J Am Oil Chem Soc (2008) 85:581–590
DOI 10.1007/s11746-008-1238-6

ORIGINAL PAPER

Protein Subunit Composition Effects on the Thermal Denaturation at Different Stages During the Soy Protein Isolate Processing and Gelation Profiles of Soy Protein Isolates

Eduarda M. Bainy · Susan M. Tosh · Milena Corredig · Lorna Woodrow · Vaino Poysa

Received: 25 December 2007 / Revised: 14 March 2008 / Accepted: 3 April 2008 / Published online: 6 May 2008
© Her Majesty the Queen in Right of Canada 2008

Abstract This study focussed on the evaluation of thermal denaturation at three different stages during soy protein isolation and the effect of subunit composition on the formation of heat-induced soy protein gels. Soy protein isolates (SPI) were prepared from 12 high protein lines, Harovinton variety and 11 derived null-lines which lacked specific glycinin (11S) and β-conglycinin (7S) protein subunits. Protein denaturation during SPI processing was monitored by differential scanning calorimetry (DSC). The results showed that hexane extraction of oil from soybean flours at 23 °C or 105 °C did cause changes in protein conformation. Rheological measurements showed that lines with different subunit compositions and 11S:7S ratio had distinctive gelation temperatures and resulted in gels with different network structures. All lines formed particulate gels at 11% protein. The 11S:7S ratio was not correlated to final stiffness, measured as the storage modulus G', of SPI gels. Lower gelation temperatures were usually observed for 7S-rich lines. The absence of A3 and the combination of A1, A2 and A4 subunits of 11S fraction may suggest the formation of stiffer gels. A more detailed study of the frequency dependence of G' for the various networks formed also indicated that differences in subunit composition influenced the network structures.

Keywords Soybean · Soy protein isolates · DSC · Gel · Gelation

E. M. Bainy · S. M. Tosh (✉)
Guelph Food Research Centre,
Agriculture and Agri-Food Canada,
N1G 5C9 Guelph, ON, Canada
e-mail: ToshS@agr.gc.ca

E. M. Bainy · M. Corredig
Department of Food Science, University of Guelph,
N1G 2W1 Guelph, ON, Canada
e-mail: ebainy@uoguelph.ca

L. Woodrow · V. Poysa
Harrow Research Centre, Agriculture and Agri-Food Canada,
N0R 1G0 Harrow, ON, Canada

Introduction

Soybeans are known for their high nutritional value with a "complete protein" composition, high polyunsaturated fatty acid content, and high content of beneficial bioactive components. In recent years, increased health consciousness has lead to increased demand for soy-containing food products.

Soybeans are rich in protein, averaging 40% of total dry matter. The major storage proteins are β-conglycinin and glycinin, also called 7S and 11S, respectively. β-Conglycinin is composed of α', α and β subunits. Glycinin is composed of an acidic peptide linked by a single disulphide bond with a basic peptide [1]. Based on subunit sequence homology, the subunits are classified into two main groups, group I (A1aB1b, A2B1a, A1bB2) and group II (A5A4B3, A3B4) [2]. Group II is subdivided on the basis of genetic analysis in group IIa (A5A4B3) and group IIb (A3B4) [3]. 11S:7S protein ratios range from 0.5 to 1.7 among soybean cultivars [4]. It has been reported that the ratio of 11S to 7S strongly affects the types of gels formed, and that glycinin forms more elastic, stiffer and harder heat-induced gels than β-conglycinin, mostly because of the higher number of disulphide bridges formed in glycinin gels [5–6]. This characteristic reflects the requirement of protein denaturation and formation of intermolecular bonds for gel formation. At neutral pH and no salt added, glycinin has a

higher onset denaturation temperature (~80 °C) than β-conglycinin (~70 °C) [7]; therefore glycinin is more heat stable than β-conglycinin under the same conditions of ionic strength and pH [8].

The functional characteristics of SPI reported in the literature are frequently very different from what is found in commercially prepared SPI. Industrially produced SPI usually has much lower solubility and poor gel forming ability caused by denaturation and aggregation during processing (unpublished data). Commercially, throughout the world, soybean oil is extracted from flaked soybeans using hexane. Residual hexane is removed using superheated hexane at 135–175 °C followed by direct heating or steam. Protein is extracted from the resulting "white flake" preparation to make defatted flours and grits and protein concentrates and isolates [9] Exposure to non-polar solvent (hexane) at high temperatures and pressures is thought to be the cause of this change in functional properties. Therefore, it may be possible to make improvements to the processing conditions which will enhance the gelling ability of SPI.

This research focused on an established Canadian tofu-type variety, Harovinton; and 11 derived soybean null-lines which have high protein content differing in 7S and 11S subunit composition. Previously published work has focussed on the effect of protein subunit composition effects on tofu quality of these lines [10] and amino acid composition [11]. Very little is known about the changes in heat-induced gelation properties when breeding soybeans with varying protein subunits. Further studies need to be performed, however, to understand how the changes in protein composition affect the protein functionality of soybean lines during processing. Therefore, investigating the processing functionalities of these lines will assist in the breeding of new soybean varieties with specific food uses. Furthermore, an understanding of the changes in protein denaturation during three different stages of SPI processing—before defatting (full fat flour), after defatting (defatted flour) and after soy protein isolation (SPI)—is still lacking in the current literature.

The first objective of this study was to evaluate differences in the thermal denaturation of Harovinton and 11 null-lines at three different stages during SPI processing. In commercially available SPI, a reduction in protein solubility and functionality is frequently observed. Hexane extraction of oil at the increased temperatures and pressures commonly used in industry may contribute to this loss of functionality by causing denaturation. To investigate whether the high temperature and pressure was a factor, two different defatting treatments were performed. The oil was extracted both at high pressure (6.67 MPa, 105 °C) and also at ambient pressure and temperature (23 °C) to evaluate whether hexane-extraction was causing any losses in protein functionality. Soy protein isolates (SPI) were the final products analyzed. The second objective was to evaluate the effect of subunit composition on the heat-induced gels formed with SPI prepared from the 12 soybean lines.

Materials and Methods

Materials

The soybean lines used in this study included the established Canadian tofu type variety, Harovinton, and 11 lines which lacked specific glycinin and β-conglycinin protein subunits (null-lines; Table 1). These lines were developed by crossing and backcrossing Harovinton with a Japanese line lacking all glycinin subunits and the α′ subunit of β-conglycinin. Seeds of Harovinton and the 11 lines were grown at the Greenhouse and Processing Crops Research Centre (GPCRC), Harrow, Ontario in 2005. Full-fat soybean flours, soybean flour defatted at high pressure (6.67 MPa, 105 °C) and soybean flour defatted at ambient pressure and temperature (23 °C) were prepared at GPCRC. These two sets of extraction conditions were chosen as the effect of extraction temperature on the protein denaturation is of interest for manufacturing SPI with desirable functional properties. Soybean flour defatted at 23 °C to prevent heat denaturation of soy proteins, was the starting material to prepare SPI from the 12 soybean lines.

Fat Extraction

Soybean seeds (30–35 g) were ground to a fine powder before defatting using a Knifetec Mill (Foss, Eden Praire, MN), water-cooled grinder equipped with a sharp blade, which prevents samples from being heat degraded. Defatting at high temperature followed the Dionex Application Note No. 325 (Extraction of oils from oilseeds by accelerated solvent extraction, ASE). Briefly, 10–12 g ground soybean flour was loaded in the Dionex ASE 200 (Dionex Corp., Sunnyvale, CA) per sample cell. Three sequential extractions of 10 min each 6.67 MPa were performed using hexane as solvent. The increase in pressure caused the temperature to rise to 105 °C. Safety precautions specified by the manufacturer of the extractor were followed.

For defatting at low temperature (23 °C), the ground sample (10 g) was mixed with 100 ml hexane using a orbital shaker (DS-500, VWR, Scientific) at 70 rpm for 1 h in a fume hood. Hexane was decanted, discarded and the previous operation was performed two more times. Defatted flours were dried in the fumehood overnight and refrigerated.

Table 1 Protein subunit profile of parent variety Harovinton and 11 soybean lines lacking various β-conglycinin and glycinin subunits

Name	Soybean genotypes									
	Cultivar names	Subunits		% Protein	%11S[b]	%7S[b]	Ratio	β-Conglycinin		Glycinin		
	Genotype designation	Absent subunits	Present subunits				11S/7S	% ($\alpha + \alpha'$)[b]	% (β)[b]	% (A3)[b]	% (A1, A2, A4)[b]	% (Basic)[b]
Harovinton[a]	Harovinton	–	–	43.5	40.40	31.13	1.295	24.02	7.11	5.21	15.73	17.93
Line 2	SQ98-0110-3-1	A3	α, α′, β, A1A2, A4	41.5	32.64	37.78	0.865	29.62	8.17	0.86	13.81	16.35
Line 3	SQ97-0263-54-1-5	α′, A4	α, β, A1A2, A3	44.6	38.85	33.04	1.180	20.10	12.94	5.12	14.02	18.38
Line 4	SQ98-0105-6-1	α′, A3	α, β, A1A2, A4	44.4	42.36	28.35	1.495	19.61	8.73	2.44	18.85	18.32
Line 5	SQ97-0263-71-1-3	A1A2, A4	α, α′, β, A3	41.9	22.81	47.15	0.485	38.84	8.30	6.80	2.59	12.36
Line 6	SQ98-0105-1-1b	A3, A4	α, α′, β, A1A2	43.3	20.52	44.38	0.465	36.57	7.81	1.41	6.54	11.01
Line 7	SQ97-0263_21-7-2	α′, A3, A4	α, β, A1A2	43.0	30.53	40.21	0.760	29.35	10.86	2.71	11.73	15.37
Line 8	SQ97-0263_3-10-1	α′, A1A2, A3	α, β, A4	45.4	28.37	38.97	0.730	27.65	11.31	1.67	12.83	10.99
Line 9	SQ970252_S17-2-1	A1A2, A3, A4	α, α′, β	42.9	11.70	56.76	0.205	43.88	12.88	1.31	2.50	6.08
Line 10	SQ970252_S17-2-3	A1A2, A3, A4	α, α′, β	42.0	14.92	55.44	0.270	43.59	11.85	2.49	3.39	7.08
Line 11	SQ97-0263_3-1a	α′, A1A2, A3, A4	α, β	42.7	12.87	48.83	0.265	34.00	14.83	0.97	1.64	9.31
Line 12	SQ98-0112-S7-1	A1A2	α, α′, β, A3, A4	45.5	28.20	43.28	0.650	33.73	9.55	4.67	10.97	8.96

[a] Parent line

[b] Percentage of total protein

Preparation of Soy Protein Isolate

SPI was prepared in the laboratory by Tris–HCl solubilisation followed by acid precipitation based on previously published methods [12] with slight modifications. Flours defatted at room temperature (23 °C) were suspended in a 100 mM Tris–HCl buffer of pH 8.0 in a 1:10 ratio (w/v), and stirred at room temperature for 1 h. The insoluble fraction was removed by centrifugation (12,000 g, 30 min, 10 °C) using a Beckman Coulter Model J2-21 (Follerton, CA) and recovered using porcelain filter with a filter paper (Qualitative P8, Fisher Scientific, Pittsburgh, PA). The supernatant, which contains mostly dissolved protein, was adjusted to pH 4.8 with 2 M HCl to induce protein precipitation. After 2 h at 4 °C, the dispersion was centrifuged as described above. The precipitate was resuspended with 10 mM sodium acetate buffer of pH 4.8 in a 1:8 ratio (w/v) and recentrifuged (as above) to remove any entrapped soluble materials. The final precipitate (SPI) was suspended with ultrapure water, adjusted to pH 7.5 with 1 M NaOH, dialysed overnight and freeze dried. Isolates were stored at −20 °C until used. Protein content of SPI was determined by the Dumas combustion method (Leco FP-528 Mississauga, ON) using conversion factor 6.25 for protein (approved method 46-30 AACC, 2000).

Gel Electrophoresis

The protein compositions of the parent variety, Harovinton, and its 11 derived lines were identified using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was performed with 12.5% (v/v) bisacrylamide gel with 4% (v/v) stacking gel in a BioRad mini-Protein electrophoresis (BioRad Laboratories, Hercules, CA). The electrophoresis buffer was 125 mM Tris, 5 M urea at pH 6.8, 0.2% (v/v) SDS, 20% (v/v) glycerol, and 0.01% (w/v) bromophenol blue. Defatted soybean flour (6 mg) was mixed with 420 µl extraction buffer composed of 50 mM Tris, 5 M urea at pH 8.0, 0.2% (v/v) SDS, 2% (w/v) 2-mercaptoethanol and incubated for 1 h. The samples were then mixed with 420 µl electrophoresis buffer. This solution was heated at 95 °C for 5 min with constant shaking. Aliquots of 6 µl of prepared sample were loaded into each well. The electrophoretic separation was conducted at 200 V for 50 min. The gels were stained with Coomassie blue solution for 30 min with constant shaking, and then destained twice with 45% (v/v) ultrapure water, 45% (v/v) methanol and 10% (v/v) acetic acid solution for 1 h and once with 22.5% (v/v) methanol and 5% (v/v) acetic acid solution for 12 h. The gels were scanned (Sharp JX-330 scanner, Amersham Biosciences, Quebec), and the integrated intensities of electrophoresis protein bands were analyzed with Image Master ID Elite software (Version

2.0, Amersham Biosciences). The protein composition was calculated by dividing the area of the individual peaks by the sum of the areas under all of the peaks including non-storage proteins (for example lectins and lipoxygenase). 11S/7S ratio was calculated by dividing the sum of the areas from the densitometer scan of the electrophoresis results for 11S proteins divided by the sum of the areas for the 7S proteins.

Differential Scanning Calorimetry

Full-fat flour, flour defatted at 105 °C, flour defatted at 23 °C and SPI of the 12 lines were analyzed for differences in their thermal denaturation using differential scanning calorimetry (2920 modulated DSC, TA Instrument, New Castle, DE). Samples of aqueous dispersions (~60 mg) were placed in sealed aluminum pans and analyzed at 2 °C/min from 30 to 115 °C; an empty aluminum pan was used as a reference. Denaturation temperature (T_D in °C) and thermal denaturation enthalpies (ΔH in Joules per gram of protein) were calculated from the endothermic curves. T_D is the intercept of the extrapolated slope of the peak and the baseline; ΔH is the area under the endothermic curve using the Universal Analysis 2000 software supplied by TA Instruments. Determinations were performed in triplicate.

Rheological Measurements

Gel formation was followed by dynamic rheological measurements in a stress controlled rheometer (AR 2000, TA instruments, UK) to determine differences in the viscoelastic properties of the isolates during heating, cooling and holding. The geometry used was an aluminium standard-size recessed end concentric cylinder (15 mm × 14 mm × 42 mm) with a gap of 4 mm. To induce gelation, samples of 11% protein (volume was 8 ml) were heated at 1 °C/min from 25 to 90 °C, kept at 90 °C for 30 min, and then cooled to 25 °C at 1 °C/min. An angular frequency (ω) of 6.3 rad/s (1 Hz) and a constant maximum strain of 0.01 were kept within the temperature ramps. Frequency sweep tests were carried out after the temperature ramp from 10 to 0.01 Hz with a constant applied strain of 0.01 at 25 °C. Stress sweep tests were conducted after frequency sweeps from 0.001 to 300 Pa with a constant applied frequency of 1 Hz confirming that measurements were within the linear viscoelastic range. Frequency dependence of the storage modulus (G') and loss modulus (G'') were determined by the slope of log G' and log G'' respectively as a function of log ω to evaluate differences in the mechanical properties of gels. A thin layer of mineral oil (approximately 0.5 ml) was used on top of the samples to prevent evaporation. Determinations were performed in triplicate.

Statistical Analysis

Statistical evaluation was conducted using SAS (Version 8.0, Cary, NC). General Linear Model (GLM) and Least Squares Means (LSMEANS) procedures were carried out to determine significant differences among the 12 soybean lines. GLM was also conducted to determine significant differences among four different products (full-fat flour, flours defatted at 105 °C, flours defatted at 23 °C and SPI).

Results and Discussion

Characterisation of Soybean Lines

The protein composition of the 12 soybean lines was characterised using SDS-PAGE gel electrophoresis and results are shown in Fig. 1. Genotype designation, 11S:7S ratio and percentage protein composition are shown in Table 1. It was confirmed that Harovinton contains all the β-conglycinin and glycinin subunits. The 11S:7S ratio calculated from the electrophoresis gels ranged from 0.2 to 1.5, with the 11S null-lines showing just trace amounts of 11S subunits. Lines 9, 10 and 11 are the 11S null-lines. Line 11 also lacks the α' subunit of 7S. Lines 3, 4, 7 and 8 lack the β-conglycinin subunit α', and also some glycinin subunits. Lines 2, 5, 6 and 12 have the subunit α', but some 11S subunits are absent. The 11S null-lines and line 3 have the highest content in β-subunit of 7S (~13%). The 7S (α and α'-subunits) content is the highest (~41%) for lines 5, 6, 9 and 10. The basic subunit of glycinin averages ~15% for all lines except for 11S-null lines and line 12, which have the lowest content (~8%). Harovinton and lines 3, 5 and 12 had the highest content of 11S (A3) with about 5%. 11S (A1, A2, A4) average ~14% for Harovinton and lines 2, 3, 4, 7, 8 and 12, while the other lines have only ~3%. Although the quantity of different protein subunits in the null lines was greatly suppressed none of them were eliminated. This would suggest that the genetic variation acted at the level of protein expression or post-translational modifications rather than sequence which codes for the protein. The result is that the 12 varieties used in this work provided a wide spectrum in variation of subunit composition.

Thermal Denaturation

To evaluate how different protein subunits were affected by SPI processing, full-fat flours (with minimal processing), flours defatted at ambient and moderate (6.67 MPa) pressures which resulted in different extraction temperatures (23 and 105 °C, respectively) and SPI were analyzed using DSC. DSC was used to identify changes in

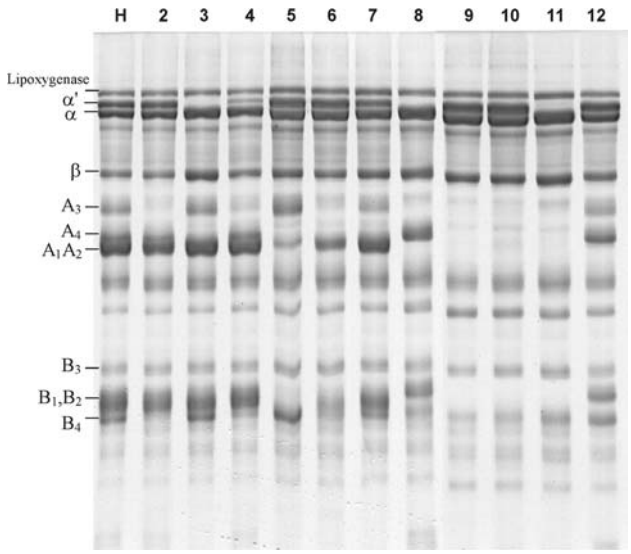

Fig. 1 SDS-PAGE profiles of 12 soybean lines with various β-conglycinin (7S) and glycinin (11S) polypeptide compositions. Parent variety Harovinton is identified as an *H* and derived 11 null-lines are identified as number 2–12. The major storage polypeptides are indicated and labelled at the *left* of the gel: β-conglycinin polypeptides (α',α and β) and glycinin polypeptides (*A*: acidic and *B*: basic). The other band identified is lipoxygenase

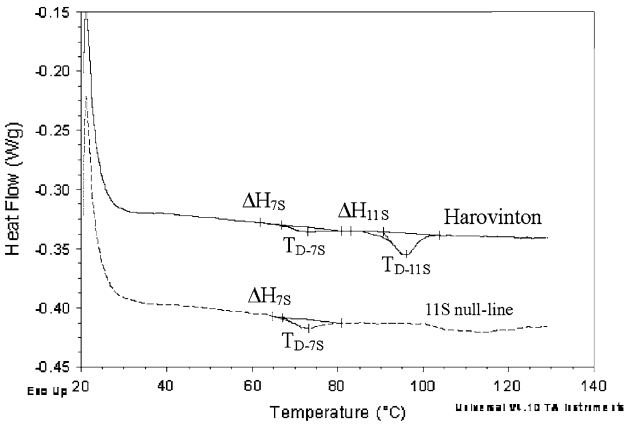

Fig. 2 DSC-thermogram of the parent variety Harovinton (*full line*) and Line 9 which is a 11S null-line (*broken line*). Denaturation temperatures ($T_{D\text{-}7S}$) and enthalpy of denaturation (ΔH_{7S}) are shown in the first endothermic peak. 11S denaturation temperature ($T_{D\text{-}11S}$) and enthalpy of denaturation (ΔH_{11S}) are shown in the second endothermic peak. 11S null-lines lack the second endothermic peak as shown

functionality with processing that may affect the applicability of these products in the food industry.

DSC-thermograms of full-fat flours of the parent variety Harovinton and an 11S null-line (Line 9) are shown in Fig. 2. Two endothermic peaks caused by heat denaturation were observed for lines containing β-conglycinin and glycinin. A single endothermic peak was obtained for 11S null-lines because the glycinin content was too low to be detected by the DSC.

Enthalpies of denaturation (ΔH) and denaturation temperatures (T_D) are shown in Tables 2 and 3, respectively. Statistical analysis showed that within lines, the values of ΔH and T_D were significantly different ($P < 0.01$) among the four processing conditions studied (i.e. flour, flour defatted at low and high temperature, SPI). ΔH was also highly significantly different ($P < 0.0001$) among the 12 lines in all conditions (Table 2). These variations found in the denaturation temperatures and enthalpies among the lines are most likely caused by the differences in heat stability given by particular subunits present in these lines.

Lines 5–12, which contain lower 11S:7S ratio, had higher ΔH_{7S}. The enthalpy was corrected for protein content in the sample and represents the amount of energy required to denature a gram of protein. For instance, line 9 had the highest 7S content and this was reflected in the higher enthalpies of denaturation of 7S compared to other lines. The enthalpies of denaturation range from 0.2 to 3.0 J/g protein for 7S and from 0.2 to 6.0 J/g protein for 11S. These results indicate that 11S is more heat stable than 7S, as previously reported [7].

Denaturation temperature of 7S protein ($T_{D\text{-}7S}$) in the full-fat flours are not significantly different ($P > 0.05$) between lines (Table 3). However, the 11S denaturation temperatures ($T_{D\text{-}11S}$) are significantly different between the lines, shown in Table 3. This indicates that the variation in the subunit composition of 11S strongly affects the temperature of denaturation, while the subunit composition of 7S did not affect it, at least for the subunit combinations (with or without α') used in this work. A previous study [13] indicated that the β-subunit of 7S had a stronger effect on the temperature of denaturation than did the α' subunit. $T_{D\text{-}7S}$ ranged from 72 to 76 °C and $T_{D\text{-}11S}$ varied from 90 to 99 °C for the three different stages during SPI processing. Table 3 shows that isolates generally had a lower denaturation temperature for both 7S and 11S, but they usually had higher enthalpy of denaturation than the other conditions. The ΔH_{11S} of SPI-line 6 was the only line that did not follow this behaviour. Although the enthalpy changes may be mostly caused by the differences in the protein amounts in the various materials, these results indicate that other components (e.g. fat, carbohydrates) present in different content among the four processed samples or differences in the way the proteins are assembled into larger structures may affect the temperature of denaturation of the samples.

Tables 2 and 3 show that, for most lines, there is an increase in the denaturation temperatures and enthalpies of denaturation of 7S protein. Denaturation temperatures and enthalpies for 7S in flours defatted at high temperature are significantly different from those defatted at room temperature ($P < 0.001$). The ΔH_{7S} of the flours defatted at

Table 2 Enthalpy of denaturation ΔH (J/g protein) for the 7S and 11S proteins of full-fat flour, flour defatted at 105 and 23 °C and soy protein isolates

	ΔH_{7S} (J/g protein)				ΔH_{11S} (J/g protein)			
	Full-fat flour	DF-105 °C[A]	DF-23 °C[B]	SPI[C]	Full-fat flour	DF-105 °C[A]	DF-23 °C[B]	SPI[C]
Harovinton	0.38 ± 0.04^{c}	0.29 ± 0.02^{a}	0.70 ± 0.05^{b}	0.87 ± 0.12^{a}	1.50 ± 0.08^{e}	2.18 ± 0.05^{c}	1.99 ± 0.40^{d}	5.96 ± 0.37^{f}
Line 2	0.31 ± 0.01^{b}	0.17 ± 0.14^{a}	0.53 ± 0.06^{a}	0.99 ± 0.13^{a}	0.92 ± 0.03^{d}	1.38 ± 0.25^{b}	1.24 ± 0.22^{c}	4.75 ± 0.47^{e}
Line 3	0.34 ± 0.05$^{b–c}$	0.31 ± 0.10^{a}	0.65 ± 0.08^{b}	0.81 ± 0.08^{a}	1.38 ± 0.12^{e}	1.55 ± 0.47^{b}	2.06 ± 0.11^{d}	4.94 ± 0.28^{e}
Line 4	0.18 ± 0.03^{a}	0.32 ± 0.07^{a}	0.50 ± 0.05^{a}	0.93 ± 0.05^{a}	1.45 ± 0.07^{e}	2.54 ± 0.17^{d}	2.20 ± 0.02^{d}	4.97 ± 0.07^{e}
Line 5	0.38 ± 0.01^{c}	0.69 ± 0.07$^{b–c}$	1.01 ± 0.13^{d}	2.39 ± 0.12^{d}	0.25 ± 0.04^{a}	0.58 ± 0.05^{a}	0.47 ± 0.05^{a}	2.16 ± 0.08^{c}
Line 6	0.56 ± 0.02^{d}	0.84 ± 0.14$^{c–d}$	1.12 ± 0.18$^{d–e}$	2.75 ± 0.20^{e}	0.48 ± 0.02^{b}	0.63 ± 0.10^{a}	0.62 ± 0.08$^{a–b}$	0.26 ± 0.04^{a}
Line 7	0.35 ± 0.01$^{b–c}$	0.63 ± 0.13^{b}	0.84 ± 0.05^{c}	1.35 ± 0.09^{b}	0.90 ± 0.09$^{c–d}$	1.38 ± 0.08^{b}	1.27 ± 0.10^{c}	3.84 ± 0.40^{d}
Line 8	0.53 ± 0.09^{d}	0.79 ± 0.14$^{c–d}$	0.86 ± 0.01^{c}	2.38 ± 0.15^{d}	0.41 ± 0.07^{b}	0.76 ± 0.03^{a}	0.87 ± 0.05^{b}	1.55 ± 0.11^{b}
Line 9	0.67 ± 0.07^{e}	1.21 ± 0.18^{f}	1.30 ± 0.12^{f}	3.36 ± 0.18^{f}	–	–	–	–
Line 10	0.50 ± 0.02^{d}	0.94 ± 0.07$^{d–e}$	1.14 ± 0.08$^{d–e}$	1.98 ± 0.17^{c}	–	–	–	–
Line 11	0.74 ± 0.04^{f}	0.99 ± 0.03^{e}	1.18 ± 0.02$^{e–f}$	2.69 ± 0.16^{e}	–	–	–	–
Line 12	0.55 ± 0.05^{d}	0.61 ± 0.18^{b}	0.75 ± 0.04$^{b–c}$	2.19 ± 0.24$^{c–d}$	0.79 ± 0.02^{c}	0.91 ± 0.11^{a}	0.87 ± 0.18^{b}	2.71 ± 0.53^{c}

Data are means of triplicate measurements ± the standard deviation. Means in a column with different letters are significantly different ($P < 0.05$)

[A] Flour defatted at 105 °C

[B] Flour defatted at 23 °C

[C] Soy protein isolates

23 °C are higher than the ones defatted at 105 °C (Table 2). This behaviour was not seen for the T_{D11S} and ΔH_{11S} as denaturation temperatures and enthalpies for 11S in flours defatted at high temperature are not significantly different from those defatted at room temperature ($P > 0.05$). These results are a clear indication of conformational change of the 7S storage proteins.

Relationships between the enthalpies of denaturation of 7S and 11S globulins versus 11S:7S ratios were observed as expected. The 11S:7S ratio increased with and increase in the enthalpy of 11S denaturation and decreased with an increase in the enthalpy of 7S denaturation for the four conditions.

Although conformational changes in the protein were observed during the processing of SPI, neither the high temperature nor the low temperature hexane extractions can account for the degree of denaturation found in industrially produced SPI. Commercially available SPI frequently has no measurable DSC peak which would indicate extensive denaturation (data not shown). The other major difference between the process used here and commercial processing is the drying method. Freeze drying was used for these samples, whereas industrial processes use heat to dry the SPI. An investigation of the effect of drying methods on SPI solubility and functionality may be useful for improving SPI quality.

Gelling Properties of Soy Protein Isolates

To determine the changes in viscoelastic behaviour of the SPI during gel formation, the storage (G') and loss (G'') moduli were measured during heating. A typical example of a heat-induced gel curve of SPI dispersions at pH 7.0 as a function of time is shown in Fig. 3. The elastic modulus of the gels (G'), increases with heating, and continues to increase during cooling as previously reported [14]. Unfolding of the proteins during heating allows for the formation of intermolecular disulphide bonds between cystine residues. A further increase in the G' occurs during the cooling step of soy protein gels, as the proteins rearrange and further associate through non-covalent interactions [14]. Figure 3 shows that for the Harovinton line, the gelation cross point, which was defined as the point when the loss tangent ($\tan \delta = G''/G'$) equals 1 at a frequency of 1 Hz, was reached at 90 °C ($t = 72$ min). This temperature coincides with the $T_{D\text{-}11S}$ of Harovinton SPI at ~91 °C, the temperature at which the 11S begins to unfold.

Protein denaturation is a prerequisite for gelation of globulins [15] which leads to the association of unfolded proteins with exposed reactive residues, and subsequent formation of a gel network. Consequently, it is possible to predict that, since 7S denatures at a lower temperature than 11S, 7S will form a gel at a lower temperature (~75 °C) than 11S (~90 °C). This postulation agrees with the results reported in Table 4; for instance, line 5 (containing a high % of 7S) showed an earlier gel point, at a lower temperature, than lines containing high 11S content. This was also shown in the 11S null-lines (lines 9, 10 and 11).

The storage modulus (G') at the end of the heating and cooling experiment was not correlated with the 11S:7S

Table 3 Denaturation temperature T_D (°C) for the 7S and 11S proteins of full-fat flour, flour defatted at 105 and 23 °C and soy protein isolates

	$T_{D\text{-}7S}$ (°C)				$T_{D\text{-}11S}$ (°C)			
	Full-fat flour	DF-105 °C[A]	DF-23 °C[B]	SPI[C]	Full-fat flour	DF-105 °C[A]	DF-23 °C[B]	SPI[C]
Harovinton	73.63 ± 0.81^{a-c}	75.31 ± 0.41^{a-b}	75.37 ± 0.85^{b}	71.58 ± 0.69^{a}	96.79 ± 0.15^{c-d}	98.37 ± 0.34^{a-b}	98.63 ± 0.29^{a-b}	91.03 ± 0.76^{b}
Line 2	72.75 ± 0.16^{a-b}	75.29 ± 0.52^{a-b}	73.47 ± 1.89^{a}	71.52 ± 0.92^{a}	96.46 ± 0.41^{c}	97.53 ± 0.95^{a}	97.55 ± 0.50^{a}	91.28 ± 0.84^{b}
Line 3	73.38 ± 0.41^{a-c}	74.56 ± 0.25^{a}	74.18 ± 0.76^{b}	74.37 ± 0.25^{d}	96.08 ± 0.22^{b-c}	98.06 ± 0.87^{a-b}	98.28 ± 0.50^{a-b}	91.60 ± 0.50^{b-c}
Line 4	72.56 ± 1.01^{a}	74.94 ± 0.32^{a-b}	72.94 ± 0.71^{a}	72.58 ± 0.39^{b}	96.16 ± 0.10^{b-c}	97.66 ± 0.78^{a}	98.17 ± 0.13^{a}	89.88 ± 0.13^{a}
Line 5	73.08 ± 0.34^{a-c}	74.21 ± 0.92^{a}	73.32 ± 0.43^{a}	72.06 ± 0.03^{a-b}	97.42 ± 0.31^{d}	98.57 ± 0.05^{b}	98.57 ± 0.25^{a-b}	94.46 ± 0.12^{d}
Line 6	72.99 ± 0.24^{a-b}	75.51 ± 0.165^{b}	75.05 ± 0.43^{b}	72.69 ± 0.52^{b}	95.65 ± 0.97^{a-b}	99.03 ± 0.72^{b}	99.02 ± 0.74^{b}	95.06 ± 0.51^{d-e}
Line 7	72.90 ± 0.19^{a-b}	75.60 ± 0.20^{b}	73.77 ± 1.21^{a}	73.44 ± 0.33^{c-d}	95.20 ± 0.73^{a}	98.85 ± 0.31^{b}	98.17 ± 0.41^{a}	92.26 ± 0.30^{c}
Line 8	72.87 ± 0.22^{a-b}	75.01 ± 0.56^{a-b}	73.05 ± 0.39^{a}	74.13 ± 0.36^{d}	96.22 ± 0.55^{b}	98.04 ± 0.22^{a-b}	97.84 ± 0.25^{a}	95.41 ± 0.06^{e}
Line 9	73.98 ± 0.33^{c}	75.25 ± 0.53^{a-b}	74.79 ± 0.62^{b}	72.70 ± 0.40^{b}	–	–	–	–
Line 10	73.16 ± 0.39^{a-c}	74.63 ± 0.52^{a}	74.67 ± 0.33^{b}	72.64 ± 0.26^{b}	–	–	–	–
Line 11	73.33 ± 1.08^{a-c}	75.34 ± 0.63^{a-b}	72.58 ± 0.45^{a}	73.20 ± 0.10^{c}	–	–	–	–
Line 12	72.67 ± 0.37^{a}	75.36 ± 0.84^{a-b}	73.41 ± 0.46^{a}	72.78 ± 0.08^{b-c}	96.27 ± 0.65^{b-c}	98.08 ± 0.36^{a-b}	97.75 ± 0.34^{a}	94.80 ± 0.43^{d-e}

Data are means of triplicate measurements ± the standard deviation. Means in a column with different letters are significantly different ($P < 0.05$)

[A] Flour defatted at 105 °C

[B] Flour defatted at 23 °C

[C] Soy protein isolates

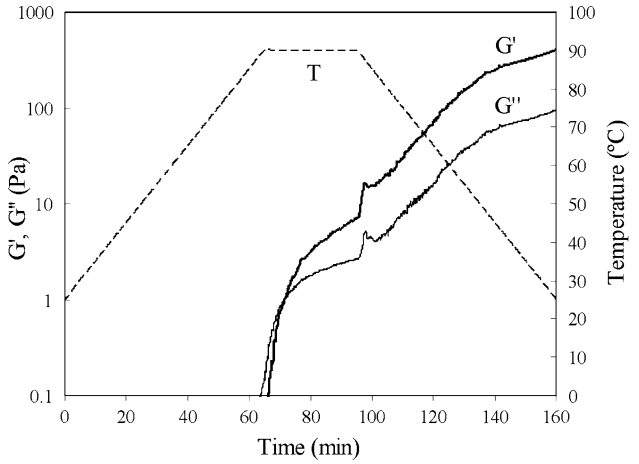

Fig. 3 Elastic moduli (G') and viscous moduli (G'') of soy protein isolate (Harovinton) dispersion at 11% protein in ultrapure water at pH 7 as a function of time during heating and cooling ramps. The *broken line* (T) represents the temperature change. Values are the average of three independent replicate experiments

ratio in the lines ($R^2 < 0.50$). This is somewhat surprising, as it has been previously reported that glycinin forms stiffer gels than β-conglycinin [6, 16]. It was also previously reported [10] that while the Group IIb (A3B4) subunit conferred greater gel firmness, the Group IIa (A5A4B3) subunit resulted in less firm tofu. However the tofu was produced by acid gelation, a different mechanism which is results in different interactions between the proteins.

Gelation differences of SPI gels may also be caused by differences in the concentration of the sulphur-containing amino acid, cysteine, among soybean genotypes as previously reported [17]. 11S protein has three to four times more methionine and cysteine per unit protein than 7S globulin [18]; therefore 11S has more cysteine residues which contribute to stronger cross linking during the gel formation.

The final G' value was the highest for lines 2, 6 and 8. The lines with the highest final G' showed a gelation point (with times between 75 and 98 min) later than the other lines and, at the gelation time, showed the lowest value of G' (Table 4). Lines 2, 6 and 8 have very low (less than 2%) A3 subunit content (Table 1). The A3 subunit has fewer cysteine residues than the other acidic subunits [17] which would affect its ability to form intermolecular disulphide linkages during gelation of the protein and contribute to the development of an elastic network. Figure 4 shows that there is a correlation between the content of 11S subunits and the final G' of the gels ($R^2 = 0.967$) when the A3 subunit content is less than 2% of the total protein (lines 2, 6 8, 9, and 11).

Amongst these three lines, line 6 has low 11S content, and showed a distinct gelation behaviour. Line 6 had the

Table 4 Temperature, time and storage modulus of gelation cross point and storage, loss modulus and loss tangent of final gels

Line	Cross point T (°C)[A]	Cross point time (min)[B]	Cross point G' (Pa)[C]	Final G' (Pa)[D]	Final G'' (Pa)[D]	tan δ[E]
Harovinton	90.0 ± 0.0^{e}	72 ± 1$^{e–f}$	1.00 ± 0.06^{c}	404 ± 472^{a}	94 ± 111^{a}	0.230 ± 0.008^{f}
Line 2	89.9 ± 0.2^{e}	88 ± 7^{g}	0.50 ± 0.10^{a}	7283 ± 1506^{c}	1047 ± 110^{c}	0.148 ± 0.036$^{a–c}$
Line 3	86.2 ± 0.4^{d}	61 ± 0$^{b–c}$	0.86 ± 0.01$^{b–c}$	423 ± 111^{a}	56 ± 15^{a}	0.132 ± 0.006$^{a–b}$
Line 4	90.2 ± 0.2^{e}	65 ± 0$^{c–d}$	0.84 ± 0.03$^{b–c}$	410 ± 57^{a}	63 ± 7^{a}	0.153 ± 0.004$^{a–c}$
Line 5	76.1 ± 0.4^{b}	51 ± 0^{a}	0.80 ± 0.04^{b}	234 ± 95^{a}	27 ± 9^{a}	0.118 ± 0.019^{a}
Line 6	86.8 ± 2.2^{d}	98 ± 2^{h}	0.46 ± 0.05^{a}	1117 ± 517$^{a–b}$	233 ± 106$^{a–b}$	0.209 ± 0.002$^{d–f}$
Line 7	90.0 ± 0.0^{e}	68 ± 0$^{d–e}$	0.90 ± 0.06$^{b–c}$	127 ± 15^{a}	26 ± 3^{a}	0.206 ± 0.013$^{d–f}$
Line 8	90.0 ± 0.0^{e}	75 ± 4^{f}	0.39 ± 0.18^{a}	3160 ± 4611^{b}	466 ± 625^{b}	0.181 ± 0.043$^{c–e}$
Line 9	74.2 ± 0.1^{a}	49 ± 0^{a}	1.31 ± 0.17^{d}	277 ± 108^{a}	43 ± 7^{a}	0.166 ± 0.051$^{b–d}$
Line 10	82.2 ± 2.7^{c}	57 ± 3^{b}	1.01 ± 0.06^{c}	189 ± 86^{a}	36 ± 18^{a}	0.185 ± 0.034$^{c–e}$
Line 11	76.4 ± 1.1^{b}	51 ± 1^{a}	0.82 ± 0.08$^{b–c}$	157 ± 24^{a}	33 ± 5^{a}	0.212 ± 0.004$^{e–f}$
Line 12	90.0 ± 0.1^{e}	67 ± 1$^{d–e}$	0.53 ± 0.28^{a}	224 ± 72^{a}	49 ± 13^{a}	0.224 ± 0.019$^{e–f}$

Data are means of triplicate measurements ± the standard deviation. Means in a column with different letters are significantly different ($P < 0.05$)

[A] Temperature when gelation cross point occurs

[B] Time in minutes when gelation cross point occurs

[C] Gelation cross point when G' equals G''

[D] Storage (G') and loss (G'') modulus in Pascal

[E] Loss tangent (tan δ) = Final G''/Final G'

longest gelling time, as the gelation cross point occurred during the cooling step where most of G' development occurred. This delayed gelation in lines 2, 6 and 8 appeared to be associated with a higher final G'.

However, the final G' of gels made from SPI with A3 subunit content higher than 2% is not significantly different from the parent variety (Fig. 4). The behaviour of SPI made from line 4 suggests that the role of the A3 subunit actually inhibits the development of stiffness. Line 4 is nominally A3 null, but 2.44% of the total protein is A3. Although line 4 has the highest A1 + A2 + A4 protein subunit content (18.85% of total protein), the presence of the A3 subunits apparently prevents the development of stiffness comparable to line 2 (13.81% A1 + A2 + A4).

Additionally, There is no significant difference between the final stiffness of lines which contain very low levels of 11S subunits (lines 9, 10 and 11) and those with high 11S subunit content where the A3 subunit makes up more than 2% of the total protein (parent variety, lines 3, 4, 5, 7 and 12). This would suggest that the 7S proteins may be the major structural element in the gels made from SPI containing more than 2% A3 subunit. For these lines the holding temperature of 90 °C may not be sufficient to unfold the 11S proteins.

The present results are somewhat in disagreement with previously published data on gel hardness: soy proteins with higher A3 content formed harder tofu [10, 19] and soy cultivars lacking the A4 subunit (identified in the paper as A5) also formed harder and more solid-like tofu gels than

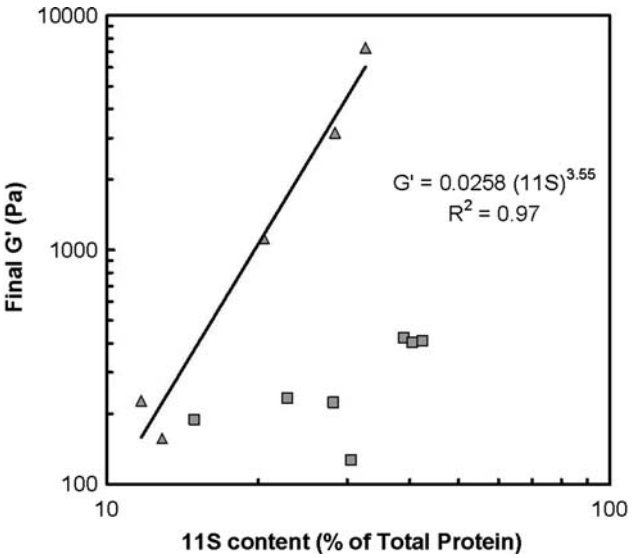

Fig. 4 Correlation of final G' of 11% SPI gels with 11S protein content for lines containing less than 2% A3 subunit (as a percentage of total protein), *triangles*. No correlation was found between final G' and 11S protein content for lines where the A3 subunit content was greater than 2% of total protein, *squares*

those prepared from varieties with the A4 subunit [10, 20]. However it is important to note that the development of the G' and the small deformation rheological properties may not fully relate with large deformation data (hardness). In addition, many other factors are involved in protein aggregation under acidic conditions during tofu making,

which are different from the present study which dealt with heat-induced aggregation of soy proteins.

Line 9, containing the highest 7S content, showed the highest G' at the crossover point and a low temperature of gelation (Table 4). This is a clear indication that in lines containing high 7S, the 7S contributed to the gel structure. Line 4, with the highest 11S:7S ratio showed an intermediate crossover point value when compared to the other lines.

Lines 1, 3 and 4 had similar 11S:7S ratio and the final G' values ranged over about 400 Pa. Although line 7 has a similar protein composition to line 8, it has a very distinct gelation profile from that of line 8. The gelation time and the value of G' at the crossover point were significantly different between the two lines. The development of stiffness was a lot slower in line 8 than in line 7 the final G'' was much higher in line 8. While line 8 has A4, line 7 lacks A4 and has higher content of A3 than line 8. Once again, the content of A3 and A4 subunits seems to influence network formation.

The loss tangent (tan δ) describes the viscoelastic nature of the gel networks formed. tan δ is much lower for an elastic network than for a particulate or weak network. The values of tan δ summarised in Table 4 range from 0.118 to 0.20 and show that at 11% protein the gel formed contained a significant viscous (energy loss) component. This again suggests a particulate or weak network structure.

Viscoelastic Properties of SPI Gels

Frequency sweep tests were performed immediately after the temperature ramps. In all the samples G' was higher than G'' throughout the range of the frequency sweep indicating that a gel network had formed in all of the solutions. G' showed some degree of frequency dependent with lower G' values being measured at low frequency than at high frequency, because at longer experimental time scales more protein-protein bonds have time relax during the periodic deformation.

The viscoelastic characteristics of final SPI gels can be mathematically represented by using the power-type model ($G' = K\omega^n$) correlating the elastic modulus (G') and frequency (ω). K and n are regression coefficients relating G' and ω (Table 5). The storage modulus dependency with frequency was significantly different ($P < 0.05$) among lines as the slope of the plot of log G' as a function of log frequency (which corresponds to n value in the power law model). The values calculated for n ranged from 0.062 to 0.152 for the different lines. From a structural point of view, perfectly elastic gel networks (independent of the frequency of oscillation) are identified by a zero slope for G', whereas particulate gels show positive slopes [21]. The positive slopes of the SPI gels again demonstrate that the gels are weak or particulate type gels. There was a positive correlation ($R^2 = 0.998$) between the values found for the consistency coefficient k and the final G' (Fig. 5), These correlations suggest that the basic structure of the protein gels is similar, with increasing numbers of interaction points causing the increase in stiffness. There was also between the behavioural index, n, and the tan δ ($R^2 = 0.68$) as shown in Fig. 6, demonstrating the similarity of viscoelastic behaviour in the different gels.

Table 5 Regression output of equation $G' = k\omega^n$ for soy protein isolate (SPI) gels of the 12 soybean lines

Line	k	n	R^2
Harovinton	317.12[a]	0.131[f]	0.997
Line 2	6093.73[c]	0.094[c–d]	0.976
Line 3	381.71[a]	0.070[a–b]	0.992
Line 4	358.56[a]	0.087[b–c]	0.998
Line 5	213.22[a]	0.062[a]	0.991
Line 6	833.94[a–b]	0.152[g]	0.974
Line 7	105.91[a]	0.115[e–f]	0.993
Line 8	2509.46[b]	0.120[e–f]	0.981
Line 9	243.13[a]	0.080[b–c]	0.993
Line 10	158.27[a]	0.105[d–e]	0.993
Line 11	133.64[a]	0.109[d–e]	0.991
Line 12	181.34[a]	0.129[f]	0.997

Values were calculated from the slope of the log (G') versus log frequency and are means of three independent experiments. Means in a column with different letters are significantly different ($P < 0.05$)

Treatment of full-fat soy flour with hexane, either at room temperature or at elevated temperatures used in industry, did cause changes in the thermal denaturation temperatures and enthalpies for 7S storage protein. Three

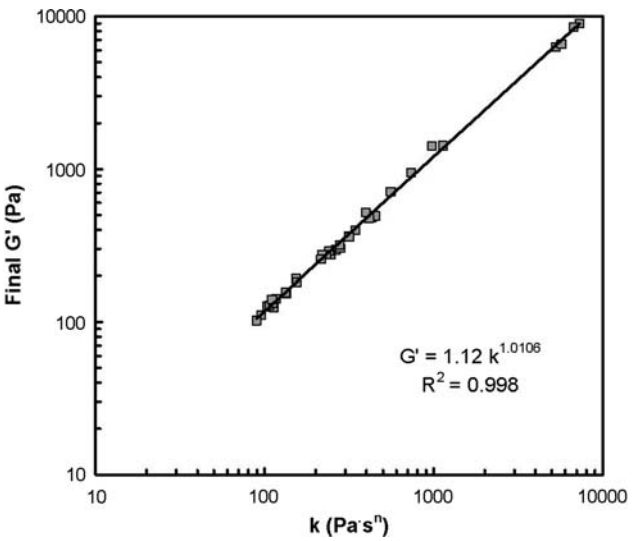

Fig. 5 Correlation of the final G' of 11% SPI gels with the consistency coefficient, k

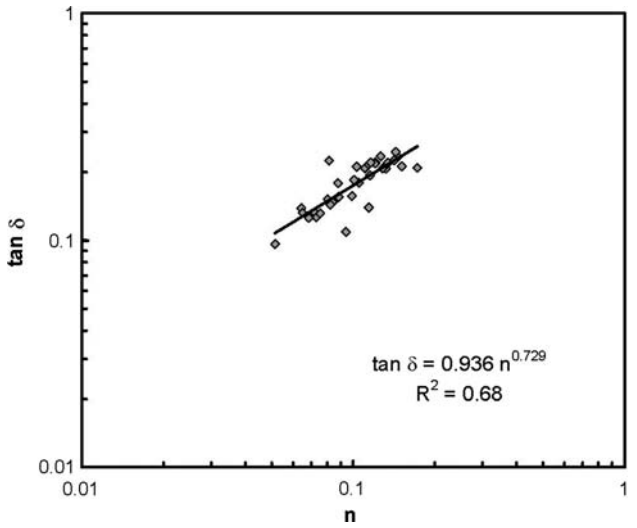

Fig. 6 Correlation of the tan δ of 11% SPI gels with the behaviour index, n

lines exhibited significantly higher stiffness in gels made from 11% SPI solutions and they may be useful lines in developing SPI for use as a gelling agent. The presence of the A3 subunit appears to hinder development of a coherent gel network. The correlation of the consistency index, k, suggests that the basic structure of all of the gels is similar.

Acknowledgments The authors thank the Ontario Soybean Growers and Agriculture and Agri-Food, Canada (Matching Investment Initiatives) for the funding provided and Amir Malaki Nik for the SDS-Page gel.

References

1. Liu K (1999) Soybeans: chemistry, technology and utilization, 1st edn. Aspen, Gaithersburg, pp 25–77
2. Nielsen NC, Dickinson CD, Cho TJ, Thanh VH, Scallon BJ, Fischer RL, Sims TL, Drews GN, Goldberg RB (1989) Characterization of the glycinin gene family in soybean. Plant Cell 1:313–328
3. 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.). Breed Sci 46:11–15
4. Wright DJ (1987) The seed globulins: part II. In: Hudson BJF (ed) Developments in food proteins. Elsevier, London, pp 81–157
5. Nakamura T, Utsumi S, Mori T (1986) Interactions during heat-induced gelation in a mixed system of soybean 7S and 11S globulins. Agri Bio Chem 50:2429–2435
6. Renkema JMS, Knabben JHH, Vliet TV (2001) Gel formation by β-conglycinin and glycinin and their mixtures. Food Hydrocoll 15:407–414
7. Hermansson AMJ (1978) Physicochemical aspects of soy proteins structure formation. Text Stud 9:2–33
8. Puppo MC, Añón MC (1999) Soybean protein dispersions at acid pH. Thermal and rheological properties. J Food Sci 64:50–56
9. Lusas EW (2004) Soybean processing and utilization. In: Boerma HR, Specht JE (eds) Soybeans: improvement, production, and uses. ASA/CSSA/SSSA, Madison, pp 949–1045
10. Poysa V, Woodrow L, Yu K (2006) Effect of soy protein subunit composition on tofu quality. Food Res Int 39:309–317
11. Zarkadas CG, Gagnon C, Poysa V, Khanizadeh S, Cober ER, Chang V, Gleddie S (2007) Protein quality and identification of the storage protein subunits of tofu and null soybean genotypes, using amino acid analysis, one- and two-dimensional gel electrophoresis, and tandem mass spectrometry. Food Res Int 40:111–128
12. Renkema JMS, Lakemond CMM, de Jongh HHJ, Gruppen H, van Vliet T (2000) The effect of pH on heat denaturation and gel forming properties of soy proteins. J Biotechnol 79:223–230
13. Maruyama N, Katsube T, Wada Y, Oh MH, Barba de la Rosa AP, Okuda E, Nakagawa S, Utsumi S (1998) The roles of the N-linked glycans and extension regions of soybean β-conglycinin in folding, assembly and structural features. Eur J Bioch 258:854–864
14. Renkema JMS, van Vliet T (2002) Heat-induced gel formation by soy proteins at neutral pH. J Agric Food Chem 50:1569–1573
15. Kinsella JE (1976) Functional properties of proteins in foods: a survey. CRC Crit Rev Food Sci Nutr 23:323–395
16. Riblett AL, Herald TJ, Schmidt KA, Tilley KA (2001) Characterization of β-conglycinin and glycinin soy protein fractions from four selected soybean genotypes. J Agric Food Chem 49:4983–4989
17. Khatib KA, Herald TJ, Aramouni FM, MacRitchie F, Schapaugh WTJ (2002) Characterization and functional properties of soy β-conglycinin and glycinin of selected genotypes. Food Sci 67:2923–2929
18. Kitamura K (1995) Genetic improvement of nutrition and food processing quality in soybean. Jpn Agric Res Quart 29:1–8
19. Nakamura T, Utsumi S, Kitamura K, Harada K, Mori TJ (1984) Cultivar differences in gelling characteristics of soybean glycinin. Agric Food Chem 32:647–651
20. Nishinari K, Kohyarna K, Zhang Y, Kitamura K, Sugimoto T, Saio K, Kawamura Y (1991) Rheological study on the effect of the A5 subunit on gelation characteristics of soybean proteins. Agric Biol Chem 55:351–355
21. Ross-Murphy SB (1984) Rheological methods. In: Chan HWS (ed) Biophysical methods in food research. Alden Press, Oxford, pp 138–199