Food Anal. Methods (2016) 9:385–392
DOI 10.1007/s12161-015-0205-8

Comparison of Two Small-Scale Processing Methods for Testing Silken Tofu Quality

Aijun Yang[1] **· Andrew T. James**[1]

Received: 23 April 2015 / Accepted: 18 May 2015 / Published online: 27 May 2015

Abstract In developing a simple, reliable, small-scale method to assess silken tofu quality in our soybean improvement program, we examined two processing methods and two coagulants, glucono-δ-lactone (GDL) or nigari (magnesium chloride) in two experiments. Silken tofu was prepared from a commercial soybean variety (expt 1) or seven soybean varieties (V1–V7) which were grown and harvested together (expt 2). The soybeans were soaked overnight (the soak method, with 55 g soybeans) or ground dry first (the dry method, with 60 g soybeans) before processing. The quality of the silken tofu was evaluated and compared among varieties and coagulant-processing methods and their interactions. Moisture and protein content in soymilk and soybean seeds, soymilk yield and protein and solid recovery in soymilk were determined. Compared with the dry method, the soak method allowed faster soymilk extraction, produced soymilk with lower solid and higher protein content and firmer silken tofu with either GDL or nigari as coagulant. Depending on whether nigari or GDL was used as coagulant, the soak method also produced silken tofu with the highest or the lowest water loss which correlated strongly and negatively with tofu hardness ($r=-0.93$***). Differences were detected among varieties for the key quality attributes. Taken together, the soak method with GDL as coagulant would be the preferred combination to use to assess tofu quality.

Keywords Tofu processing methods · Coagulants · Silken tofu quality · Soybean varieties

✉ Aijun Yang
aijun.yang@csiro.au

1 CSIRO Agriculture Flagship, 306 Carmody Road, St Lucia, Brisbane, Qld 4067, Australia

Introduction

Soybean (*Glycine max*) seeds are processed into an extraordinary range of food and feed ingredients and products. There is great variation in attributes of the seed and in the processing performance of seed into ingredients and products. Tofu is a traditional food product made from whole soybean and is a curd made by coagulation of the protein and oil in soymilk. It is effectively a water-based gel formed through the addition of calcium or magnesium salts or acids to heated soymilk, with soy lipids and proteins trapped in its gel networks (Kohyama et al. 1995). The two main types of tofu are silken which is set in a container, or hard which is pressed. There is wide variation in the basic procedure used to make tofu, but the main steps are producing a slurry of beans with water, then cooking and separating the soluble material from the insoluble. A coagulant such as glucono-δ-lactone (GDL), magnesium chloride or calcium sulphate is then added to the soluble 'milk' component to form a curd (Cui et al. 2004).

Tofu is, nonetheless, an elaborately transformed product manufactured via a range of traditional and modern methodologies. The diversity of manufacturing processes and their interaction with attributes of the raw soybean seed are a challenge which needs to be addressed in an improvement program targeting development of cultivars with improved tofu making potential. There has been much conjecture and some conflicting results published pertaining to variation in attributes of soybean seed and resultant variation in yield and quality of tofu manufactured. Some of this variation is due to non-genetic effects, such as insect damage during the maturity of the crop, adverse weather conditions at the time of grain maturity, damage caused during harvest, handling, storage or storage time. For example, there can be substantial year-to-year or batch-to-batch variation in tofu quality from a single cultivar (Kijima 1997). Pre- and post-harvest handling and storage also affect tofu making potential of the soybean as it can affect protein solubility (Hou et al. 1997). Poor protein solubility may result from beans being stored at too high temperature and moisture content or too low moisture content (Saio 1997; Sasaki 1997), or from damage during harvesting resulting in many split beans (Hou et al. 1997).

There is strong evidence that choice of cultivar strongly affects tofu yield and quality (Cui et al. 2004; Jin and Gai 1995; Qian et al. 1999b). In the early stages of varietal improvement for tofu quality, desirable traits of soybean seeds such as yellow seedcoat and yellow hilum, large round seed, high protein content and moderate oil and sugar contents can be addressed reasonably easily (Cui et al. 2004; Gai et al. 2000; Qian et al. 1999a; Taira et al. 1990). Once these attributes are largely achieved, further improvements are less tangible and require tofu to be made from the beans and its quality to be evaluated. Seeds of differing cultivars need to be grown, harvested and stored as similarly as possible so that varietal differences can be measured with minimal confounding effects (Cui et al. 2004).

Variation in manufacture technique is also considered likely to affect quality and yield of tofu (Beddows and Wong 1987; Cai and Chang 1999). Generally, the traditional method for solubilising the material from soybean seeds is to soak the beans first, then grind the wet beans into a slurry, heat the slurry and separate the soluble material or milk from the remaining material. However, it was discovered that, as a result of reduced lipoxygenase activity, a more bland product could be produced if the beans were ground dry before being blended with hot water to solubilise the material, (Saio 1997). There are advantages in using the dry-grind method with GDL as the coagulant in small-scale evaluation (Evans et al. 1997). These are mostly associated with the convenience of grinding the beans in advance and in higher repeatability of GDL in coagulation. However, nearly all manufacturers of tofu in Australia use an overnight soak of beans as it gives a higher solubility of protein (Saio 1997) and use either nigari or a nigari-GDL mix to coagulate the soymilk because of the better flavour. Recent results from Australia have shown that both variety and processing conditions affect silken tofu quality (Yang and James 2013). In developing and refining a suitable, small-scale method to make silken tofu for use in our soybean improvement program for human consumption, we adapted the dry method (Evans et al. 1997) to our laboratory and compared it with a more commonly used soak method. Two main types of coagulants were also examined in order to determine an appropriate coagulant-processing method combination to use.

Materials and Methods

In expt 1, a commercial soybean (*Glycine max*) variety commonly used in Australia for manufacture of silken tofu was used. The seeds were harvested in Queensland in 2008 and had a moisture content of 9.2 % and a protein content of 41.8 %. In expt 2, samples from seven soybean varieties (V1–V7) that were grown and harvested together in 2009 at Cecil Plains, Queensland, Australia (27° 34′ S, 151° 12′ E) were chosen on the basis of their general acceptability for human consumption. The harvested beans were kept together in an air-conditioned laboratory (~21 °C) and used within 12 months. The moisture content of the soybeans was approximately 8.0–8.4 %. GDL (reagent grade) and nigari (containing mostly magnesium chloride, food-grade ingredient from the Melbourne Ingredient Depot, Victoria, Australia) were used as coagulants. Three commercial silken tofu products were also purchased from retail outlets and evaluated.

Preparation of Soymilk

For small-scale silken tofu making, we developed a small-scale adaptation of the commonly used wet grind technique (the soak method) and a dry method which was adapted to suit our laboratory from that published by Evans et al. (1997) (the dry method). In the soak method, 55 g dry soybean seeds were soaked in around 300 ml deionised water at 15 °C for ~16 h. The soaked beans were then drained and rinsed with deionised water. Fresh deionised water was added to the soaked beans to a total weight of 400 g, and the content was homogenised in a domestic blender (Breville Optiva) on high setting for 3 min. The slurry was transferred into a plastic tub and cooked at around 98 °C for 15 min. The hot slurry was poured slowly into a domestic centrifugal juice extractor (Breville Juice Fountain Plus) running on high setting to extract soymilk. The soymilk was collected, and its weight was recorded. The bean/water ratio was 1:6.27.

For the dry method, we substantially simplified the instruments and steps required from those reported by Evans et al. (1997) and reduced the amount of ground soybean used by 25 % from 80 to 60 g. We eliminated the use of antifoam agent as we did not find it necessary in our preliminary studies. Instead of using a steam hose to heat the soybean slurry, the slurry was cooked in a water bath which enabled a greater number of samples to be prepared simultaneously under near-identical cooking treatment. The degassing and filtering of resulting soymilk were also found to be non-essential. Briefly, soybean seeds were ground in a domestic coffee grinder (Breville) for 90 s then transferred to a pre-warmed blender to which 400 g fresh boiling water was added. The content was then homogenised on high setting for 3 min. The hot slurry was poured through a funnel into a commercial juice extractor running on high setting to extract soymilk. The soymilk was collected, and its weight was recorded. The bean/water ratio was 1 to 6.67. For the dry method in expt 1, the same bean/water ratio used in the soak method (1:6.27) was also examined for its impact on tofu texture.

The soymilk yield was expressed as the percentage of total solid in soymilk per 100 g raw soybeans on dry basis. The protein recovery was the percentage of total protein in soymilk per 100 g raw soybeans on dry basis.

Preparation of Silken Tofu

Our development work showed that over the coagulant concentration range of 0.1 to 1.0 %, texture peaked at around 0.3 % for both GDL and nigari before it started declining (data not shown). The final coagulant concentration of 0.3 % in soymilk was therefore chosen to examine an acid, GDL, and a magnesium salt, nigari, in forming silken tofu. Soymilk, 220 ml, was decanted into a round plastic container and was allowed to cool to below 30 °C before 7 ml of 9.73 % coagulant solution was added and stirred thoroughly. The container was covered with a lid pierced with a hole to permit gas escape. The container was placed in an 85 °C water bath for 35 min for curd formation to occur. The tofu was cooled and stored at 4 °C overnight before being analysed. Tofu was made in three replicates for each variety and combination of coagulant and processing method.

The procedure for both the dry and soak methods to make silken tofu is summarised in a flowchart in Fig. 1.

Seed Weight, Moisture, Solid and Protein Determination

One hundred raw soybean seeds were weighed, and the weight (g/100 seeds) for each variety was recorded as the seed weight. The moisture and solid content in soymilk and ground soybean seeds were determined by placing weighed samples of soymilk (~10 g) or ground soybeans (~3 g) in an oven at 105 °C for 24 h. The dried soymilk was then ground, and the nitrogen content for dried soymilk and soybean seed was analysed using LECO TrueSpec CHN analyser (Leco Corp., St Joseph, MI, USA) (Australian Laboratory Handbook of Soil and Water Chemical Methods 1992). The total crude protein content was obtained by multiplying total nitrogen content with a conversion factor of 6.25.

Tofu Colour

The colour of tofu was measured using a Minolta colorimeter (model CR300, Minolta CO., Osaka, Japan) and expressed in CIE L^*, a^*, b^* values where the value ranges from 0 (dark black) to 100 (white) for L, $+a^*$ (red) to $-a^*$ (green) and $+b^*$ (yellow) to $-b^*$ (blue). The meter was calibrated with a standard white tile using the followings parameters: Y=92.3, x=0.3134 and y=0.3195. Tofu was equilibrated to room temperature (~21 °C) before colour was measured. Two colour measurements were made on each tofu slab, and the mean was used for each of the three replicates.

Tofu Texture

Following the colour measurement, tofu texture was determined on a texture analyser (TA.XT2 Plus, Stable Micro System, UK) fitted with a 30-kg load cell. An aluminium probe (35 mm i.d.) compressed the centre of the tofu slab from 30 to 15 mm (50 % compression). The compressing speed was 1 mm/s, the pre-test speed was 2 mm/s and the post-test speed was 10 mm/s. The peak force (kg) reached during the compression was recorded and taken as the hardness of the tofu and used as a measure for silken tofu texture.

Water Loss

After the texture measurement, the tofu was weighed with the container before the whole tofu slab was placed onto a flat mesh which was positioned over a plastic container. Water was allowed to drain for 20 min, and the amount of water drained was recorded. Water loss was calculated as the percentage of the amount of water drained over the total weight of tofu and used as a measurement of the water holding capacity of tofu.

Statistical Analysis

All measurements in this study were replicated three times. The data were subject to analysis of variance (ANOVA) to examine the effect of processing methods, coagulants or varieties and their interactions. The means were separated using the least significant difference (LSD) at the 5 % significance level. A simple Pearson correlation coefficient method was used to test for the potential correlations among various variables.

Results and Discussion

Experiment 1

This experiment was conducted to examine the two types of processing methods, the soak and the dry, for soymilk properties and the key silken tofu quality attribute, texture. The results are presented in Table 1. There were significant differences ($P<0.05$) between the processing methods in the yield and solid and protein content of soymilk. The dry methods produced more soymilk which contained more solid than the soak method even when a lower bean/water ratio was used. The protein content, however, was significantly higher in the soymilk from the soak method. The solid content of 10.89 % for the soak method was lower than we expected. We tested a range of bean/water ratios for both the dry and the soak method in the preliminary studies aiming for a solid content of 11.5–12 % in soymilk. It was found that 60 g dry ground

Fig. 1 Flowchart for making silken tofu using a dry or a soak method

The Dry Method	The Soak Method
Grind soybean seeds in a coffee grinder	Soak soybeans (55 g) in dH_2O at 15°C for 16 h
↓	↓
Rinse the blender with boiling dH_2O	Drain and wash the soaked beans
↓	↓
Add ground soybean (60 g) and boiling dH_2O (400 g)	Add fresh dH_2O (to 400 g total weight)
↓	↓
Homogenise the beans and water in a blender for 3 min	Homogenise the soaked beans and water in a blender for 3 min
↓	↓
Extract soymilk in a juice extractor	Cook the soybean slurry at 98°C for 15-20 min
↓	↓
Record soymilk wt and cool	Extract soymilk in a juice extractor
↓	↓
Add coagulant and stir thoroughly	Record soymilk wt and cool
↓	↓
Curd formation at 85°C for 35 min	Add coagulant and stir thoroughly
↓	↓
Store at 4°C until analysis	Curd formation at 85°C for 35 min
	↓
	Store at 4°C until analysis

soybean in 400 g water (a ratio of 1 to 6.67, *w*/*w*) for the dry method and 55 g dry soybean in 400 g water (a ratio of 1 to 6.27, *w*/*w*) for the soak method was an optimum and practical amount of soybean and water to use in making silken tofu in

Table 1 Characteristics of soymilk and silken tofu processed using a soak, a dry or a dry method with the same bean/water ratio as the dry method (sDry) (expt 1, n=4)

Soymilk					Silken tofu				
	Bean/water ratio	Yield (g/g bean)	Solid (%)	Protein (%)		L^*	a^*	b^*	Hardness (kg)
Soak	1: 6.27	4.85 b	10.89 c	47.31 a	Soak-GDL	82.6 a	0.47 b	10.7 d	0.304 a
					Soak-nigari	81.3 c	0.62 a	11.2 c	0.291 a
Dry	1: 6.67	5.55 a	11.83 b	44.72 b	Dry-GDL	81.2 c	0.28 c	11.2 c	0.238 b
					Dry-nigari	82.7 a	0.02 d	11.6 bc	0.191 c
sDry	1: 6.27	5.32 a	12.64 a	44.65 b	sDry-GDL	81.9 bc	0.27 c	11.8 ab	0.251 b
					sDry-nigari	82.6 a	0.12 d	12.0 a	0.186 c

Means with the same lowercase letters within columns are not significantly different (P>0.05) our laboratory. As expected, the soymilk from the dry method with a higher bean to water ratio (1:6.27), the same as the soak method, contained significantly more solid (12.6 %) than that from the dry method with a lower bean to water ratio (1 : 6.67, 11.8 %, $P<0.05$). Their protein content, however, was similar.

These results suggest that the same processing method, irrespective of bean to water ratio, would produce soymilk with different solid content but similar protein content. Under the processing conditions tested, the soak method produced soymilk which, despite containing significantly less solid, had substantially more protein than the dry method regardless of the bean to water ratio used in the dry method. The soak method also produced silken tofu with firmer texture ($P<0.05$), suggesting that it was the protein, and not the solid, content of the soymilk that determined the texture of silken tofu. It would therefore appear that, while more solid was extracted into soymilk from ground soybeans in the dry methods, not all the solids were fully solubilised and contributed to subsequent gel matrix formation. Overnight soaking of soybean seeds in the soak method, on the other hand, seemed to result in a more efficient solubilisation and extraction process for the proteins in the seed, as reported by Saio (1997).

Experiment 2

Physical and Chemical Properties of Soybeans and Soymilk

Table 2 shows the properties of soybean seeds of the seven varieties used in this experiment and soymilk produced from these varieties using the two processing methods. Samples from the varieties selected for this study had a very wide range in seed weight (9.6 to 23.6 g/100 seeds, $P<0.05$). Except for V5, all varieties had seed size within the range that is generally accepted by tofu manufacturers in Australia. The protein content in the soybean seed ranged from 42.4 to 45.7 % ($P<0.05$) with the lower end of that range being considered marginal for acceptance by tofu manufacturers. Although the range in seed moisture content was statistically significant (8.02 to 8.39 %, $P<0.05$), the practical differences were quite small.

There were significant varietal differences ($P<0.05$) in the solid and protein content of soymilk for both processing methods although no varietal differences were observed in soymilk yield and solid and protein recovery for either method. When the data from the seven samples were combined, the two processing methods differed significantly in the soymilk produced ($n=21$, $P<0.05$). As observed in expt 1, the dry method produced more soymilk which contained less protein and more solid and, as a result, had higher solid and protein recovery than the soak method. Grinding the beans first led to more solids being extracted into the soymilk (12.0 vs 11.6 %, $P<0.05$) although a lower proportion of these solids were proteins as evidenced by the lower protein content of the soymilk from the dry method (45.6 vs 48.5 %, $P<0.05$). The solid content in soymilk was within a narrow range for both the dry (11.8–12.3 %) and the soak (11.3–11.9 %) method and close to our target and within the range for commercial silken tofu (9.6–12.6 %). The lower solid (57.7 vs 51.7 %) and protein (64.3 vs 60.4 %) recovery of soymilk from the soak method was likely due to the lower amount of soymilk produced for a given weight of soybean from the soak method (4.87 vs 5.24 g, $P<0.05$) and lower solid content in this soymilk (11.6 vs 12.0 %, $P<0.05$) than the dry method. In the preliminary studies, we observed batch to batch variations in soymilk yield even when the same blender and extractor were used. The solid content of soymilk, however, was relatively constant despite this variation in volume. These results on the soymilk

Table 2 Characteristics of soybean seeds and soymilk processed using a dry or soak method (expt 2, $n=3$)

	Soybean seeds			Soymilk protein (% DM)		Soymilk solid (%)		Soymilk wt (g/g soybean)		Solid yield (%)		Protein recovery (%)	
Variety	Seed wt (g/100)	Protein (% DM)	Moisture (%)	Dry method	Soak method	Dry method	Soak method	Dry method	Soak method	Dry method	Soak method	Dry method	Soak method
1	16.97 d	44.1 c	8.39 a	46.1 ab	47.5 c	11.8 c	11.9 a	5.29	4.69[a]	57.4	51.0[a]	65.5	58.9[a]
2	23.61 a	42.4 d	8.22 b	45.3 bc	47.6 c	12.1 b	11.3 d[a]	5.60	4.95[a]	61.9	51.4[a]	71.1	61.3[a]
3	19.53 c	43.0 d	8.37 a	44.7 c	48.7 b[a]	12.1 b	11.7 b[a]	5.31	4.74[a]	58.7	50.7[a]	65.6	61.2
4	20.42 b	44.4 bc	8.25 b	44.6 c	47.6 c[a]	11.8 c	11.4 cd[a]	5.12	4.95	55.5	51.8[a]	61.0	59.3
5	9.64 e	45.3 a	8.02 c	46.5 ab	50.8 a[a]	11.9 bc	11.5 c[a]	5.08	4.95	55.5	52.4	61.4	62.2
6	19.76 c	45.2 ab	8.06 b	45.5 abc	48.4 bc[a]	12.0 bc	11.7 b[a]	5.17	4.94	56.9	52.7[a]	63.1	60.4
7	19.71 c	45.7 a	8.04 c	46.3 ab	48.8 b[a]	12.3 a	11.7 b[a]	5.12	4.85	57.7	52.0[a]	62.1	59.1
Pooled ($n=21$)	18.52	44.3	8.19	45.6	48.5[a]	12.0	11.6[a]	5.24	4.87[a]	57.7	51.7[a]	64.3	60.4[a]

For variety comparisons, means with the same lowercase letters within a column are not significantly different ($P>0.05$)

[a] The two methods differed significantly for the attribute within a variety or the average of seven varieties ($P<0.05$)

yield, solid content and solid and protein recovery of soymilk were comparable to those reported by others using the dry-grind method to make silken tofu (Evans et al. 1997; Yang and James 2013).

Tofu Texture

Of the many attributes of importance for tofu quality, texture is considered the most important quality parameter by tofu manufacturers. While there were no significant differences (P>0.05) in texture resulting from variety × coagulant-method interactions, there existed substantial varietal and method differences (Table 3). Silken tofu made with V2, V1 and V5 using the soak method ranked higher than that from the other four varieties using either GDL or nigari as coagulant. With the dry method, there appeared to be two distinct groups for texture with V2, V5 and V7 giving firmer tofu texture than the other four varieties for both GDL and nigari. Compared with the dry method, the soak method clearly produced silken tofu with much firmer texture (0.30 vs 0.13 kg, P<0.05), particularly when GDL was used. It is likely that the higher protein content of the soymilk produced by the soak method contributed to the firmer texture. These results agreed with many studies in the literature reporting that the protein content in soymilk is of prime importance in producing tofu with firm texture (Evans et al. 1997; James and Yang 2014; Saio 1985; Shen et al. 1991; Wang and Chang 1995). The combination of the soak method with GDL as coagulant produced silken tofu with the hardest texture (0.381 kg, P<0.05), followed by that of the soak method with nigari (0.218 kg, P<0.05) while the dry method produced much softer tofu with either GDL (0.138 kg) or nigari (0.123 kg). With GDL, the texture of silken tofu from the soak method was about 2.8 times that from the dry method. The impact of nigari on texture was, however, less profound with tofu from the soak method being about 1.8 times firmer than that from the dry method. The texture of commercial 'silken tofu' products varied greatly, and the hardness value of what we believed to be genuine silken tofu (i.e. tub set without any pressing) ranged from 0.36 to 0.54 kg. The soak method with GDL as coagulant therefore produced silken tofu that was similar to commercial products in texture.

Water Loss of Tofu

Water holding capacity is another important parameter for silken tofu as the coagulated curd is not pressed in the manufacturing process. Curds that hold more water are therefore more valuable to the manufacturer. There were significant interactions between variety and processing-coagulant methods (Table 3, P<0.05) for water loss. For example, while V2, V5 and V7 had lower water loss than V1, V3, V4 and V6 in the dry method with either GDL or nigari (P<0.05), they ranked differently in the soak method depending on whether GDL or nigari was used for coagulating. The effect of processing methods and coagulants on water loss of tofu was clearly shown in Table 3. Silken tofu made with the soak-GDL combination had the lowest water loss (10.8 %, P<0.05), followed by that with the dry-GDL (14.0 %) or nigari (15.4 %) combination with silken tofu made with the soak-nigari combination having the highest water loss (18.0 %, P<0.05). The higher water loss in tofu using the soak-nigari combination confirmed our visual observation that silken tofu produced using this procedure clearly contained more water some of which was accumulated around the tofu slab. The commercial silken tofu products we tested all had water loss of less than 5 %.

Water loss of tofu correlated strongly and negatively with tofu texture (r=−0.93, P<0.0001, n=12) with tofu having the lowest water loss also displaying the firmest texture confirming recently reported results (James and Yang 2014; Yang and James 2013, 2014). This negative relationship

Table 3 Hardness and water loss of silken tofu prepared using a dry or soak method with 0.3 % GDL or nigari (expt 2, n=3)

	Hardness (kg)				Water loss %			
Variety	Dry-GDL	Dry-nigari	Soak-GDL	Soak-nigari	Dry-GDL	Dry-nigari	Soak-GDL	Soak-nigari
1	0.106 b^{3}	0.081 d^{3}	0.404 b^{1}	0.271 a^{2}	17.1 a^{1}	18.8 a^{1}	9.9b c^{3}	16.1 c^{2}
2	0.178 a^{3}	0.141 b^{3}	0.476 a^{1}	0.275 a^{2}	12.1 c^{2}	13.2 c^{2}	5.3 d^{3}	16.6 c^{1}
3	0.117 b^{3}	0.109 c^{3}	0.365 bc^{1}	0.203 bc^{2}	14.4 b^{2}	15.5b c^{2}	13.4 a^{2}	18.9 b^{1}
4	0.104 b^{2}	0.101 c^{2}	0.324 c^{1}	0.162 c^{2}	15.6 ab^{3}	18.0 ab^{2}	14.3 a^{3}	21.4 a^{1}
5	0.180 a^{3}	0.154 b^{3}	0.396 b^{1}	0.244 ab^{2}	12.3 c^{2}	12.7 c^{2}	8.1 c^{3}	16.7 c^{1}
6	0.102 b^{3}	0.103 c^{3}	0.374 bc^{1}	0.168 c^{2}	16.0 ab^{2}	17.0 ab^{2}	13.3 a^{3}	20.1 ab^{1}
7	0.177 a^{2}	0.171 a^{2}	0.328 c^{1}	0.203 bc^{2}	10.4 c^{3}	12.6 c^{2}	11.4 b^{23}	16.5 c^{1}
Pooled (n=21)	0.138^{c}	0.123^{c}	0.381^{a}	0.218^{b}	14.0^{b}	15.4^{b}	10.8^{c}	18.0^{a}

For variety comparisons, means with the same letter within a column are not significantly different (P>0.05). For method-coagulant combinations within each variety, means with the same superscript within a row for hardness or water loss are not significantly different (P>0.05) between tofu texture and water loss was clearly illustrated in Fig. 2 with the ranked means for the seven varieties. This would appear to indicate that water retained in tofu could have formed a strong gel by binding to the proteins and other constituents of soymilk. Information on the relationship between tofu texture and water holding in the literature is rather limited. However, both significantly positive (Lim et al. 1990) and negative (Mujoo et al. 2002) correlations between tofu texture and tofu moisture have been reported while Kim and Wicker (2005) found no effect of tofu moisture content on tofu hardness.

Tofu Colour

The colour of soybean products is an important trait in the selection of soybean for the food industry. Desirable silken tofu should be white or light creamy white, i.e. with high L^* value and low a^* and b^* values. As tofu becomes yellow (high b^* value) with storage, soybean lines that produce yellow products will be discriminated against as consumers will perceive it as being aged stock. Tofu colour was evaluated using the *CIE* $L^*a^*b^*$ colour system in this study, and the results (Table 4) showed that silken tofu made with nigari tended to have higher a^* and b^* values than that with GDL for both the dry and the soak method while their L^* values were similar ($P<0.05$). This would indicate a light creamy colour for tofu made with nigari, agreeing with those reported in the literature (Khatib et al. 2002; Obatolu 2008). The lightness of tofu, L^* value, correlated negatively with a^* value ($r=-0.83$, $P<0.05$) although no significant differences were found between processing methods and coagulants used for L^* value or b^* value. There were no significant differences ($P>0.05$) in colour resulting from variety × coagulant-method interactions.

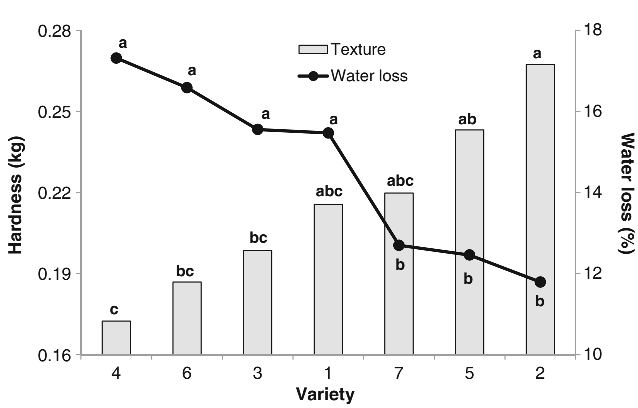

Fig. 2 Relationship between tofu texture and water loss in seven soybean varieties grown in Australia ($n=12$, $r=-0.93$, $P<0.001$). Values were the means of the combinations of two production methods and two coagulants, in three replicates. Means with the same letter for each graph type are not significantly different ($P>0.05$)

Table 4 Colour of silken tofu prepared using a dry or soak method with 0.3 % GDL or nigari (expt 2, $n=3$)

Variety	L^*				a^*				b^*			
	Dry-GDL	Dry-nigari	Soak-GDL	Soak-nigari	Dry-GDL	Dry-nigari	Soak-GDL	Soak-nigari	Dry-GDL	Dry-nigari	Soak-GDL	Soak-nigari
1	81.2 ab	81.0 b	80.8 a	80.2	0.96 bcd^{2}	0.4 b^{3}	1.65 a^{1}	1.13 a^{2}	10.8 bc^{12}	11.3 bc^{1}	10.4 b^{2}	11.1 b^{1}
2	81.4 a^{12}	82.0 a^{1}	79.7 b^{3}	80.8 ^{2}	0.87 cd^{1}	0.3 bc^{2}	0.91 b^{1}	1.04 a^{1}	11.1 b^{1}	11.4 bc^{1}	10.4 b^{2}	11.5 b^{1}
3	81.0 ab^{12}	81.7 ab^{1}	80.7 a^{12}	80.6 ^{2}	1.38 b^{1}	0.38 bc^{3}	0.93 b^{2}	0.64 ab^{23}	10.7b c^{3}	11.4 bc^{12}	10.9 a^{23}	11.5 b^{1}
4	81.1 ab	81.4 ab	81.0 a	80.9	1.29 bc^{1}	0.30 bc^{3}	0.87 b^{12}	0.56 b^{23}	10.4 c^{2}	11.0 c^{1}	10.2 b^{2}	11.0 b^{1}
5	80.4 bc	81.0 bc	81.2 a	80.9	0.56 d^{1}	0.09 c^{2}	0.92 b^{1}	0.56 b^{1}	11.6 a^{2}	12.1 a^{1}	10.9 a^{3}	11.4 b^{2}
6	81.5 a	81.1 ab	80.7 a	80.6	1.36 b^{1}	0.62 b^{2}	0.86 b^{2}	0.55 b^{2}	9.4 d^{3}	10.0 d^{2}	10.1 b^{2}	11.2 b^{1}
7	79.5 c^{12}	80.2 c^{1}	78.6 c^{2}	80.1 ^{1}	1.87 a^{1}	1.27 a^{2}	1.66 a^{12}	0.67 ab^{3}	10.9 b^{2}	11.6 b^{1}	11.0 a^{2}	11.7 a^{1}
Pooled ($n=21$)	80.9	81.2	80.2	80.4	1.18^{a}	0.49^{b}	1.11^{a}	0.74^{ab}	10.7^{b}	11.3^{a}	10.6^{b}	11.3^{a}

For variety comparisons, means with the same letter within a column are not significantly different ($P>0.05$). For method-coagulant combinations within each variety, means with the same superscript within a row for L^*, a^* or b^* are not significantly different ($P>0.05$)

Conclusions

Both the dry and the soak processing methods, together with either GDL or nigari as coagulant, reported in this study are able to detect the difference in major quality parameters of silken tofu prepared from a range of soybean varieties. Varieties ranked similarly for texture, the most important quality attribute of silken tofu irrespective of the method. However, the soak method combined with GDL as coagulant produced silken tofu with firmer texture and superior water holding capacity most closely approximated to commercial products. This combination would therefore be our preferred choice to use in our soybean improvement program. In addition, the soak procedure allows faster soymilk extraction, produces soymilk with higher protein content and is the predominant method used by tofu manufacturers.

Acknowledgments Work reported here was conducted under the auspices of the Australian Soybean Breeding Program funded in part by the Grains Research and Development Corporation, Australia.

Compliance with Ethical Standards Funding: Grains Research and Development Corporation, Australia (grant number GRDC CSP00157).

Conflict of Interest Aijun Yang declares that she has no conflict of interest. Andrew James declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

References

Australian Laboratory Handbook of Soil and Water Chemical Methods (1992) Eds GE Rayment and FR Higginson. Inkata Press. Section 6B3 p36

Beddows CG, Wong J (1987) Optimization of yield and properties of silken tofu from soybeans. 1. water-bean ratio. Int J Food Sci Technol 22:15–21

Cai TD, Chang KC (1999) Processing effect on soybean storage proteins and their relationship with tofu quality. J Agric Food Chem 47:720–727

Cui Z, James AT, Mizazaki S, Wilson RF, Carter TE (2004) Breeding specialty soybeans for traditional and new soyfoods. In: Liu K (ed) Soybeans as functional foods and ingredients. AOCS Press, Champaign, pp 264–322

Evans DE, Tsukamoto C, Nielsen NC (1997) A small scale method for the production of soymilk and silken tofu. Crop Sci 37:1463–1471

Gai JY, Qian HJ, Ji DF, Wang MJ (2000) A study on the inheritance of dried tofu output of soybean. Acta Genet Sin 27:434–439

Hou HJ, Chang KC, Shih MC (1997) Yield and textural properties of soft tofu as affected by coagulation method. J Food Sci 62:824–827

James AT, 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 Sci 65:268–273

Jin J, Gai J (1995) A study on genetic variation in tofu yield, quality and processing traits of soybean landraces. J Nanjing Agric Univ 18:5–9

Khatib KA, Aramouni FM, Herald TJ, Boyer JE (2002) Physicochemical characteristics of soft tofu formulated from selected soybean varieties. J Food Qual 25:289–303

Kijima H (1997) Manufacture of tofu. In: Watanabe T (ed) Science of tofu. Food J CO, Ltd Kyoto, Japan, pp 14–29

Kim Y, Wicker L (2005) Soybean cultivars impact quality and function of soymilk and tofu. J Sci Food Agric 85:2514–2518

Kohyama K, Sano Y, Doi E (1995) Rheological characteristics and gelation mechanim of todu (soybean curd). J Agric Food Chem 43: 1808–1812

Lim BT, Deman JM, Deman L, Buzzell RI (1990) Yield and quality of toru as affected by soybean and soymilk characteristics - calcium sulphate coagulant. J Food Sci 55:1088–1092

Mujoo R, Trinh DT, Ng PKW (2002) Evaluation of soybean varieties for soymilk and tofu production potential using laboratory-developed procedures. Food Sci Biotechnol 11:470–476

Obatolu VA (2008) Effect of different coagulants on yield and quality of tofu from soymilk. Eur Food Res Technol 226:467–472

Qian H, Gai J, Ji D, Wang M (1999a) Correlations of tofu yield and quality with seed nutrients and processing traits. J Chin Cereals Oils Assoc 14:35–39

Qian H, Yu D, Wang M, Song Q, Gai J (1999b) Genetic variation among landraces and inheritance of soymilk and tofu processing-related traits. In: Kaufman HE (ed) Proceedings of VI World Soybean Research Conference, Chicago, pp 481–482

Saio K (1985) Tofu processing characteristics of Japanese domestic soybeans. Report of the National Food Research Institute 128–149

Saio K (1997) What are soybeans? In: Watanabe T (ed) Science of tofu. Food J CO, Ltd Kyoto, Japan, pp 77–103

Sasaki I (1997) Material soybeans. In: Watanabe T (ed) Science of tofu. Food J CO, Ltd Kyoto, Japan, pp 68–76

Shen CF, Deman L, Buzzell RI, Deman JM (1991) Yield and quality of tofu as affected by soybean and soymilk characteristics - glucono-delta-lactone coagulant. J Food Sci 56:109–112

Taira H, Tanaka H, Saito M (1990) Quality of soybean seeds grown in Japan. 20: Differences in sugar contents of soybeans cultured by upland and drained paddy field. J Jpn Soc Food Sci 37:602–611

Wang CCR, Chang SKC (1995) Physicochemical properties and tofu quality of soybean cultivar Proto. J Agric Food Chem 43:3029–3034

Yang A, James AT (2013) Effects of soybean protein composition and processing conditions on silken tofu properties. J Sci Food Agric 93: 3065–3071

Yang A, James AT (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 Sci 65:259–267