



Interactions of protein content and globulin subunit composition of soybean proteins in relation to tofu gel properties



Andrew T. James, Aijun Yang*

CSIRO Agriculture, 306 Carmody Road, St Lucia, Qld 4067, Australia

ARTICLE INFO

Article history:

Received 9 June 2015

Received in revised form 5 August 2015

Accepted 6 August 2015

Available online 7 August 2015

Keywords:

Soybean protein content

Globulin subunits

Tofu gel quality

ABSTRACT

The content and globulin subunit composition of soybean proteins are known to affect tofu quality and food-grade soybeans usually have higher levels of proteins. We studied the tofu quality of soybeans with high (44.8%) or low (39.1%) protein content and with or without the 11S globulin polypeptide, 11SA4. Both protein content and 11SA4 significantly affected tofu gel properties. Soybeans containing more protein had smaller seeds which produced significantly firmer (0.663 vs. 0.557 N, $p < 0.001$) tofu gels with creamier colour. The absence of 11SA4 was positively correlated with seed size, tofu hardness and water holding capacity and led to significant changes to the profile of storage protein subunits, which may have contributed to the improvement in tofu gel properties. These results suggest that, in combination with higher protein content, certain protein subunits or their polypeptides can also be targeted in selecting soybeans to further improve soy food quality.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Soybeans offer high quality and affordable protein and oil for humans and animals. Soy foods including tofu and soymilk have traditionally been staple foods in many parts of Asia and their consumption in western countries has been increasing due to their potential health benefits. Tofu is essentially a water-based protein gel formed by the addition of a coagulant to soymilk. The yield and quality, texture in particular, of this gel are therefore affected by the protein content in the seed. Tofu texture is known to be positively affected by the protein content in the seed (Cai & Chang, 1999; Kim & Wicker, 2005; Schaefer & Love, 1992; Shen, Deman, Buzzell, & Deman, 1991; Stanojevic, Barac, Pesic, & Vucelic-Radovic, 2011) and higher protein content has been an aim in the selection and development of soybeans intended for food use. There is, however, a strong negative correlation between seed protein content and harvest yield (Cui, James, Mizazaki, Wilson, & Carter, 2004), limiting the extent to which protein content can be increased.

In addition to protein content, globulin subunit composition of the two major groups of storage proteins in soybeans, glycinin (11S globulins) and β -conglycinin (7S globulins), also play a major role in determining soybean protein quality and its functionality in soy foods although their reported effects on tofu quality have not

been consistent. Glycinin and β -conglycinin account for approximately 60–75% of the total soybean seed proteins (Nielsen et al., 1989). They are structurally different and possess rather different properties including molecular makeup, isoelectric points and amino acid composition, all of which could affect their functionality including heating stability and gel strength in a food system such as the gel network in tofu. Tofu quality has been reported to be affected by different levels of 11S and 7S globulins (Cai & Chang, 1999; Cai, McCurdy, & Baik, 2002; Kim & Wicker, 2005) and the subunit compositions of 11S and 7S globulins (Nakamura, Utsumi, Kitamura, Harada, & Mori, 1984; Poysa, Woodrow, & Yu, 2006; Stanojevic et al., 2011). We have also shown recently that the absence of a particular 11S globulin polypeptide, 11SA4, increased tofu firmness (James & Yang, 2014; Yang & James, 2013, 2014), confirming the results reported by others (Nishinari et al., 1991; Poysa et al., 2006). Tezuka, Taira, Igarashi, Yagasaki, and Ono (2000), however, found 11SA4 absence may affect the breaking stress differently depending on the coagulant. Poysa et al. (2006) evaluated 20 soybean varieties of various subunit compositions and concluded that 11SA4 had a negative or no effect on tofu hardness depending on growing year and coagulant used.

Using a local variety Bunya which contains modest level of seed proteins (38–40%) and lacks 11SA4, we have been able to demonstrate that the positive contribution of the absence of this globulin polypeptide could compensate in part for the lower seed protein content on tofu quality (James & Yang, 2014). To further elucidate the impact of globulin subunits, or lack of it, and their interactions

* Corresponding author.

E-mail address: Aijun.Yang@csiro.au (A. Yang).

with protein content on the gelling properties of tofu, we compared seed characteristics, storage protein subunit composition and tofu properties of four groups of soybeans: genotypes with high or low protein content and with or without 11SA4. The proteins in the bands separated by the classic SDS–PAGE were also analysed using nano-LC–MS/MS.

2. Materials and methods

Eleven soybean (*Glycine max*) samples were selected, based on their protein content and the presence of 11SA4, from a large varietal trial conducted at Gatton (27°33'S, 151°17'E), Queensland, Australia. They had high (~44.8%) or low (~39.1%) protein content and were with or without 11SA4. They were grown in three replicates and harvested in 2013 and the harvested seeds were kept together in an air-conditioned laboratory (~21 °C) before use. The moisture content of the soybeans was approximately 9%.

2.1. Seed characteristics

Seed weight and moisture and protein content were determined as described previously (Yang & James, 2013). Briefly, seed weight (g/100 seeds) was determined by weighing one hundred raw soybean seeds. The moisture and solid content in the seed and soymilk were determined by placing weighed samples of ground soybeans (~3 g) or soymilk (~10 g) in an oven at 105 °C for 24 h. The dried soymilk was then ground and the nitrogen content for dried soymilk and dried ground seed was analysed using LECO TrueSpec CHN analyser (Leco Corp., St Joseph, MI, USA). The total crude protein content was obtained by multiplying total nitrogen content with a conversion factor of 6.25.

The seed proteins were extracted from 10 mg of the dried, ground soybean with 200 μ L 0.03 M Tris–HCl, pH 8.0, containing 10 mmol L⁻¹ dithiothreitol (DTT). The 11S and 7S globulins and their subunits and polypeptides were separated using 14% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) under the conditions previously reported (Yang & James, 2013). The gels were stained overnight with Coomassie brilliant blue R250. After destaining, the gels were scanned and analysed using Quantity One software version 4.2.1 (BioRad Australia). Molecular weight markers (PageRuler Prestained Protein Ladder, Fermentas) covering 10–170 kDa were used and bands representing 7S and 11S globulin subunits and polypeptides were identified by their relative mobility against the protein ladder and published data.

2.2. Nano-HPLC, mass spectrometry and protein identification

Fourteen of the separated bands which represented major globulin subunits or polypeptides were manually excised from the gel and the gel pieces were de-stained with 50 mM NH₄HCO₃ in 50% (v/v) acetonitrile (ACN) and dried for 10 min with 100% ACN and then under vacuum for 30 min. Gel pieces were rehydrated in 30 μ L 50 mM NH₄HCO₃ containing 30 ng/ μ L sequencing-grade trypsin (Promega) and incubated for 1 h at 4 °C. After brief centrifugation, excess trypsin solution was removed and samples were incubated at 37 °C for 16 h. On the following day, 30 μ L 50% (v/v) ACN with 1% (v/v) formic acid was added to the gel pieces which were incubated for 1 h at 37 °C. The supernatant containing the peptides was collected after brief centrifugation. This was repeated once. The combined supernatant was dried under vacuum before reconstituted in 20 μ L 1% (v/v) formic acid.

The extracts were analysed by LC–MS/MS on a Shimadzu Prominence Nano HPLC (Japan) coupled to a Triple TOF 5600 mass spectrometer (ABSCIEX, Canada) equipped with a nano

electrospray ion source. The peptide extract, 10 μ L, was injected onto a 50 mm $\times$ 300 μ m C18 trap column (Agilent Technologies, Australia) at 60 μ L/min. The samples were de-salted on the trap column for 5 min using 0.1% formic acid at 60 μ L/min. The trap column was then placed in-line with the analytical nano HPLC column (150 mm $\times$ 100 μ m 300SBC18, 3.5 μ m, Agilent Technologies, Australia) for mass spectrometry analysis. Linear gradients of 2–80% solvent B over 40 min at 500 nL/min flow rate, followed by a steeper gradient from 80% to 96% solvent B in 1 min and held for 5 min, were used for peptide elution. Solvent A consisted of 0.1% formic acid and solvent B contained 90% acetonitrile with 0.1% formic acid. The ionspray voltage was set to 2200 V, declustering potential (DP) 100 V, curtain gas flow 25, nebuliser gas 1 (GS1) 12 and interface heater at 160 °C. Full scan TOF–MS data were acquired over the mass range 300–1800 and MS/MS 80–1400 for product ion. The data was acquired and processed using Analyst TF 1.6.1 software (ABSCIEX, Canada).

Raw mass spectra data files were submitted to ProteinPilot software (v.4.5, AB SCIEX) employing the Paragon Algorithm for peptide and protein identification. Separate searches were conducted against the Universal Protein Knowledge Base (UniProtKB, release 2014_09) for *Glycine max* and Fabaceae, each combined with a database of common contaminants. The search parameters were defined as iodoacetamide modified for cysteine alkylation and trypsin as the digestion enzyme. The criteria for positive protein identification were proteins with a ProteinPilot™ unused score $\geq$ 2, $\geq$ 95% confidence and with a minimum of three unique peptides.

2.3. Properties of soymilk and silken tofu

Silken tofu was prepared using a soak procedure described previously (Yang & James, 2013). Briefly, the beans were soaked in deionised water at 15 °C for around 16 h to allow full imbibition. The soaked beans were drained and rinsed before being weighed to determine the quantity of water imbibed. Fresh deionised water was then added and the content homogenised. The slurry was placed in a water bath at 98 °C for 15 min. The soymilk was extracted by centrifugation from the hot slurry and collected and its weight recorded. The solid content of the soymilk was derived from soymilk moisture content. The solid yield of soymilk was calculated as the percentage of total solids in soymilk over those in seeds. Soymilk was allowed to cool to room temperature before a coagulant was added and curd was then placed in an 85 °C water bath for 35 min. The tofu was stored at 4 °C overnight before analyses. The coagulant used was nigari (containing mostly magnesium chloride, food-grade ingredient from The Melbourne Ingredient Depot, Victoria, Australia) and was added to the soymilk at a concentration of 0.3%.

Tofu quality attributes including colour, water loss and tofu hardness were determined as previously described (Yang & James, 2013). Briefly, the colour of tofu was measured using a Minolta Chroma Meter (model CR300, Minolta CO., Osaka, Japan) and expressed in CIE L^* , a^* , b^* values. Tofu hardness was determined with a texture analyser (TA.XT2 Plus, Stable Micro System, UK) and the peak reading recorded during a 75% penetration test was taken as the hardness value in N. The percentage of water loss over the total weight of tofu during a 30 min period of draining was used as a measurement of water holding capacity of silken tofu.

2.4. Statistical analysis

All measurements were performed in three replicates. The data were subject to analysis of variance (ANOVA) according to the general linear procedure of SAS™ (SAS Inc., 1997) to examine the effect of protein content, 11SA4 and their interactions. The means were

separated using the least significant difference at the 5% significance level.

3. Results

Typical SDS–PAGE profiles for the four groups of soybeans with high or low protein content and with or without the globulin polypeptide 11SA4 are shown in Fig. 1. The bands were identified according to the molecular weight of the protein ladder used and also well-established data in the literature. The percentage of major subunits for β -conglycinin (7S) and glycinin (11S) within each band was calculated using densitometry. The major protein(s) identified in the SDS–PAGE bands using nano-LC/MS/MS and data base searches are presented in Table 1.

The mean squares from the analysis of variance revealed some significant impacts that the protein content or the absence of 11SA4 had on seed characteristics and soymilk and tofu quality (Table 2). Protein content significantly influenced seed weight, seed imbibition, soymilk proteins and solid content, tofu colour and tofu gel hardness. No significant effects of protein content on seed moisture and oil content and tofu gel water loss were observed. The effect of protein content on the profile of seed storage proteins was limited to 7S α' , total 7S and total 7S+11S. In comparison, and as expected, the absence of 11SA4 highly significantly influenced the profile of seed storage proteins, with the notable exception of total 7S+11S. Significantly, lack of 11SA4 affected seed size, tofu gel hardness and water loss. Except for seed size and the proportion of total 7S, however, there were no significant protein content $\times$ 11SA4 interactions.

The mean values for seed characteristics and tofu gel quality attributes for the four groups: high protein+11SA4, high protein–11SA4, low protein+11SA4 and low protein–11SA4, and the pooled means for high or low protein contents and for those

with or without 11SA4 are presented in Table 3. It is clear that at either high or low protein level, the absence of 11SA4 was consistently associated with significantly larger seed size ($p < 0.05$). It also significantly improved the tofu gel hardness and water holding capacity when the data for high and low protein content were pooled, although the improvement was only statistically significant at the high protein level for tofu hardness and at the low protein level for water holding capacity. It is interesting to note with the pooled data that the tofu gels from the groups lacking 11SA4 were firmer (0.617 vs. 0.557, $p < 0.05$) despite having lower protein content in the soymilk (46.38 vs. 47.21, $p < 0.05$). With or without 11SA4, the soybeans with high protein content consistently had significantly higher solid and protein content in the soymilk and also produced firmer tofu gels with a creamier and slightly pinker colour. When the data for 11SA4 were pooled, the soybeans with high protein content also had smaller seeds, lower seed oil content and a higher soymilk solid yield. However, there was no effect of protein content on the water holding capacity of the tofu gels.

Table 4 presents the mean values of the proteins separated with SDS–PAGE including 7S and 11S subunits, lipoxygenases and lectin for the four groups with high or low protein content and with or without 11SA4 as well as their pooled means for high or low protein contents and for those with or without 11SA4. As expected, relative to protein content, 11SA4 had a far greater effect on the subunit composition of the major storage proteins. At both protein levels, the absence of 11SA4 resulted in a higher proportion of 7S β and 11SA1/2, 11SB1/2, total 7S subunits and a lower proportion of 11SA1/2/4, 11SB4, total 11SB and total 11S, resulting in a considerably lower ratio of 11S to 7S (1.49 vs. 1.87, $p < 0.001$). Proportionally, the proteins “destined” for the 11SA4 seemed to have been redistributed to 7S β and 11SA1/2. Despite these changes, there was no difference in the total 7S+11S due to the absence of 11SA4. In comparison, higher protein content resulted

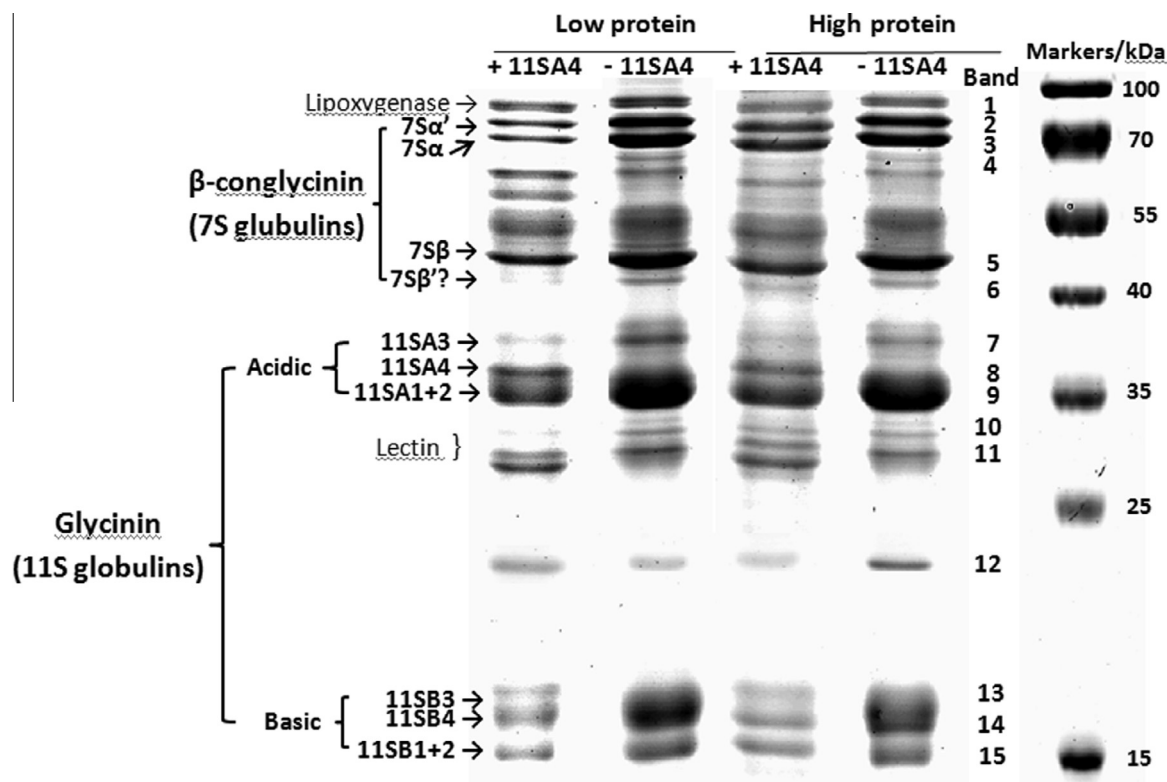


Fig. 1. Typical sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE, 14%) profiles of soybean seed proteins at high or low protein level, and with or without 11SA4. The bands were identified based on the protein ladder used, our previous work and the literature.

Table 1

Proteins in the major bands separated by SDS–PAGE and identified using nano-LC-MS/MS and data base searches.

Band#	Protein#	Accession#	Homologous proteins	Species	pI	MW (kDa)	Protein sequence coverage (%) ^a	Matched peptides ^b
1	1	D6PAW7	Lipoxygenase	<i>Glycine max</i>	6.12	96.8	71.7	65
2	1	Q4LER6	Beta-conglycinin alpha prime subunit	<i>Glycine max</i>	5.50	72.2	72.6	175
3	1	Q94LX2	Beta-conglycinin alpha subunit	<i>Glycine max</i>	5.12	70.3	77.5	164
4	1	I1L860	Sucrose binding protein	<i>Glycine soja</i>	6.08	58.0	66.3	89
5	1	F7J077	Beta-conglycinin beta subunit	<i>Glycine max</i>	5.88	50.5	85.7	108
6	1	Q94LX2	Beta-conglycinin subunit fragment	<i>Glycine max</i>	5.12	70.3	66.6	64
7	1	Q7GC77	Glycinin A3B4 subunit (Gy5)	<i>Glycine max</i>	5.52	58.2	65.2	115
8	1	P04776	Glycinin G1 (Gy1)	<i>Glycine max</i>	5.89	55.7	85.3	51
9	1	P04776	Glycinin G1 (Gy1)	<i>Glycine max</i>	5.89	55.7	83.0	156
10	1	P05046	Lectin	<i>Glycine max</i>	5.65	30.9	85.6	89
11	1	P05046	Lectin	<i>Glycine max</i>	5.65	30.9	79.0	62
12	1	Q94LX2	Beta-conglycinin alpha subunit	<i>Glycine max</i>	5.12	70.3	54.5	36
12	2	Q549Z4	Proglycinin A2B1	<i>Glycine max</i>	5.46	54.4	73.4	45
12	3	I1N747	Oleosin	<i>Glycine max</i>	8.90	23.6	57.5	39
12	4	Q9AVK8	Allergen Gly m Bd 28 K	<i>Glycine max</i>	5.73	52.9	27.1	29
12	6	Q7GC77	Glycinin A3B4 subunit	<i>Glycine max</i>	5.52	58.2	43.3	20
13	1	Q549Z4	Proglycinin A2B1	<i>Glycine max</i>	5.46	54.4	60.4	133
13	3	Q7GC77	Glycinin A3B4 subunit	<i>Glycine max</i>	5.52	58.2	39.1	56
13	4	Q39898	Kunitz trypsin inhibitor	<i>Glycine max</i>	4.86	21.3	78.3	39
14	1	Q549Z4	Proglycinin A2B1	<i>Glycine max</i>	5.46	54.4	59.0	50
14	2	Q9SB11	Glycinin A5A4B3	<i>Glycine max</i>	5.17	63.8	48.9	30
14	3	P13917	Basic 7S globulin	<i>Glycine max</i>	8.68	46.4	53.2	68
14	5	Q9XER5	Seed maturation protein PM22	<i>Glycine max</i>	5.16	16.7	63.8	14
15	1	Q9S9D0	Glycinin G4 subunit	<i>Glycine max</i>	5.38	63.7	49.5	48
15	2	Q549Z4	Proglycinin A2B1	<i>Glycine max</i>	5.46	54.4	56.5	44

^a With ≥95% confidence.^b The number of matched peptides for the protein with 95% confidence.**Table 2**

Mean squares from the analysis of variance for seed and soymilk characteristics and tofu quality of four soybean groups.

	11SA4	Protein	Protein x 11SA4	Error
df	1	1	1	19
Seed wt	78.02***	52.46***	3.27*	0.64
Seed moisture	15.79**	1.44	0.01	1.48
Seed protein	0.12	200.45***	0.50	1.89
Seed oil	2.54*	0.59	0.01	0.35
Seed imbibition	0.00	0.01***	0.001	0.001
Seed globulins				
LOX	3.87*	10.03***	0.49	0.55
7Sα'	0.003	7.49***	1.60	0.46
7Sα	9.13*	7.15	4.97	1.90
7Sβ	63.83***	0.07	0.77	0.73
7S subunit fragment	0.21	0.31	0.14	0.33
11SA3	0.87	0.48	0.03	0.42
11SA4	311.67***	0.26	0.69	0.71
11SA1+2	142.31***	1.88	0.12	2.73
11SA1+2+4	32.89***	3.54	1.42	1.87
Lectin	14.77**	13.06**	0.19	1.44
11SB3	0.35	0.87	0.05	1.32
11SB4	90.46***	1.33	1.61	2.62
11SB1+2	12.23***	0.31	0.02	0.83
Total 11SB	29.43**	7.02	1.39	2.53
Total 7S	110.17***	32.50**	15.94*	2.70
Total 11S	104.44***	14.77	0.03	3.75
7S+11S	0.08	91.03***	14.61	4.83
11S: 7S ratio	1.23***	0.07	0.08	0.02
Soymilk protein	4.13*	126.36***	0.91	0.57
Soymilk solid	0.04	0.91***	0.13	0.06
Solid yield	4.60	20.03*	0.18	4.15
Tofu colour				
L	7.61**	0.58	0.0002	0.75
a	0.21*	2.83***	0.006	0.05
b	0.55	4.79***	0.14	0.22
Tofu hardness	0.03*	0.20***	0.0001	0.004
Tofu water loss	50.16*	6.26	3.00	7.61

* $p < 0.05$.** $p < 0.01$.*** $p < 0.001$.

in a significantly higher proportion of 7Sα' and, more interestingly, total 7S+11S (70.57% vs. 67.26%). A possible 7S subunit, temporarily termed 7Sβ', has been reported in earlier work to be part of the 7S globulins (Coates, Medeiros, Thanh, & Nielsen, 1985; Morita, Fukase, Yamaguchi, Fukuda, & Morita, 1996) although its structure and properties have not been defined. Based on the limited results from literature, the band (#6) immediately below 7Sβ was possibly 7Sβ' although our LC–MS/MS work and data base searches indicated it was likely a 7S subunit fragment.

4. Discussion

The increasing consumption of soy foods in recent years, due mainly to the consumer's increased awareness of their potential health benefits, has resulted in higher demand for quality whole-bean based soy products and soy ingredients. Tofu is a whole-bean based soy food and its key quality attributes, textural hardness and water holding capacity, are important for tofu manufacturers. As protein content impacts positively on these tofu quality attributes, selection for higher protein content has therefore been an important goal for soybean variety destined for soy foods. Our results again confirmed the positive effect of soybean protein content on the textural hardness of tofu gels, agreeing with many reports in the literature (Evans, Tsukamoto, & Nielsen, 1997; James & Yang, 2014; Saio, 1985; Schaefer & Love, 1992; Shen et al., 1991; Wang & Chang, 1995).

However, the strong negative relationship between soybean seed yield and protein content limits the extent to which protein content can be increased without penalising harvest yields. Many other factors can affect the quality and yield of tofu gels, including the content and proportion of glycinin (11S) and β-conglycinin (7S), the major storage proteins in soybean seeds and their subunit composition (Cai & Chang, 1999; Cai et al., 2002; Ji, Cai, & Chang, 1999; Khatib, Aramouni, Herald, & Boyer, 2002; Mujoo, Trinh, & Ng, 2003; Nakamura et al., 1984; Saio, Kamiya, & Watanabe, 1969; Saio & Watanabe, 1978; Stanojevic et al., 2011; Yang &

Table 3

Effect of protein content and 11SA4 on the properties of soybean seed, soymilk and tofu gel.

	High protein+11SA4 (n = 3)	High protein–11SA4 (n = 3)	Low protein+11SA4 (n = 2)	Low protein–11SA4 (n = 3)	Protein, pooled		11SA4, pooled	
					High (n = 6)	Low (n = 5)	With (n = 5)	Without (n = 6)
<i>Seed characteristics</i>								
Seed wt (g/100)	18.45c [#]	22.54b	21.93b	24.63a	20.50	23.28 ^{***}	20.19	23.59 ^{***}
Moisture (%)	9.47c	10.7ab	9.74bc	11.24a	10.08	10.49 ^{**}	9.60	10.97 ^{***}
Protein (%)	44.91a	44.77a	38.93b	39.34b	44.84	39.14 ^{***}	41.92	42.06
Oil (%)	19.78b	20.47a	20.14ab	20.72a	20.12	20.43 [*]	19.96	20.60 [*]
<i>Soymilk properties</i>								
Imbibition	2.26a	2.27a	2.23b	2.22b	2.27	2.23 ^{**}	2.25	2.25
Solid (%)	11.20a	11.01a	10.75b	10.81b	11.11	10.78 ^{***}	10.97	10.91
Solid yield (%)	55.13a	54.25ab	53.44ab	52.86b	54.69	53.15 [*]	54.28	53.55
Protein (%)	49.69a	48.48b	44.72c	44.28c	49.09	44.50 ^{***}	47.21	46.38 [*]
<i>Tofu gel quality</i>								
Colour – L	73.07a	72.12b	72.80ab	71.86b	72.59	72.33	72.93	71.99 ^{**}
Colour – a	0.68a	0.86a	0.13b	0.26b	0.77	0.20 ^{***}	0.40	0.56 [*]
Colour – b	9.97a	10.10a	9.09b	9.47b	10.03	9.28 ^{***}	9.95	9.79
Hardness (N)	0.631b	0.695a	0.483c	0.539c	0.663	0.557 ^{***}	0.557	0.617 [*]
Water loss (%)	9.59ab	7.75b	11.06a	8.03b	8.67	9.54	10.33	7.89 [*]

For comparison between high and low protein levels or with and without 11SA4.

* $p < 0.05$.** $p < 0.01$.*** $p < 0.001$.# Means with different letters within rows are significantly different ($p < 0.05$).**Table 4**

Effect of protein content and 11SA4 on the profile of major soybean seed storage proteins (%).

	High protein+11SA4 (n = 3)	High protein–11SA4 (n = 3)	Low protein+11SA4 (n = 2)	Low protein–11SA4 (n = 3)	Protein, pooled		11SA4, pooled	
					High (n = 6)	Low (n = 5)	With (n = 5)	Without (n = 6)
LOX	4.61c [#]	5.52b	5.95ab	6.37a	5.07	6.16***	5.28	5.95*
7S α'	6.68a	6.24a	5.30b	5.73b	6.46	5.51***	5.99	5.99
7S α	6.51a	6.81a	4.81b	6.63a	6.66	5.72	5.66	6.72*
7S β	8.58b	11.02a	8.37b	11.40a	9.80	9.88	8.47	11.20***
7S subunit fragment	4.13	4.09	4.07	3.77	4.11	3.92	4.09	3.93
11SA3	4.28	4.66	4.58	4.84	4.47	4.71	4.43	4.75
11SA4	7.53a	<1.0b	7.06a	<1.0b	4.37	4.19	7.30	1.26***
11SA1+2	11.49b	15.47a	10.90b	15.10a	13.47	13.00	11.19	15.28***
11SA1+2+4	19.03a	16.67b	17.96a	16.42b	17.85	17.19	18.49	16.54***
Lectin	14.98a	13.52b	16.06a	14.90a	14.25	15.48**	15.52	14.21**
11SB3	6.84	6.96	6.44	6.72	6.90	6.58	6.64	6.84
11SB4	8.35a	5.52b	8.41a	4.70b	6.93	6.55	8.38	5.11***
11SB1+2	6.77b	8.01a	6.63b	7.78a	7.39	7.20	6.70	7.90***
Total 11SB	21.96a	20.49b	21.47a	19.19b	21.23	20.33	21.71	19.84***
Total 7S	25.89b	28.16a	22.53c	27.53a	27.02	25.03**	24.21	27.85***
Total 11S	45.26a	41.82b	44.01a	40.45b	43.54	42.23	44.64	41.14***
7S+11S	71.15a	69.98a	66.54b	67.99b	70.57	67.26***	68.84	68.98
11S/7S	1.78b	1.49c	1.96a	1.49c	1.63	1.72	1.87	1.49***

For comparison between high and low protein levels or with and without 11SA4.

* $p < 0.05$.** $p < 0.01$.*** $p < 0.001$.# Means with different letters within rows are significantly different ($p < 0.05$).

James, 2013, 2014). We have reported previously that the absence of 11SA4 could partially compensate for any negative effects of the lower seed protein content (below 40%) on tofu quality (James & Yang, 2014). The present results further demonstrated that the lack of this polypeptide positively influences the textural hardness and water holding capacity of tofu gels. This was particularly evident in the high protein group, where the improvement in textural hardness was statistically significant, allowing the group with high protein content and without 11SA4 to clearly outperform the other three groups in producing firmer tofu gels. The positive effect of the absence of 11SA4 on tofu texture can also be affected by the coagulants used during processing as well as seed production environment (Nishinari et al., 1991; Poysa et al., 2006; Tezuka et al.,

2000). In addition to specific globulin subunits, the total amount of the two groups of storage proteins, 7S+11S, has been reported to be higher in soybeans of higher protein content (Krishnan, Natarajan, Mahmoud, & Nelson, 2007; Yaklich, 2001), in line with our results here.

Water holding capacity is important for silken tofu manufacturers as the coagulated curd is not pressed during the process and its ability to hold extra amount of water is therefore of practical benefit. The results reported here confirmed those from our previous studies that soybeans null in 11SA4 produce tofu with much improved water holding capacity, for a specific protein level (James & Yang, 2014; Yang & James, 2013, 2014). They offer further support that a stronger gel is likely to be formed when more water

is trapped in the gel network through interaction with the proteins and other constituents in the system, suggesting that a positive structure-function relationship among soybean proteins results from removing 11SA4.

Taken together, our results demonstrate that, in combination with higher protein content, the removal of the 11S globulin polypeptide, 11SA4, could be exploited to further improve the textural hardness and water holding capacity of tofu gels in the selection of food-grade soybean varieties.

Acknowledgements

Research reported here was funded in part by the Grains Research and Development Corporation in Australia under the auspices of the National Soybean Breeding Program. We thank Dr. T. Nguyen for technical assistance, Dr Y. Li for statistical analyses and Mr A. Jones for nano-LC-MS/MS analysis.

References

- 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.
- Cai, T. D., & Chang, K. C. (1999). Processing effect on soybean storage proteins and their relationship with tofu quality. *Journal of Agricultural and Food Chemistry*, 47(2), 720–727.
- Coates, J. B., Medeiros, J. S., Thanh, V. H., & Nielsen, N. C. (1985). Characterization of the subunits of β -conglycinin. *Archives of Biochemistry and Biophysics*, 243(1), 184–194.
- Cui, Z., James, A. T., Mizazaki, S., Wilson, R. F., & Carter, T. E. (2004). Breeding specialty soybeans for traditional and new soyfoods. In K. Liu (Ed.), *Soybeans as functional foods and ingredients* (pp. 264–322). Champaign Illinois: AOCS Press.
- Evans, D. E., Tsukamoto, C., & Nielsen, N. C. (1997). A small scale method for the production of soymilk and silken tofu. *Crop Science*, 37(5), 1463–1471.
- James, A. T., & Yang, A. (2014). Influence of globulin subunit composition of soybean proteins on silken tofu quality. 2. Absence of 11SA4 improves the effect of protein content on tofu hardness. *Crop & Pasture Science*, 65(3), 268–273.
- Ji, M. P., Cai, T. D., & Chang, K. C. (1999). Tofu yield and textural properties from three soybean cultivars as affected by ratios of 7S and 11S proteins. *Journal of Food Science*, 64(5), 763–767.
- Khatib, K. A., Aramouni, F. M., Herald, T. J., & Boyer, J. E. (2002). Physicochemical characteristics of soft tofu formulated from selected soybean varieties. *Journal of Food Quality*, 25(4), 289–303.
- Kim, Y., & Wicker, L. (2005). Soybean cultivars impact quality and function of soymilk and tofu. *Journal of the Science of Food and Agriculture*, 85(15), 2514–2518.
- Krishnan, H. B., Natarajan, S. S., Mahmoud, A. A., & Nelson, R. L. (2007). Identification of glycinin and beta-conglycinin subunits that contribute to the increased protein content of high-protein soybean lines. *Journal of Agricultural and Food Chemistry*, 55(5), 1839–1845.
- Morita, S., Fukase, M., Yamaguchi, M., Fukuda, Y., & Morita, Y. (1996). Purification, characterization, and crystallization of single molecular species of beta-conglycinin from soybean seeds. *Bioscience Biotechnology and Biochemistry*, 60(5), 866–873.
- Mujoo, R., Trinh, D. T., & Ng, P. K. W. (2003). Characterization of storage proteins in different soybean varieties and their relationship to tofu yield and texture. *Food Chemistry*, 82(2), 265–273.
- Nakamura, T., Utsumi, S., Kitamura, K., Harada, K., & Mori, T. (1984). Cultivar differences in gelling characteristics of soybean glycinin. *Journal of Agricultural and Food Chemistry*, 32(3), 647–651.
- Nielsen, N. C., Dickinson, C. D., Cho, T. J., Thanh, V. H., Scallan, B. J., Fischer, R. L., ... Goldberg, R. B. (1989). Characterization of the glycinine gene family in soybean. *Plant Cell*, 1(3), 313–328.
- Nishinari, K., Kohyama, K., Zhang, Y., Kitamura, K., Sugimoto, T., Saio, K., & Kawamura, Y. (1991). Rheological study on the effect of the A5-subunit on the gelation characteristics of soybean proteins. *Agricultural and Biological Chemistry*, 55(2), 351–355.
- Poysa, V., Woodrow, L., & Yu, K. (2006). Effect of soy protein subunit composition on tofu quality. *Food Research International*, 39(3), 309–317.
- SAS Inc. (1997). SAS/STAT Software: changes and enhancements through release 6.12. NC: Cary.
- Saio, K. (1985). Tofu processing characteristics of Japanese domestic soybeans. *Report of the National Food Research Institute*, 47, 128–149.
- Saio, K., Kamiya, M., & Watanabe, T. (1969). Food processing characteristics of soybean-11s and soybean-7s proteins. I. Effect of difference of protein components among soybean varieties on formation of tofu-gel. *Agricultural and Biological Chemistry*, 33(9), 1301–1308.
- Saio, K., & Watanabe, T. (1978). Differences in functional properties of 7s and 11s soybean proteins. *Journal of Texture Studies*, 9(1–2), 135–157.
- Schaefer, M. J., & Love, J. (1992). Relationships between soybean components and tofu texture. *Journal of Food Quality*, 15(1), 53–66.
- Shen, C. F., Deman, L., Buzzell, R. L., & Deman, J. M. (1991). Yield and quality of tofu as affected by soybean and soymilk characteristics – glucono-delta-lactone coagulant. *Journal of Food Science*, 56(1), 109–112.
- Stanojevic, S. P., Barac, M. B., Pesic, M. B., & Vucelic-Radovic, B. V. (2011). Assessment of soy genotype and processing method on quality of soybean tofu. *Journal of Agricultural and Food Chemistry*, 59(13), 7368–7376.
- Tezuka, M., Taira, H., Igarashi, Y., Yagasaki, K., & Ono, T. (2000). Properties of tofus and soy milks prepared from soybeans having different subunits of glycinin. *Journal of Agricultural and Food Chemistry*, 48(4), 1111–1117.
- Wang, C. C. R., & Chang, S. K. C. (1995). Physicochemical properties and tofu quality of soybean cultivar Proto. *Journal of Agricultural and Food Chemistry*, 43(12), 3029–3034.
- Yaklich, R. W. (2001). Beta-conglycinin and glycinin in high-protein soybean seeds. *Journal of Agricultural and Food Chemistry*, 49(2), 729–735.
- Yang, A., & James, A. T. (2013). Effects of soybean protein composition and processing conditions on silken tofu properties. *Journal of the Science of Food and Agriculture*, 93(12), 3065–3071.
- Yang, A., & James, A. T. (2014). Influence of globulin subunit composition of soybean proteins on silken tofu quality. 1. Effect of growing location and 11SA4 and 7S alpha' deficiency. *Crop & Pasture Science*, 65(3), 259–267.