) | $T_p$ (°C) | $\Delta H$ (J/g) | $T_d$ (°C) | $T_p$ (°C) | $\Delta H$ (J/g) |
| Control | 76.44 ± 0.2[a] | 82.50 ± 0.6[a] | 1.29 ± 0.01[a] | 92.98 ± 0.7 | 98.95 ± 0.5[a] | 6.59 ± 0.28[a] |
| Adverse, 1 month | 75.91 ± 0.5[a] | 81.07 ± 0.4[ab] | 1.21 ± 0.05[a] | 92.89 ± 0.6 | 98.75 ± 0.8[a] | 6.34 ± 0.24[a] |
| Adverse, 7 months | 73.65 ± 0.3[b] | 79.50 ± 0.4[b] | 0.75 ± 0.03[b] | 92.33 ± 0.5 | 96.88 ± 0.6[b] | 3.96 ± 0.08[b] |
| Adverse, 8 months | 73.08 ± 0.5[b] | 79.22 ± 0.5[b] | 0.19 ± 0.01[c] | 92.24 ± 0.6 | 94.96 ± 0.8[c] | 2.48 ± 0.10[c] |
| Mild, 12 months | 74.54 ± 0.3[ab] | 82.17 ± 0.2[a] | 1.14 ± 0.04[ab] | 92.44 ± 0.4 | 98.15 ± 0.6[a] | 6.28 ± 0.30[a] |
| Cold, 12 months | 76.18 ± 0.4[a] | 82.73 ± 0.5[a] | 1.21 ± 0.04[a] | 92.51 ± 0.5 | 98.72 ± 0.7[a] | 6.49 ± 0.06[a] |
| Ambient, 12 months | 74.53 ± 0.2[ab] | 82.92 ± 0.3[a] | 1.18 ± 0.02[a] | 92.24 ± 0.8 | 98.53 ± 0.5[a] | 6.42 ± 0.16[a] |

[A] Data are expressed as mean ± standard deviation and are the means of two replicates. Means with different letters within the same column are significantly ($P < 0.05$) different.

condition. These results indicated that, as the protein subunits were degraded during storage time, the texture quality of the gels of the isolates became increasingly worse.

### 3.6. Analysis by differential scanning calorimetry

The thermogram of the isolates prepared from the soybean stored under the control condition shows two peaks (Fig. 8). The approximate $T_d$, $T_p$, and $\Delta H$ of 11S and 7S of the isolates from the soybeans stored under the various conditions and periods are shown in Table 1. No significant differences of the denaturation temperatures ($T_d$) were found among 7S fractions of the isolates prepared from the soybeans stored under the control condition, mild condition for 12 months, cold condition for 12 months, ambient condition for 12 months or adverse condition for 1 month; However, with the adverse storage time prolonged to 7 months or 8 months, the denaturation temperatures ($T_d$) of 7S fractions showed significant decreases ($P > 0.05$). The same effects were found for the peak temperature ($T_p$) and enthalpy change ($\Delta H$) of 7S fractions. However, Hou and Chang (2004c) found that the denaturation temperature of purified β-conglycinin did not show changes during storage under all four selected conditions. The differences between our study and Hou's might be partly due to the use of different raw materials and extraction methods.

For the 11S fractions, no significant differences of the denaturation temperatures ($T_d$) were found among 11S fractions of the isolates prepared from the soybeans stored under the four sets of conditions and the control ($P > 0.05$). Also, no significant differences of the peak temperature ($T_p$) and the enthalpy change ($\Delta H$) were found among 11S fractions of the isolates prepared from the soybeans under the control condition, mild condition for 12 months, cold condition for 12 months, ambient condition for 12 months and adverse condition for 1 month; however, with the adverse storage time prolonged to 7 months or 8 months, the peak temperature ($T_p$) and enthalpy change ($\Delta H$) of 11S fractions showed significant decreases ($P > 0.05$). This was similar to 7S.

The stabilization of the functional properties found in the isolates prepared from the soybeans stored under proper conditions for 12 months indicated that soybeans stored under proper conditions could maintain their good qualities (for making soybean products) for a long time (Hou & Chang, 2003).

## 4. Conclusion

Compared with those of the control, the protein subunits of the isolates prepared from the adverse condition, were degraded slightly after three months, degraded significantly after 6 months, and almost totally after 8 months. The relative contents of the protein subunits markedly decreased at the 7th or 8th months and some even approached zero. The relative contents of the 7S and 11S fractions began to decrease markedly at the 5th or 6th month, and the 11S/7S ratios seemed to decrease at the initial 6 months, and then increased at the 7th and 8th month. However, for the isolates from the soybeans stored under the other three conditions (mild conditions, cold conditions, and uncontrolled ambient conditions), their subunits showed no significant changes after 12 storage months. The relative contents of their 7S and 11S and subunits and their 11S/7S ratios were not markedly different from each other during 12 months of storage, and also not markedly different from that of the control and the initial three months of the adverse condition.

No significant differences were found among the protein contents of the crude flours and defatted flours and isolates prepared from the soybeans of the control and stored for 12 months under the three conditions (mild, cold and ambient). However, the protein contents of the crude flour and defatted flour prepared from the soybeans after six months of storage under adverse conditions were significantly lower than that of the control, and the initial six-month storage of the same condition and 12th month storage of the other three conditions (mild, cold and ambient). The protein contents of the isolates prepared from the soybeans stored under the adverse condition were not markedly lower until seven months of storage.

The functional properties of the isolates from the soybean stored under the adverse condition showed significant decrease after 6 months, following the degradation of subunits. Those functional properties included the nitrogen solubility index (NSI), protein disperse index (PDI), emulsifying activity, emulsifying stability, thermal stability and texture properties. Therefore, compared with the control, almost no significant changes were found among the functional properties of the protein isolates prepared from the soybeans stored under the other three conditions (mild conditions, cold conditions, and uncontrolled ambient conditions) for 12 months.

## Acknowledgements

We gratefully acknowledge the partial financial support from the National 948 Program of China (No. M2001-207), and from Program for Changjiang Scholars and Innovative Research Team in University, and from the 111 Project for this research.

## References

AACC. (1995). Approved methods of the AACC (9th ed.). Methods 46-23, and 46-24. St. Paul, MN: American Association of Cereal Chemists.

AACC. (2000). Approved methods of the AACC (10th ed.). Method 46-13. St. Paul, MN: American Association of Cereal Chemists.

Arress, E. L., Sorgentini, D. A., Wagner, J. R., & Anon, M. C. (1991). Electrophoretic, solubility, and functional properties of commercial soy protein isolates. *Journal of Agriculture and Food Chemistry, 39*, 1029–1032.

Cai, R., Mccurdy, A., & Baik, B. K. (2002). Textural property of 6 legume curds in relation to their protein constituents. *Journal of Food Science, 67*(5), 1725–1730.

Crenwelge, D. D., Dill, C. W., Tybor, P. T., & Landmann, W. A. (1974). A comparison of the emulsification capacities of some protein concentrates. *Journal of Food Science, 39*, 175–177.

Hoshi, Y., Yamauchi, F., & Shibasaki, K. (1982). Effects of relative humidity on aggregation of soybean 7S and 11S globulins. *Agricultural and Biological Chemistry, 46*(6), 1513–1517.

Hou, H. J., & Chang, K. C. (2003). Yield and textural properties of tofu as affected by the changes of phytate content during soybean storage. *Journal of Food Science, 68*, 1185–1191.

Hou, H. J., & Chang, K. C. (2004a). Structural characteristics of purified glycinin from soybeans stored under various conditions. *Journal of Agricultural and food chemistry, 52*(12), 3792–3800.

Hou, H. J., & Chang, K. C. (2004b). Storage conditions affect soybean color, chemical composition and tofu qualities. *Journal of Food Processing and Preservation, 28*(6), 473–488.

Hou, H. J., & Chang, K. C. (2004c). Structural characteristics of purified β-conglycinin from soybeans stored under four conditions. *Journal of Agriculture and Food Chemistry, 52*(26), 7931–7937.

Kitamura, K. (1995). Genetic improvement of nutritional and food processing quality in soybean. *Japan Agriculture Research Quarterly, 29*, 1–8.

Ma, C. Y. (1990). Thermal analysis of vegetable proteins and vegetable protein-based food products. In V. R. Harwalkar & C. Y. Ma (Eds.), *Thermal analysis of foods* (pp. 149–167). London: Elsevier Science Publishers LTD.

Molina, E., Papadopoulou, A., & Ledward, D. A. (2001). Emulsifying properties of high pressure treated soy protein isolate and 7S and 11S globulins. *Food Hydrocolloids, 15*, 263–269.

Murphy, P. A., Chen, H. P., Hauck, C. C., & Wilson, L. A. (1997). Soybean protein composition and tofu quality. *Food Technology, 51*. pp. 86, 88–110.

Nagano, T., Hirotsuka, M., & Mori, H. (1992). Dynamic viscoelastic study on the gelation of 7S globulin from soybeans. *Journal of Agricultural and food chemistry, 40*, 941–944.

Narayan, R., Chauhan, G. S., & Verma, N. S. (1988a). Changes in the quality of soybean during storage. Part 1-effect of storage on some physico-chemical properties of soybean. *Food Chemistry, 27*(1), 13–23.

Narayan, R., Chauhan, G. S., & Verma, N. S. (1988b). Changes in the quality of soybean during storage. Part 2-effect of soybean storage on the sensory qualities of the products made therefrom. *Food Chemistry, 30*(3), 181–190.

Nir, I., Feldman, Y., Aserin, A., & Garti, N. (1994). Surface properties and emulsification behavior of denatured soy proteins. *Journal of Food Science, 59*, 606–609.

Ortiz, S. E. M., & Wagner, J. R. (2002). Hydrolysates of native and modified soy protein isolates: structural characteristics, solubility and foaming properties. *Food Research International, 35*, 511–518.

Pearce, K. N., & Kinsella, J. E. (1978). Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *Journal of Agriculture and Food Chemistry, 26*, 716–723.

Ramanatham, G., Ran, L. H., & Urs, L. N. (1978). Emulsification properties of groundnut protein. *Journal of Food Science, 43*, 1270–1273.

Renkema, J. M. S., Lakemond, C. M. M., De Jongh, H. H. J., Gruppen, H., & Van Vliet, T. (2000). The effect of pH on heat denaturation and gel forming properties of soy proteins. *Journal of Biotechnology, 79*, 223–230.

Renkema, J. M. S., Knabben, J. H. M., & Van Vliet, T. (2001). Gel formation by β-conglycinin and glycinin and their mixtures. *Food Hydrocolloids, 15*, 407–414.

Saio, K., & Watanabe, T. (1978). Differences in functional properties of 7S and 11S soybean proteins. *Journal of Texture Study, 9*, 135–157.

Saio, K., Kobayakawa, K., & Kito, M. (1982). Protein denaturation during model storage studies of soybeans and meals. *Cereal Chemistry, 59*, 408–412.

Shewry, P. R., Napier, J. A., & Tatham, A. S. (1995). Seed storage proteins: Structures and biosynthesis. *Plant Cell, 7*, 945–956.

Wilson, K. A., Papastoitsis, G., Hartl, I., & Tan-Wilson, A. L. (1988). Survey of the proteolytic activities degrading the Kunitz trypsin inhibitor and glycinin in germinating soybeans (Glycine max). *Plant Physiology, 88*, 355–360.

Yagasaki, K., Kaizuma, N., & Kitamura, K. (1996). Inheritance of glycinin subunits and characterization of glycinin molecules lacking the subunits in soybean (Glycine max (L) Merr.). *Breeding Science, 46*, 11–15.